ISSN 1029-533X

NEPAL AGRICULTURE RESEARCH JOURNAL

Volume 8, 2007

Nepal Agricultural
Research Council (NARC)

Society of Agricultural
Scientists Nepal (SAS-N)

Nepal Agriculture Research Journal

Published annually by the Society of Agricultural Scientists Nepal (SAS-N) and Nepal Agricultural Research Council (NARC) since 1997. It could not be published in 2001, 2002 and 2003. **Subscription rates per copy is Rs200 for individual, Rs500 for institution, $15 for SAARC countries and $25 for overseas. Publication charge per article is Rs500 for non-member ($25.00 for foreigner).** SAS-N member can publish their articles free of cost. In case of articles with many authors, one of the authors must be the member of the Society for free publication in the journal.

Original (not published or submitted for publication elsewhere) research, review and feature articles written in English from members of the Society of Agricultural Scientists Nepal (SAS-N) and other interested scientists or technicians in all aspects of agricultural research particularly in the field of agriculture, animal science, agro-forestry, post harvest technology and several other topics related to agriculture will be accepted. Research note may also be published in the journal.

Editorial Board

CORRESPONDING ADDRESS
SAS-N
Bal K. Joshi
Biotechnology Unit-NARC, Khumaltar
PO Box 1135 Kathmandu, Nepal
Tel: 977-01-5521615

The Society of Agricultural Scientists, Nepal (SAS-N) is a professional organization that serves as a common forum for the agricultural scientists and researchers. SAS-N got officially registered in District Administration Office, Lalitpur on 5 June 1995 with registration no. 490/051/52.

Nepal Agricultural Research Council (NARC), established in 1991, is an apex body for agricultural research in the country.

The views expressed in this journal are those of the author/s.

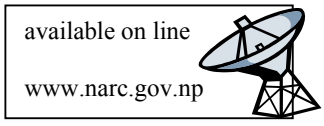
REVIEWERS OF THIS VOLUME

Binesh M. Sakha, NPRP-NARC, Khumaltar
Bishnu R. Ojha, IAAS, Rampur
Buddhi P. Sharma, NPRP-NARC, Khumaltar
Dinesh Pariyar, PFRD-NARC, Khumaltar
Gajendra S. Niroula, NPDP, Department of Agriculture, Khumaltar
Gyanu Manandhar, PPD-NARC, Khumaltar
Hari B. KC, ABD-NARC, Khumaltar
Hari K. Shrestha, NARC, Kathmandu
Hira K. Manandhar, NARC, Kathmandu
Jwala Bajracharya, ABD-NARC, Khumaltar
Khem R. Bhatarai, Department of Plant Resources, Godawari
Laxman Sherchand, Khumaltar
Madhav Joshi, AD-NARC, Khumaltar
Madhusudan P. Ghale, HRD-NARC, Khumaltar
Mana R. Kolakshepati, IAAS, Rampur
Naresh S. Thakur, SED-NARC, Khumaltar
Nava R. Devkota, IAAS, Rampur
Purushottam P. Khatiwada, HRD-NARC, Khumaltar
Ram B. KC, ARS-NARC, Pokhara
Ram C. Adhikari, NARI-NARC, Khumaltar
Ram D. Timila, PPD-NARC, Khumaltar
Rama N. Yadav, IAAS, Rampur
Sangita Shrestha, NAST, Khumaltar
Shree M. Shrestha, Agriculture Engineering Division-NARC, Khumaltar
Shreechan Shah, IAAS, Rampur
Subarna M. Pradhan, NASRI-NARC, Khumaltar
Sundar K. Shrestha, PPD-NARC, Khumaltar
Sushil Pradhan, ICIMOD, Khumaltar
Yug N. Ghimire, Outreach Research Division-NARC, Khumaltar

INSTRUCTIONS TO AUTHORS

Please see Volume 6, 2005.

Cover photo: Bhate phapar (*Fagopyrum tataricum* Gaertn. ssp *tataricum*)



Nepal Agriculture Research Journal **Vol. 8, 2007**

CONTENTS

Optimization of PCR Conditions for DNA Amplification of Common Buckwheat Using EST Primers

Bal K. Joshi*, Kazutoshi Okuno, Ryo Ohsawa and Takashi Hara

Laboratory of Plant Breeding, Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan

* Current address: Nepal Agricultural Research Council, Kathmandu, Nepal <joshibalak@yahoo.com>

ABSTRACT

Under optimal conditions the PCR reaction is very efficient; microgram quantities may be synthesized from a single molecule of substrate DNA. DNA of four lines of common buckwheat (Kyusu, Canada, Miyazaki and Botansoba) was used to optimize PCR reaction and cycling program of 26 primers for DNA amplification of common buckwheat. Annealing temperature (Ta), PCR cycle number and $MgCl_2$ concentration were considered optimum if the single clear band was observed. Of the 26 primers Ta of only 10 primers could be optimized. Three primer pairs performed best at Ta of 54^oC. The optimum concentration of $MgCl_2$ was found to be 1.5mM for all primer pairs. Similarly the number of PCR cycles was found to be 40 for all 10 primer pairs except for primer pair 57. Optimized PCR conditions were used for subsequent studies such as transferability of EST primers to other *Fagopyrum* species and construction of linkage map.

Key words: Annealing temperature, buckwheat, EST markers, optimization of PCR conditions

INTRODUCTION

The polymerase chain reaction (PCR) has become an indispensable tool of molecular biology. In the PCR technique, DNA is amplified in vitro by a series of polymerization cycles consisting of three temperature-dependent steps: DNA denaturation, primer-template annealing and DNA synthesis by a thermostable DNA polymerase. The PCR sample may be single- or double-stranded DNA or RNA (Rychlik et al 1990).

Under optimal conditions the PCR reaction is very efficient; microgram quantities may be synthesized from a single molecule of substrate DNA. Ordinarily, several experiments are required to achieve optimal conditions for PCR, even if good primers are chosen, ie those that do not form dimers by annealing of their 3'-termini and that are specific to one sequence in the substrate DNA (Rychlik et al 1990). Optimization of PCR involves testing a number of variables. Condition should produce high yields of specific DNA target sequences. Since no single set of conditions can be applied to all PCR amplifications, individual reaction component concentrations, and time and temperature parameters must be adjusted within suggested ranges for efficient amplification of specific targets (Applied Biosystem 2006).

With some combinations of primers and genomic DNA templates, a non discrete size range of amplification products, appearing as a smear as visualized on a gel could be converted to discretely sized bands by reducing the concentration of either the polymerase or the genomic DNA (Williams et al 1990). Inclusion of formamide in the PCR eliminates most of the nonspecific products and increases the efficiency of the amplification (Sarkar et al 1990). Formamide is found to be a simple and inexpensive method of increasing the specificity of PCR.

There is obviously no single set of conditions that can be applied to all PCR amplifications. For example, depending on the length and sequence of primer used, the annealing temperatures of the

PCR can differ from each other. Finding the optimal or the most stringent annealing temperature is a prerequisite for reliable results and efficient amplification of a specific target (Ishii and Fukui 2001). The requirement of an optimal PCR reaction is to amplify a specific locus without any unspecific by-products. Therefore, annealing needs to take place at a sufficiently high temperature to allow only the perfect DNA-DNA matches the reaction (Henegariu 2006). For any given primer pair, the PCR program can be selected based on the composition (GC content) of the primers and the length of the expected PCR product. In the majority of the cases, products expected to be amplified are relatively small from 0.1 to 2-3 kb (Henegariu 2006).

EST-based markers are associated with the coding regions of the genome therefore, they enhance molecular germplasm evaluation by capturing variation across transcribed regions and in genes of known function (Buhariwalla et al 2005). In this article we experimentally determine the optimal annealing temperature (TaOPT) values for several expressed sequence tag (EST) primer pairs which were designed from the cDNA sequences from bud and style of common buckwheat (*Fagopyrum esculentum*, 2n = 2x = 16) (Jyotaro Aii 2006, personal communication). Optimal $MgCl_2$ concentration and number of PCR cycles were also determined.

MATERIALS AND METHODS

Plant materials and DNA extraction

DNA samples of four lines of common buckwheat (Kyusu, Canada, Miyazaki and Botansoba) was used to optimize the annealing temperature (Ta), number of PCR cycles and $MgCl_2$ concentration for 26 primer pairs (Table 1). Leaves from 22 day old seedlings were collected in plastic bag and kept at -20^{o}C. DNA was extracted from the frozen dried leaf using the DNeasy Plant Mini Kit (Qiagen, Japan). The extracted DNA was stored at -20^{o}C and used directly without diluting for further study.

PCR reaction

Except three variables (Ta, PCR cycle and $MgCl_2$ concentration) all other PCR protocols were same. Amplification reactions were performed in 30 µl reaction volume consisting of 14.7 µl sterile distilled water, 6 µl 5 x buffer (50 mM Tris-HCl pH 8.3, 250 mM KCl, 500 µM dNTP, 0.1% Triton X-100, 0.05% Gelatin, 7.5 mM $MgCl_2$), 3 µl of each primer of 2 µM concentration, 0.3 µl Taq DNA polymerase and 3 µl template DNA. Two concentrations of $MgCl_2$ (1.5mM and 2.0mM) were tested in final PCR reaction. DNA amplifications were performed in thermal cyclers (GeneAmp PCR system 9700 and 2700, and TAKARA) using the following cycling condition: one cycle of 94^{o}C for 3 min; different cycles of 94^{o}C for 0.30 min, different annealing temperatures for 0.30 min, 72^{o}C for 0.30 min; one cycle of 72^{o}C for 7 min followed by soaking in 4^{o}C.

Analysis of PCR products

PCR products were analyzed in 2% Agarose gels containing 0.0025% ethidium bromide in 1 x TAE buffer. Electrophoresis was run at 100 V for 45 min. Gels were visualized and documented using Kodak 1D software (Kodak EDAS290). Annealing temperature, PCR cycle number and $MgCl_2$ concentration were considered optimum if the single clear band was observed. If the band was not observed, annealing temperature was decreased and in the case of multiple bands, the temperature was increased. The number of PCR cycle was increased if there was single band with low intensity and cycle number was decreased in the case of multiple and unclear bands. In some cases of very faint single band (low intensity), concentration of $MgCl_2$ and number of cycle were increased. In the multiple bands with high and low intensity the number of cycle was decreased. The experiment was repeated until the single clear band of tested primers was achieved.

Table 1. Primers used for optimizing PCR conditions (Kindly provided by Jyotaro Aii, Japan 2006)

PN	Primer	Sequence	Product length, bp	Tm	GC content, %
3	Fe_cb_0006_primer_4	GAGGCTACGGATTTTCTGCC	320	61.091	55.000
		TCCTCGTCCTCTTCCTCCTC		60.870	60.000
5	Fe_cb_0012_primer_2	CAAGCCAACAAGCTGGAGAA	307	61.481	50.000
		AATGGGAGAATGCTTAGTTGCTTAC		61.137	40.000
7	Fe_cb_0014_primer_0	CAGCATACCAATGGCAGAGAA	536	61.172	47.619
		TCGATACGATAACCGGAAACA		60.329	42.857
8	Fe_cb_0016_primer_0	TCCAAATAGTCCCAACTTTACGC	50	61.506	43.478
		GTTGTTAATGCCGATTGCCG		63.507	50.000
19	Fe_cb_0043_primer_2	GTTTCATGGCCGGTTTCATC	604	62.543	50.000
		CACGAGAAAAGGAGCACAGTC		60.043	52.381
24	Fe_cb_0052_primer_0	CGAGCAAAACATCTCATTCAGG	343	62.022	45.455
		TTTCAGCCTCACGAGTTCCA		61.917	50.000
26	Fe_cb_0061_primer_0	AATGTGGAGACGTGGGTGAG	157	60.987	55.000
		GATTTATGAGCGCCAGAGCA		61.413	50.000
28	Fe_cb_0066_primer_3	CCGAAACAGAAAGCATTACGA	401	60.250	42.857
		AAACAATGGAAGGAGGCAGG		61.370	50.000
37	Fe_cb_0089_primer_0	TCCTCCTTGAAATATCCTTCCC	448	60.609	45.455
		TCCATACAGACGCGATTCTTG		61.172	47.619
43	Fe_cs_0035_primer_0	TATTCTTTGATCTGGCCGGG	497	62.192	50.000
		CTGGTTGTGGTGGTTGTGTG		60.949	55.000
45	Fe_cs_0063_primer_2	AATGATGTCCTCCGCCACC	490	63.242	57.895
		TTAGAACTGACCAGCGGCAC		61.375	55.000
46	Fe_cs_0064_primer_0	AGCACCACCACCACCTCTTC	487	62.518	60.000
		TTCCCATACTCTTCGGTGTCCT		62.104	50.000
47	Fe_cs_0067_primer_0	ACTGGTTCTTGGTGAAGGGG	288	61.306	55.000
		CCCATTTCCCATTGGCTTT		61.934	47.368
54	Fe_cs_0171_primer_0	AGTGTTTAGGATGGCGAGAGG	231	60.635	52.381
		TCTCATGGGTACAATCGGTCA		61.313	47.619
55	Fe_cs_0173_primer_2	CTTTTCCGCCTCCTCTTCTC	578	60.455	55.000
		CCACTCTCCCTAACACTTGACC		60.031	54.545
57	Fe_cs_0177_primer_2	GCAGCCAGCAGTCGAGAAAC	428	63.553	60.000
		GACAGCGCACAATCACACAA		61.944	50.000
63	Fe_cs_0207_primer_1	GCAGCTGATTACGGCGTTC	276	61.879	57.895
		TGTTTGGTTTACTTGGCCCTC		61.239	47.619
65	Fe_cs_0214_primer_0	TCCGACATTGCGAATAACCA	651	62.331	45.000
		AGGGAGGGAGAGAGGGAAAA		61.429	55.000
68	Fe_cs_0221_primer_3	TATGAGCAAGAGCGAATGCC	225	61.413	50.000
		TGAGGGTGGATGTTTTGACC		60.758	50.000
69	Fe_cs_0222_primer_0	TGGCTGATAGCGGTGAAAGA	377	61.848	50.000
		GGATTGTGGGCTGACAAAGA		61.046	50.000
70	Fe_cs_0224_primer_0	AAGCATAGGTTGCCTTCCCA	124	61.875	50.000
		CGAGGAATCATCTCCAACCA		61.004	50.000
71	Fe_cs_0225_primer_3	TCCATCGTCATCCACCAAAC	178	61.749	50.000
		ATTGCTCGCGATTCCTCACT		61.318	52.632
73	Fe_cs_0227_primer_1	TGCACACCAAATTCACCAAG	138	60.552	45.000
		AAAGGAAAGGAGTAATGAGGAAGTG		60.375	40.000
88	Fe_cs_0272_primer_1	GGCAATCACATAAATGCAACC	100	60.,210	42.857
		GGTCGAATAATGAATACGCCAA		61.027	40.909
89	Fe_cs_0275_primer_0	TGGATTCCCAGATCAAGCAC	299	61.016	50.000
		TCCTTCAATCCCTAACCGAAA		60.778	42.857
90	Fe_cs_0279_primer_2	CACATACCAACCAGAACTCAATACA	184	60.208	40.000
		GCCCGAGTATCGTTTGCTCTC		63.371	57.143
91	Fe_cs_0287_primer_0	TCACAGACGGTATCTCCCCA	61	61.487	55.000
		GGGCATTGAGAAGGAAGGAC		60.970	55.000

Tm, Melting temperature.

RESULTS AND DISCUSSION

The four lines of common buckwheat (Kyusu, Canada, Miyazaki and Botansoa) are self compatible and inbred. Therefore, EST band should be single if the annealing temperature (Ta) of primer is optimum. Annealing temperature is based on the T_m (melting temperature) of the oligonucleotides chosen for PCR amplification. Whenever unwanted bands were observed, the annealing temperature was raised by 2°C-5°C in subsequent optimization runs. We started Ta from 62°C with 40 cycles of PCR for optimizing these parameters. Single and clear band in all four lines were considered as indicator to know the optimum Ta and number of cycles. The PCR results tested in different Ta are given in Table 2. Optimum Ta of primer pairs, 3 and 57 was known by a single experiment (Figure 1). On an average we conducted PCR 6.5 times for the same primer pair to know the optimum Ta. PCR had to be carried out 10 times to find out Ta of primer pair, 88.

As an example, some PCR results are given in Figure 2. Primer 3 showed single clear band in all four lines at Ta of 62°C. Therefore its optimum Ta is 62°C. But in other cases, some showed multiple bands, some were faint, some were missing and in some case no band was observed. For these conditions we had repeated experiments at different Ta. Among 26 primer pairs we could optimize the Ta of only 10 and others 16 primers were discarded due to difficulty in optimizing PCR conditions (Table 2). Three primer pairs performed best at Ta of 54°C. The optimum concentration of $MgCl_2$ was found to be 1.5mM for all primer pairs. Similarly the number of optimum PCR cycles was found to be 40 for all 10 primers except for primer pair 57 for which optimum cycle number was 30.

Table 2. Optimized PCR conditions of primers

SN	Primer	Ta	Cycle	$MgCl_2$	Product length, bp
1	3	62	40	1.5mM	300
2	7	58	40	1.5mM	600
3	8	66	40	1.5mM	900
4	28	60	40	1.5mM	200
5	57	62	30	1.5mM	400
6	65	60	40	1.5mM	200
7	71	54	40	1.5mM	200
8	88	52	40	1.5mM	100
9	89	54	40	1.5mM	300
10	90	54	40	1.5mM	200

The purity and yield of the reaction products depend on several parameters, one of which is the annealing temperature (Ta) (Rychlik et al 1990). At both sub- and super-optimal Ta values, non-specific products may be formed, and the yield of products is reduced (Coyne et al 2001). If the Ta is too low, non-specific DNA fragments are amplified, causing the appearance of multiple bands on agarose gels (Nakao et al 2001). If the Ta is too high, the yield of the desired product, and sometimes the purity is reduced due to poor annealing of primers (Rychlik et al 1990). Optimizing the Ta is especially critical when long products are synthesized or when a total genomic DNA is the substrate for PCR. The optimum annealing temperature is a function of the melting temperatures of the less stable primer-template pair and of the product (Rychlik et al 1990).

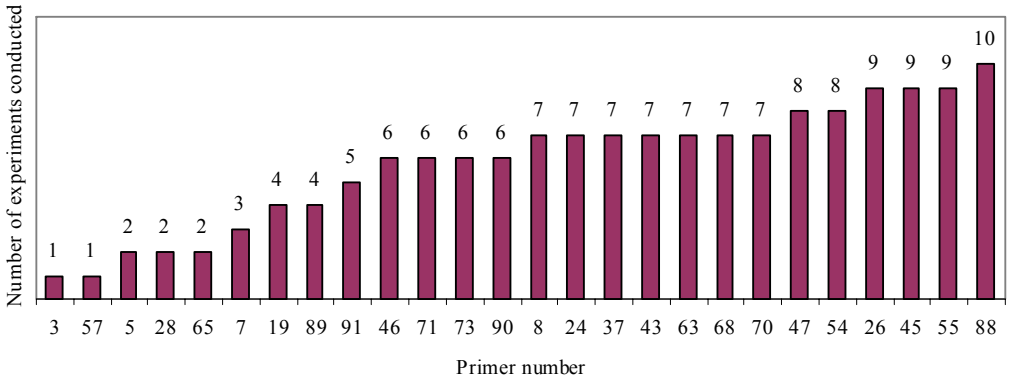


Figure 1. Number of experiments conducted for 26 primers to optimize PCR condition.

Annealing temperature is one of the most important parameters that need adjustment in the PCR reaction (Henegariu 2006). Moreover, the flexibility of this parameter allows optimization of the reaction in the presence of variable amounts of other ingredients (especially template DNA). If the same reaction is performed in the presence of a higher amount of DNA template, the low annealing temperature results in the appearance of many non-specific secondary products. Thus, it appears that by decreasing the amount of DNA template, the number of potentially non-specific sites is also decreased, making possible the drop in annealing temperature (Henegariu 2006, Yap and McGee 1991). Annealing temperature is important in finding and documenting polymorphisms. Slight mismatches, (even 1 base-pair mutations) in one of sequences bound by the two primers used to amplify a DNA locus, can be detected by slight variations in annealing temperature and/or by multiplex PCR (Henegariu 2006). For segments with G + C content significantly greater than 75%, it may be necessary to either increase the denaturation and the annealing / elongation temperatures or to add denaturants such as DMSO or formamide (Dutton et al 1993).

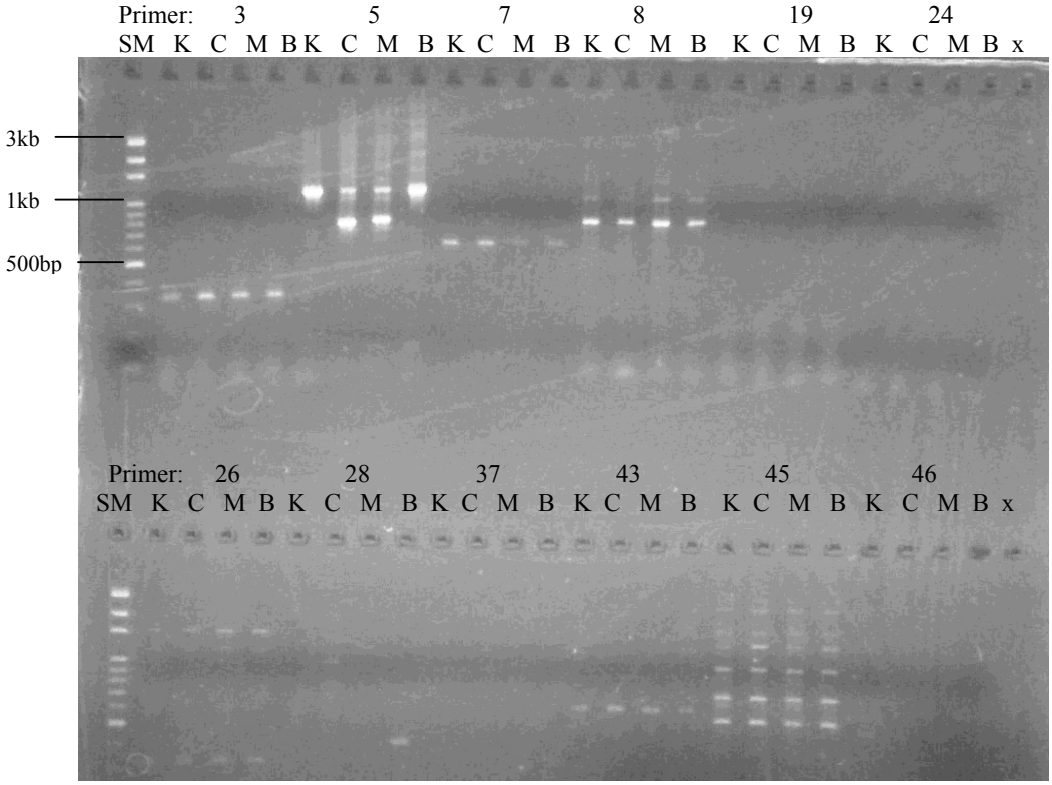


Figure 2. PCR products using different primers (indicated by primer number above the abbreviated buckwheat lines) at PCR 62^{o}C annealing temperature and 40 and 30 cycles *(SM, Size marker, K, Kyusyu, C, Canada, M, Miyazaki, B, Botansoba).*

In general, 30 cycles should be sufficient for a usual PCR reaction (Applied Biosystem 2006, Henegariu 2006). An increased number of cycles will not dramatically change the amount of product. The most obvious variation in the amount of products was around 24 cycles for ethidium bromide stained gels. 28-30 cycles are usually sufficient in a reaction. Little or no quantitative changes (ie relative amounts of PCR products) were observed with increasing cycle number up to 45 (Henegariu 2006). Little quantitative gain was noticed when increasing the number of cycles up to 60.

Generally, larger PCR volumes require lower or longer annealing temperature programs, because the temperature in larger volumes of reaction mixtures changes more slowly than that in smaller volumes (Ishii and Fukui 2001). These results indicate that annealing temperature is an important parameter in PCR for molecular research.

Optimization of PCR conditions is a critical precursor for accurate and robust large-scale marker screening and offers additional benefits for reducing the unit cost of genotyping. These optimized PCR conditions were used for further studies such as transferability of EST primers in other *Fagopyrum* species and construction of linkage map.

ACKNOWLEDGEMENT

This research work was an outcome of the training course, Sustainable use of plant genetic resources from 9 May to 3 Nov 2006, organized and supported by JICA.

REFERENCES

Applied Biosystem. 2006. PCR optimization: Reaction conditions and components. *http://www.appliedbiosystems.com/support/techtools/pcropt/*. Access 10 July 2006.

Buhariwalla HK, Jayashree B, K Eshwar and JH Crouch. 2005. Development of ESTs from chickpea roots and their use in diversity analysis of the *Cicer* genus. *BMC Plant Biology* 5(16):1-14.

Coyne VE, MD James, SJ Reid and EP Rybicki, eds. 2001. Molecular biology techniques manual. 3rd ed. PCR primer design and reaction optimization. Ed Rybicki, Department of Molecular and Cell Biology, University of Cape Town. *http://www.mcb.uct.ac.za/pcroptim.htm*. Access 10 July 2006.

Dutton CM, C Paynton and SS Sommer. 1993. General method for amplifying regions of very high G + C content. *Nucleic Acids Research* 21(12): 2953 -2954.

Henegariu O. 2006. Annealing time and temperature. *http://info.med.yale.edu/genetics/ward/tavi/p09.html*. Access 10 July 2006.

Ishii K and M Fukui. 2001. Optimization of annealing temperature to reduce bias caused by a primer mismatch in multitemplate PCR. *Applied and Environmental Microbiology* 67(8): 3753–3755.

Nakao Y, H Iwata, A Matsumoto, Y Tsumura and N Tomaru. 2001. highly polymorphic microsatellite markers in Chamaecyparis obtuse. *Can. J. For.Res.* 31:2248-2251.

Rychlik W, WJ Spencer and RE Rhoads. 1990. Optimization of the annealing temperature for DNA amplification in vitro. *Nucleic Acids Research* 18 (21): 6409-6412.

Sarkar G, S Kapelner and SS Sommer. 1990. Formamide can dramatically improve the specificity of PCR. *Nucleic Acids Research* 18(24):7465.

Williams JGK, AR Kubelik, KJ Livak, JA Rafalski and SV Tingey. 1990. DNA polymorophisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*. 18:6531-6535.

Yap EPH and JOD McGee. 1991. Short PCR product yields improved by lower denaturation temperatures. *Nucleic Acids Research* 19 (7): 1713.

Disease-free Pre-Basic Seed Potato Production through Tissue Culture in Nepal

Binesh M. Sakha*, Gyan P. Rai, Shambhu P. Dhital and Ram B. Nepal

National Potato Research Programme-Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal

ABSTRACT

Pre-basic seed potatoes are disease free potato minitubers produced by transplanting pathogen free *in vitro* potato plantlets under protected condition in aphid-proof glasshouse and/or screen house. Double antibody sand witched - enzyme linked immuno-sorbant assay is used to test six major potato viruses, namely PLRV, PVS, PVX, PVY, PVA and PVM. Thermotherapy cum meristem tip excision techniques are used to eliminate these viruses. Virus free *in vitro* potato plantlets are rapidly propagated by single nodal cuttings on modified MS media. For pre-basic seed production disease free *in vitro* potato plantlets are transplanted in the sterile sand soil substrate under glasshouse and screen house, once in autumn season and next in spring season. Since 1990, National Potato Research Program has been producing about 200,000 pre-basic seeds annually. So far, PBS of 19 different recommended and released potato cultivars has been produced. Till date 3,465,799 PBS had been produced and 3,217,666 pre-basic seeds distributed to the different seed potato growers groups, District Agriculture Development Offices, government farms/research stations, and NGOs/INGOs for subsequent basic seed potato production. After establishment of tissue culture facilities in National Potato Research Program, the productivity of potato has been increased by 71% due to utilization of pre-basic seed potatoes.

Key words: DAS-ELISA, meristem excision, potato, pre-basic seed, thermotherapy

INTRODUCTION

Potato, being vegetatively propagated crop, is very prune to seed degeneration as several potato viruses accumulate to the seed tubers overtimes resulting into its reduced yield potential. So far, six major potato viruses, namely PLRV, PVS, PVX, PVY, PVA and PVM had been reported to infect potato crops in Nepal (Akius and Kloos 1990, Ranjit et al 1994). To overcome seed tuber degeneration, seed potatoes should be replaced by high quality seed potatoes at regular intervals (Sakha and Rai 2004). A continuous source of high quality seed potatoes is, therefore, necessary in the country. For this purpose, the National Potato Research Program (NPRP) has been established well equipped tissue culture facilities to produce a limited quantity of pre-basic seed (PBS) potatoes each year. These pre-basic seed potatoes are used as source seeds for the production of high quality seed potatoes for several generations. The adopted use of high quality seed potatoes on a large scale will, therefore, help significantly increase the productivity of the potato in Nepal.

PBS potatoes are virus tested, disease free minitubers produced under aphid proof glasshouse and/or screenhouse. When multiplied using improved and recommended potato production techniques, these seeds produce large quantities of high quality seed potatoes for several generations. Cultivation with well-managed agronomic practices, these quality seeds will give a higher potato yield. The objective of PBS production is to increase productivity by replacing old degenerated seed potatoes from the major seed potato production pocket areas of the country.

MATERIALS AND METHODS

Virus elimination technique

Double Antibody Sandwitched - Enzyme Linked Immunosorbant Assay, DAS-ELISA, (Clark and Adams 1977) technology was used to assess the presence of six major potato viruses in potato

cultivars. Then, thermotherapy cum meristem tip excision technique was used to eliminate potato viruses. For virus elimination, tubers were allowed to sprout at 37^{o}C for 2-3 weeks as a thermal treatment and shoot tips were excised from the sprouts and washed in detergent water. Under laminar airflow chamber, their surface were treated with 70% Ethanol for 30 seconds, washed with sterile distilled water and then sterilized with 2% sodium hypochlorite solution for five minutes and again washed at least three times with sterile distilled water. By using a stereoscopic microscope, the apical meristem (with one or two leaf premordia, about 0.2 – 0.3 mm in diameter) was excised from the shoot tip and placed on top of a filter paper bridge on a liquid MS medium (Murashige and Skoog 1962) supplemented with 0.5 mgl^{-1} IAA, 0.4 mgl^{-1} Kinetin and 0.1 mgl^{-1} GA_3 (Mellor and Stace-Smith 1977). The meristem was then cultured in an incubation room under 20 ± 2^{o}C with proper illumination (2000 lux) and 16 h photoperiod. After few weeks, it became green and gave rise to stem and leaves. It was then transferred to a solid MS medium for proper rooting and shooting. This plantlet was multiplied into several clones by nodal cuttings.

Virus testing

Clones regenerated from meristem culture were utilized for virus testing by DAS-ELISA. In NPRP, there were testing facilities for major six potato viruses namely, PLRV, PVS, PVX, PVY, PVA and PVM. Once the virus-free clones were obtained, they were propagated by nodal cuttings for further multiplication and maintained *in vitro* as clean germplasm. Virus testing of the standing crops in the glasshouse/screenhouse was further conducted randomly after tuberization stage.

Germplasm maintenance

Virus eliminated clones were maintained with subsequent subcultures as germplasm in the tissue culture laboratory of NPRP. Majority of the potato germplasm were imported from the International Potato Centre (CIP), Lima, Peru as disease-free *in vitro* plantlets, some introduced from India and the rest were of Nepalese origin.

Rapid propagation

Virus-free *in vitro* plantlets, maintained as mother plantlets, were rapidly propagated by single nodal cuttings on modified MS solid media supplemented with 2 mgl^{-1} calcium pantothenate. With subsequent subcultures, desired numbers of *in vitro* plantlets of required cultivars were obtained for transplantation in each season. The cultures were incubated in growth chambers with culture conditions of 16 h photoperiod, 2000 lux light intensity and 20 ± 2^{o}C temperature.

PBS production under controlled condition

Four to six weeks old *in vitro* plantlets were transplanted into sterile mixture of 2:1 sand and soil substrate under aphid-proof glasshouse and screenhouse twice a year for pre-basic seed production. Special cultivation techniques such as planting at 20- × 10-cm spacing, irrigation with UV sterilized water, fertilizer application, earthing up, plant protection and haulm pulling were used between transplantation and harvest. Chemical fertilizers were applied at the rate of 200:200:120 kg N:P:K per hectare. Irrigation was stopped at least two weeks before harvest and haulms pulled a week ahead harvesting. Depending up on cultivars, minitubers were harvested three to four months after transplantation.

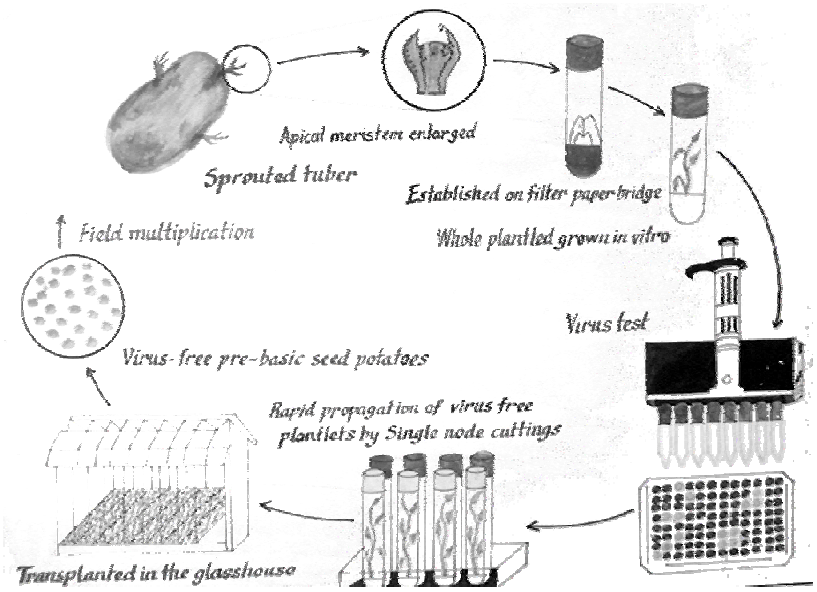


Figure 1. PBS production scheme adopted at NPRP, NARC.

PBS packaging and storage

After harvesting, PBS were graded into four different weight size categories, as >5 g, 1-5 g, 0.5-1 g and <0.5 g, and packed into screen bags with proper tagging. PBS produced during autumn season were harvested in November – December and stored in the cold store till August to October for about eight to ten months before being distributed for succeeding Tarai season field plantation. In case of spring season production, PBS were harvested in April - May and stored in the cold store till November - January for about six to eight months before being distributed for succeeding hill season field plantation.

RESULTS AND DISCUSSION

Virus elimination and germplasm maintenance

So far 12 potato cultivars had been cleaned from the major potato viruses in NPRP (Table 1). Before virus cleaning, cultivars were found to be infected with single to multiple viruses and percentage of virus elimination was higher in case of single infestation as compared to multiple infestations. Out of six major potato viruses tested through DAS-ELISA technique, PVS infestation was found to be the highest for eight clones followed by PVM infecting three clones. More than hundred potato germplasms were maintained under *in vitro* condition in the NPRP's tissue culture laboratory (NPRP 2006).

Table 1. Potato viruses eliminated cultivars in NPRP, Khumaltar

Cultivar	Eliminated viruses	Reference	Remark
Sarkari Seto	NA[†]	NA	Cleaned in 1989
Syang Dorje	NA	NA	Cleaned in 1989
Kufri Sindhuri	PVS and PLRV	Akius and Kloos 1990	
Cardinal	PVX, PVY and PVS	Shakya et al 1992	
Kathmandu Local	PVS and PVM	Ranjit et al 1994	
Tharu Local	PVS and PVM	Ranjit et al 1994	
NPI-106	PVS	Ranjit et al 1994	
Kufri Badshah	PVX	Sakha and Dhital 2006	
Jumli Local	PVX, PVS, PLRV & PVM	Sakha and Dhital 2006	
CIP 388572.1	PVS	NPRP 2006	
CIP 388572.4	PVS	NPRP 2006	
Gui Valley	PVY	Dhital et al 2006	

[†] *NA, Not available*

Rapid propagation

During rapid propagation, the explants developed into fully grown plantlets within four to six weeks depending up on cultivars. It was found that multiple explants grow rapidly as compared to single explant per culture tube. About ten to twenty thousands *in vitro* plantlets are being propagated and supplied to the glasshouse/screenhouse each season for PBS production purpose (Table 2).

Table 2. *In vitro* plantlets supplied to the glasshouse and screen house for PBS production

Year	Autumn season, n	Spring season, n	Total, n
1990/91	12,400	14,625	27,025
1991/92	10,976	13,280	24,256
1992/93	11,409	13,550	24,959
1993/94	12,546	17,599	30,145
1994/95	16,775	17,720	34,495
1995/96	16,058	8,720	24,778
1996/97	14,593	7,666	22,259
1997/98	14,094	22,264	36,358
1998/99	21,330	18,402	39,732
1999/00	21,578	18,823	40,401
2000/01	20,193	21,150	41,343
2001/02	22,600	15,170	37,770
2002/03	17,844	21,830	39,674
2003/04	22,570	15,606	38,176
2004/05	26,345	21,479	47,824
2005/06	24,894	17,655	42,549
Total	286,205	265,539	551,744

PBS production and distribution

Pre-basic seed potatoes were produced for the first time in Nepal during 1989/90 (Akius et al 1990). Since then PBS has been produced twice a year, once during autumn season and next during spring season. So far PBS of 19 cultivars has been produced in NPRP (Table 3). Out of them Cardinal, Desiree, Kufri Jyoti, Khumal Seto-1 and NPI-106 found suitable for both seasons. Depending up on the demand, PBS of five to ten cultivars are produced each season. There are altogether 20 benches of 12.88 m^2 area each in the glasshouse and eight benches of 14.28 m^2 area each in the screenhouse for PBS production in NPRP. So far about 200,000 PBS are being produced each year.

Table 3. Potato cultivars suitable for autumn and spring season plantation

SN	For Tarai season/autumn plantation	For hill season/spring plantation
1	Cardinal†	Cardinal†
2	Desiree†	Desiree†
3	Kufri Sindhuri†	Kufri Jyoti
4	Kufri Jyoti	NPI-106
5	NPI-106	Khumal Seto-1
6	Khumal Seto-1	I-1124
7	Janakdev†	CFM 69-1
8	Khumal Rato-2†	CFJ 69-1
9	BR 63-65†	Syang Dorje
10	Tharu Local	Sarkari Seto
11	Kufri Badshah	Kathmandu Local†
12	Perricholi†	Jumli Local†

† *Red skinned cultivars, rest are white skinned.*

Study of PBS production during last five years revealed that total number of PBS production during spring season is about 24% higher than that produced during autumn season (Table 4). However, production of larger sized (>1 g) PBS was much more (58.3%) during autumn season as compared to 37.6% only during spring season. Thus, production of higher number of potato tubers per unit area results in the decrease in average tuber size, and vice versa. It was found that *in vitro* plantlets produced about five minitubers per plantlet with the mean tuber size of 2.8 g (Sakha and Rai 2004).

Table 4. Size distribution of PBS produced during 2001/02 to 2005/06 in NPRP, Khumaltar

Year	Autumn PBS					Spring PBS				
	Production, n	Size distribution, %				Production, n	Size distribution, %			
		>5 g	1-5 g	0.5-1 g	<0.5 g		>5 g	1-5 g	0.5-1 g	<0.5 g
2001/02	110,331	17.9	48.6	23.0	10.5	131,003	8.5	37.4	33.0	16.4
2002/03	67,945	22.3	42.2	25.2	10.4	192,113	3.2	33.3	46.6	16.9
2003/04	104,289	7.1	40.3	44.0	8.5	103,186	7.7	42.4	48.2	11.7
2004/05	100,170	6.8	47. 7	37.6	7.8	90,760	7.8	32.1	40.3	19.7
2005/06	84,718	12.1	49.1	24.3	14.5	62,445	2.0	23.9	48.6	25.6
Mean	93,491	13.2	45.1	30.8	10.3	115,901	5.8	31.8	43.3	18.1

Initially PBS was distributed mainly to seed potato growers and seed potato grower groups (SPGs) through Field Coordinators in each Developmental Regions. After the establishment of Memorandum of Understanding (MoU) between Nepal Agriculture Research Council (NARC) and Department of Agriculture (DoA) in 1997, PBS are being distributed to the seed potato producers through District Agriculture Development Offices in collaboration with Potato Development Program, Department of Agriculture (Sakha and Dhakal 2006). Accordingly, 80% PBS has been distributed to the seed potato producers groups and the rest 20% to the Government farms, research stations, NGOs and INGOs within the country. Till date 3,365,799 PBS had been produced and 3,217,666 PBS had been distributed so far (Table 5).

Table 5. Production and distribution of pre-basic seed potatoes after the inception of tissue culture facilities in NPRP, NARC

Year	Production target, n	Production, n	Distribution†, n
1990/91	100,000	135,860	149,550
1991/92	100,000	108,722	123,696
1992/93	100,000	84,991	103,207
1993/94	200,000	222,987	86,000
1994/95	200,000	440,533	197,095
1995/96	200,000	297,268	400,802
1996/97	200,000	283,240	237,342
1997/98	200,000	162,843	203,525
1998/99	200,000	142,780	254,985
1999/00	200,000	208,496	140,057
2000/01	200,000	231,119	195,393
2001/02	200,000	241,334	226,624
2002/03	200,000	260,058	238,201
2003/04	200,000	207,475	239,167
2004/05	200,000	190,930	233,568
2005/06	150,000	147,163	188,454
Total		3,365,799	3,217,666

† *PBS, produced in the preceding year distributed during succeeding year.*

Utilization of pre-basic seed potatoes

PBS has been utilized for the production of high quality basic seed potatoes. Depending up on the environmental condition of the plantation areas and cultivation practices, PBS could be used to produce quality basic seeds for several generations: at least 3-4 years in plain areas, 5-6 years in mid-hills and 8-10 years in high hills. Depending up on the size of PBS, field plantation should be done at 60- × 10-cm, 60- × 15-cm and 60- × 20-cm spacing for PBS of <1 g, 1-5 g and >5 g sizes, respectively (Sakha and Dhakal 2006). Size of PBS, environment and cultivar were the three main factors affecting both multiplication rate and productivity. Average multiplication rates of basic seed-1 tubers produced per unit PBS were found to be 4.9 for the hills and 5.2 for the Tarai with average production of 116 g and 171 g of basic seed-1 per tuberlet in the hills and Tarai, respectively (Schulz et al 1998).

After the establishment of tissue culture facilities in NPRP in 1990, the continuous use of PBS potatoes as source seeds resulted in the significant increase in productivity of potato (Figure 2).

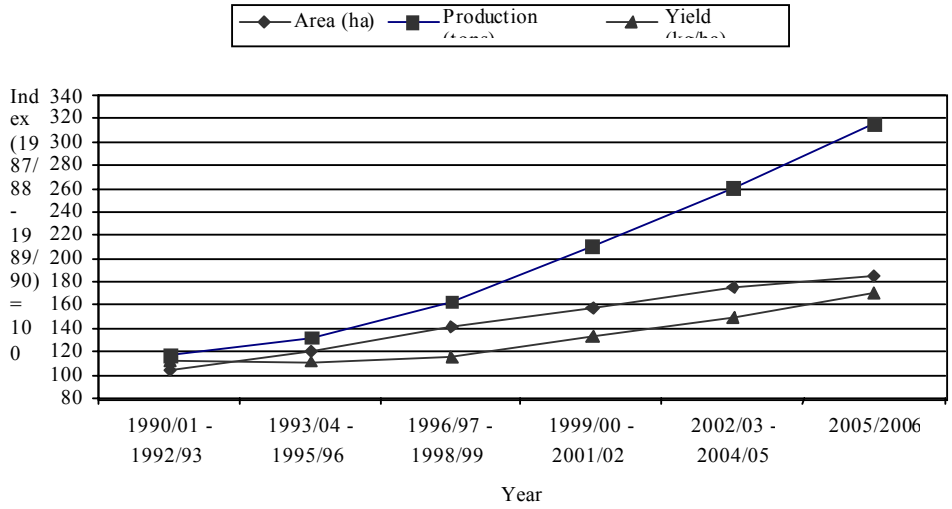


Figure 2. Index of potato area, production and yield in Nepal.
Base Year (3 Years Avg.) 1987/88 - 1989/90 = 100

Source: ABPSD, 2006.

CONCLUSION

PLRV, PVS, PVX, PVY, PVA and PVM are known to be the major potato viruses of economic importance in Nepal. National Potato Research Program under Nepal Agricultural Research Council has facilities to test these viruses. Since 1990, NPRP has been producing about 200,000 PBS annually. So far, PBS of 19 different recommended and released potato cultivars has been produced. Till date 3,465,799 PBS had been produced and 3,217,666 PBS distributed to the different seed potato growers groups, District Agriculture Development Offices, Government Farms/Research stations, NGOs/INGOs and others for subsequent basic seed potato production within the country.

REFERENCES

ABPSD. 2006. *Statistical information on Nepalese agriculture 2005/06 (2062/063)*. Agri-Business Promotion and Statistics Division, Ministry of Agriculture and Cooperatives, Government of Nepal, Kathmandu Nepal.

Akius M and JP Kloos. 1990. Viral diseases spread and detection in Nepal. **In**: *Proceeding of 11th European Association for potato Research (EAPR)*, Edinburgh, UK. Pp. 66-67.

Akius M, JP Kloos and BK Bhomi. 1990. Production of disease-free potato in Nepal: Current situation. **In**: Proceeding of 11th European Association for potato Research (EAPR), Edinburgh, UK. Pp. 297-298.

Clark MF and A Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34:475-783.

Dhital SP, BM Sakha and HT Lim. 2006. Utilization of shoot cuttings for elimination of PLRV and PVY by thermotherapy and chemotherapy from potato (*Solanum tuberosum* L.). *Nepal J. of Sci. and Tech.* 7:13-19.

Mellor FC and R Stace-Smith. 1977. Virus-free potatoes by tissue culture. **In**: *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture* (J Reinert and YPS Bajaj, eds). Springer, Berlin Heidelberg, New York, Pp. 616-635.

Murashige T and F Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*. 15:473-497.

NPRP. 2006. *Annual Report 2005/06 (2062/63)*. National Potato Research Programme (NPRP), Nepal Agriculture Research Council, Khumaltar, Lalitpur, Nepal.

Ranjit M, GP Rai, A Manandhar and V Pandey. 1994. Virus testing and elimination of viruses from some local cultivars of potato (*Solanum tuberosum*) in Nepal. **In**: *Proceedings of 4th Asian Potato Association (APA) Triennial Conference,* 5-7 Jul. 1994 (ET Rasco, FB Aromin and CH Balaltro, eds). Daekwonryeong, Korea. Pp. 95-99.

Sakha BM and GP Rai. 2004. Performance of *in vitro* plantlets and stem cuttings for pre-basic seed potato production under glasshouse condition in Nepal. **In**: *Proceeding of the 4th National Horticulture Research Workshop,* 2-4 March 2004 held at Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal. Pp. 186-189.

Sakha BM and SP Dhakal. 2006. *Basic seed potato production technology from pre-basic seed potatoes* (In Nepali). National Potato Development Program, Khumaltar, Nepal.

Sakha BM and SP Dhital. 2006. Eradication of viruses by thermotherapy and meristem-tip culture from potato (*Solanum tuberosum* L.) for pre-basic seed potato production in Nepal. *J. Pt. Breeding Gp.* 1:26-32.

Schulz S, GJ Wells, BK Baniya, TP Barakoti, G Kharel, B Saha and B Thapa. 1998. Decentralized on-farm seed potato production from pre-basic minitubers: A case study from Nepal. *Experimental Agriculture*. 34: 487-495.

Genotype × Environment Interaction and Stability Analysis for Grain Yield of Mid-hill Rice Genotypes

Hari K. Upreti[1], Sudarshan Bista[1], Surya N. Sah[2] and Ramesh Dhakal[3]

[1] Agriculture Botany Division-NARC Khumaltar <hari_upreti@yahoo.co.in>
[2] ARS-NARC, Pakhribas
[3] ARS-NARC, Dailekh

ABSTRACT

Genotype x Environment interaction limits the effectiveness of selection when selection is based only on mean yield. This G × E interaction was studied for grain yield in 7 genotypes of mid-hill rice in five different environments across the Nepal. Significant difference was observed among genotype (G), environment (E) and interaction (G × E) but could not identify the stable high yielding genotypes for diverse environments. Therefore, stability parameters were calculated and analyzed. On the basis of stability parameters, two genotypes, NR 10414, NR 10492 and NR 10515 were found to be most stable over different environments. NR 10353 was identified as suitable genotypes with high grain yield for favorable environment.

Key words: G × E interaction, grain yield, mid-hill rice, stability

INTRODUCTION

Rice is the main stay of Nepalese farmers and prefers to grow whenever possible. Hill rice contributes about 25% both in area and production (MoAC 2006). Rice yield is very low in this region due to diverse growing conditions in terms of altitude, soil type and depth, rainfall pattern and irrigation facility and input availability and crop management practices. Varietal adaptability to environmental fluctuations is important for the stabilization of crop production both over regions and years (Singh and Narayan 1993). Hence, several potential genotypes have to evaluate at different environments and years before selecting certain desirable genotypes. The relative performance of different genotypes often varies from one environment to another ie genotype environment interaction exist (Rashid et al 2002), and presence of G × E interaction in any genetical study leads to overestimation of genetical and statistical parameters (Sharma 1998) which makes the breeder difficult to decide which genotypes should be selected.

Significant G × E interaction results from changes in the magnitude of the differences among genotypes in different environments or from changes in relative ranking of the genotypes ie two types of G × E interaction exist (a) a non cross-over G × E interaction, in which the ranking of genotypes remains constant across environments and the interaction is significant because of changes in the magnitude of the response, or (b) a crossover G × E interaction, in which significant changes in rank occurs from one environment to another where one genotype may be chosen for one environment and other genotype for other. Therefore, plant breeders look for non-crossover type of interaction when selecting genotype for wider adaptation (Won et al 1998). The study of G × E interaction provides useful information to identify stable genotypes over a range of environments or specifically adapted to low and high environment (Reddy et al 1998). Testing genotypes at more location is considered important than testing in more years for stability studies (Joshi et al 2003). The present investigation was therefore, under taken to study G × E interaction and stability analysis to identify stable high yielding genotypes over different situations of mid hill areas of Nepal.

MATERIALS AND METHODS

Seven rice genotypes including two standard checks (Khumal-4 and Khumal-11) were evaluated in farmers' fields using a farmer as a replication during 2005. These trials were conducted at five locations with two replication in each site in the mid hill areas viz. Dhankuta and Dolakha in eastern, Khumaltar in central, Lamjung in western and Dailekh in mid western part of Nepal. Status and pedigrees of testing materials are given in Table 1. The plot size was 10 m^2 and spacing was 20- × 15-cm. The recommended packages of practices were followed to raise the good crop at all the locations. Although all agro-morphological data were recorded, only the grain yield data is used in this study. Grain yield was recorded on plot basis and converted to ton ha^{-1} before analysis.

Table 1. Status and pedigree of the testing materials

Genotype	Pedigree	Status
NR 10353-8-2-1	Jumli Marshi / IR 36	Pipeline
NR 10414-34-2-3	Gyamja / YR 3825-11-3-2-3-1	Pipeline
NR 10492-7-2-2	IR 36 / Khumal-5	Pipeline
Khumal-4	IR 28 / Pokhreli Masino	Released
NR 10491-57-2-1	Khumal-4 / NR 10188-7-1	Pipeline
NR 10515-69-1	Taichung 176 / Yongeng 3	Pipeline
Khumal-11	Akiyudaka / Barkat	Released

Statistical Analysis

The analysis of variance combine over locations was carried out to detect the differences among genotypes, environments and genotype × environment interaction using the statistical package MSTATC. Stability parameters were analyzed using Hanson's Composite Parameters of Stability Model (1970). This model is simple and suitable when number of genotypes and environments are small in which genotypic stability (Di) for a genotype is the deviation of its expected yield ($\hat{E}_{ij}$) from its stable yield ($\hat{S}_{ij}$).

$$Di = [\sum_{j}^{e} (\hat{E}_{ij} - \hat{S}_{ij})^2]^{0.5}$$

$$\hat{E}_{ij} = (x_{ij}+X-gi-y_j) \text{ and } \hat{S}_{ij} = bi\ (y_j-X)$$

Therefore,

$$Di = \sum_{j}^{e} (x_{ij}+X-gi-y_j)-bi\ (y_j-X)^2$$

Where,

x_{ij} = Mean of the i^{th} genotype over replications at the j^{th} environment.
gi = Mean of the i^{th} genotype over replication and over environments.
y_j = Mean of the j^{th} environment over replication and over genotypes.
X = Experimental mean over all genotypes over replication and over all environment.
bi = Regression coefficient of i^{th} genotype on the environmental index (Ij) as calculated by Eberhart and Russel (1966).

$$bi = \sum_{j}^{e} x_{ij} \times I_j / \sum_{j}^{e} I_j^2$$ where Ij = Environmental index.

RESULTS AND DISCUSSION

Analysis of variance combined over environments showed the highly significant differences among genotypes (G), environments (E) and G × E interaction. Mean genotype yield ranged from 4.1 to 5.8 t ha^{-1}.The highest yielding cultivars were NR 10353, NR 10414, NR 10515 and NR 10492 (Table 2). The environment effects were significant for the grain yield and it was the highest in Khumaltar followed by Dhankuta, Dolakha and the lowest in Lamjung. This indicates that environmental conditions at the testing sites were varied in terms of soil type and depth, altitude, rainfall and other factor affecting the crop yield.

Table 2. Grain yield (t ha^{-1}) of tested genotypes in farmer's field trials combined over location, 2005

Genotype	Dhankuta	Dolakha	Khumaltar	Lamjung	Dailekh	Mean	Di
NR 10353-8-2-1	5.5	4.1	9.8	5.1	4.3	5.8	3.1
NR 10414-34-2-3	4.9	5.0	7.3	4.7	4.6	5.3	2.2
NR 10492-7-2-2	5.8	3.8	6.6	4.0	4.5	4.9	2.2
Khumal-4	4.3	4.2	7.4	3.8	3.3	4.6	2.6
NR 10491-57-2-1	4.4	4.5	4.4	4.1	3.4	4.1	0.9
NR 10515-69-1	5.2	4.8	7.0	3.5	4.8	5.0	2.3
Khumal-11	5.9	3.9	7.0	1.7	4.8	4.7	3.3
Mean	5.1	4.3	7.1	3.8	4.2	4.9	
	Environment		Genotype	G × E	CV, %		
F-test	**		**	**	17.26		
LSD (5%)	0.89		1.31	1.75			

***, Highly significant. Di, Genotypic stability.*

The significant G × E interaction effect demonstrated that the genotypes responded differently to the variation in the environmental condition of the testing sites and grain yield fluctuated accordingly. This indicates the necessity of multi-location testing of genotypes before recommending for general cultivation. The highest grain yield across the locations was produced by NR 10353 which ranked was first in Khumaltar and Lamjung only. NR 10414 gave the highest grain yield of 5.0 t ha $^{-1}$ in Dolakha which was at par with NR 10353 across the locations. Similarly, Khumal-11 ranked first in Dhankuta and gave the lowest yield in Dolakha and Lamjung. Likewise, NR 10491 performed average in all the locations but its mean yield across the location was less than grand mean or experimental yield and could not considered as stable variety.

Thus, analyses of variance combine over locations alone unable to give clear concept in identifying stable high yielding genotypes across the locations. Hence, stability parameters in addition to mean grain yield over location were calculated.

The ideal genotype as proposed by Hanson (cited by Sharma 1998) would have the lowest Di value with higher mean yield than the grand mean. Lower the Di values smaller is the departure from the stable mean. Thus in this study, genotype NR 10491 has the lowest Di (0.9) but its mean yield (gi) was less than experimental yield or grand mean. Therefore, this genotype could not be considered as desire one. In the same line, NR 10414 and NR 10492 showed low Di followed by NR 10515 with higher mean grain yield than the experimental yield. Hence, these genotypes could be considered as stable over the environments and recommended for general cultivation if other parameters of varietal selection favors. However, cultivar NR 10353 with moderately higher genotypic stability (Di of 3.1) with the highest mean yield of 5.8 t ha^{-1} could be chosen for favorable environment or high input condition of cultivation.

The results of this investigation demonstrated that the genotypic stability (Di) value with corresponding per se mean performance through light on the relative stability pattern of each genotype. Genotypes NR 10414, NR 10492, and NR 10515 are relatively stable in all location and NR 10353 is suitable in favorable environment.

REFERENCES

Joshi BK, KP Shrestha and S Bista. 2003. Yield stability analysis of promising rice genotypes in mid hills of Nepal. **In:** *Proceedings 23rd National summer crops research workshop*, NRRP, Hardinath, Dhanusha. Pp 109-116.

MoAC. 2006. *Statistical information on Nepalese agriculture 2005/06*. Agri-business Promotion and Statistics Division, Ministry of Agriculture and Co-operatives, GON, Singh Durbar, Kathmandu.

Rashid A, G Hazara, N Javed, MS Nawaz and GM Ali. 2002. Genotype x environmental interaction and stability analysis in mustards. *Asian Journal of Plant Sciences*, 1(5); 591-592.

Reddy JN, D Pani and JK Roy. 1998. Genotype x environment interaction for grain yield of low-land rice cultivars. *Indian J. Genet.* 58 (2): 209-213.

Sharma JR. 1998. *Statistical and biometrical techniques in plant breeding*. New Age International Limited publishers, New Delhi.

Singh P and SS Narayan. 1993. *Biometrical techniques in plant breeding*. Kalyani publishers, New Delhi.

Won JG, T Yoshida and Y Uchimura. 1998. Genotype × environmental interactions of selected lines in water direct seeded rice. *http://www.di.dion.ne.jp.* Access 20 September 2005.

Evaluation of Open Pollinated Normal Maize Varieties in the Eastern Mid Hill of Nepal

Surya N. Sah[1]* and Diwakar Sharma[2]

[1] Agriculture Research Statation-NARC, Pakhribas, Nepal
[2] National Maize Research Program-NARC, Rampur, Nepal

ABSTRACT

Full season maize genotypes were evaluated in on station coordinated varietal experiments during summer season at Agriculture Research Station (ARS), Pakhribas in three consecutive years 2003, 2004 and 2005. The experiments were laid out in randomized complete block design (RCBD) with three replications. The objective of this study was to identify high yield potential normal maize genotypes suitable for the existing cropping patterns in the eastern mid hill. Genotypes were evaluated in coordinated varietal trials (CVT) in 2003, 2004 and 2005 and in intermediate yield trial (IYT) in 2004. Five common genotypes including local and standard check were evaluated in the CVT 2003 and in the CVT 2004, another five common genotypes tested in the CVT 2004 and in the CVT 2005 and six common genotypes of the IYT 2004 and CVT 2005 were analyzed over year. Genotype P501-SARCO-F1/P502-SARCO-F1 produced significantly higher grain yield followed by DRACOSYN- F1/DRBCOSYN-F1 in the 1st two years and found about one week earlier than Mankamana-3. Across 00502 and Across 9942/Across 9944 were found superior with respect to grain yield and phenotypic characters like husk cover, plant aspect and ear aspect both in CVT 2005 and IYT 2004 across the years and the genotype Across 9942/Across 9944 was found tolerant to turcicum leaf blight (TLB) in 2005.

Key words: Eastern Nepal, evaluation, genotypes, maize

INTRODUCTION

Maize (*Zea mays* L.) (2n = 2x = 20) is the main cereal crop of hilly areas and the second most important crop in Nepal. It serves as main diet of more than 55% of the people who live in the hilly regions (Baniya et al 2003). It is grown for food, feed and fodder in Nepal. It is mostly grown as a rainfed crop in the hills. The total cultivated area under maize in the country is 864885 ha, of which the eastern hill covers 178239 ha. The average productivity (1.66 t/ha) of the eastern hill is less than the national average productivity 2.0 t/ha (CBS 2005). National Maize Research Program, Rampur has released 17 open pollinated maize varieties for general cultivation for different agro ecological zones. Among these, 7 varieties have been recommended for the Mid Hill. More than 86% hill farmers prefer to grow white maize (ARSD 2001/02). Out of those varieties Mankamana-1, Mankamana-3 as well as newly released Shitala and Deuti, all white colored varieties are getting popularity among mid hill farmers of the eastern Nepal. However, the varieties, which are good for today, might not remain the same for long because of the reoccurrences of new biotypes of pathogen on account to the changing environment. Farmers need varieties having not only white colored but also flint type grains for good storing capability, strong stem, stay green characteristics, system accountability and stress tolerant. Therefore, there is a continuous need of studies to identify demand driven suitable cultivars in Eastern Mid Hill of Nepal.

MATERIALS AND METHODS

A co-coordinated varietal trial was conducted at ARS, Pakhribas for three consecutive years 2003, 2004 and 2005. Including the standard and the local checks, nine entries in CVT 2003; CVT 2004; 16 entries in IYT 2004 and 11 entries in CVT 2005 were tested in RCBD with three replications. The unit plot size was of four rows of three meter length in CVT and two rows of three meter length

in IYT with the spacing of 25- × 75-cm. The planting dates were 15 April 2003, 3 May 2004, 28 April 2004 in IYT, 28 April 2005. Fertilizers were applied at the rate of 120:60:30 NPK kg/ha. Half dose of N and full dose of P and K were supplied as a basal dose at planting and the rest half nitrogen was given as a side top dressing during the knee high stage of plant. Compost @ 15 ton/ha was also applied uniformly through broadcasting immediately before land preparation each year. Malathion 5% dust was applied @ 20 kg/ha to protect the seed and germinating plants from soil insects. One set of five genotypes in the CVT 2003 and in the CVT 2004, 2nd set of five genotypes in the CVT 2004 and in the CVT 2005, 3rd set of six genotypes in IYT 2004 and in CVT 2005 including local and standard varieties were common tested entries across the years. The analysis of variances was performed for individual and over year for the traits recorded in trials using Genstat (1996). Ear aspect, plant aspect and husk cover were scored in 1 to 5 scale where 1 stands for excellent, 2 for, good, 3 for fair, 4 for poor and 5 for very poor. Similarly, Turcicum leaf blight (TLB) was also scored in 1-5 scales. Least significance difference (LSD) was used for comparison of means.

RESULTS AND DISCUSSION

Grain yield

The genotypes which were common in the CVT 2003 and in the CVT 2004 differed significantly over year in grain yield. In both years P501-SRCO-F1/P502-SRCO-F1 produced significantly higher grain yield (5443 kg/ha) than Manakamana-3 (3805 kg/ha), farmers' variety (3703 kg/ha) and ZM 421 (3661 kg/ha). DRACOSYN-F1/DRBCOSYN-F1 produced higher grain yield than local variety and ZM 421 and performed similar to P501-SRCO-F1/P502-SRCOF1 and Manakamana-3 (Table 1). P501-SRCO-F1/P502-SRCO-F1 and DRACOSYN-F1/DRBCOSYN-F1 genotypes were also identified better by Sah et al (2004), Sharma et al (2004), Adhikari et al (2004) and Sherchan (2004). Both the genotypes produced significantly higher grain yield than Manakamana-3 and local check as stated in ARSP (2004). They also produced significantly higher grain yield than Manakamana-2 (2243 kg/ha), however, they were found at par with Manakanama-3 and local check (3458 kg/ha) and grain yield performance of ZM 421 was found similar to P501-SRCO-F1/P502-SRCO-F1 and DRACOSYN-F_1/DRBCOSYN-F1 at Pakhribas (ARSP 2005). The result of combined analysis of five common genotypes of CVT conducted in 2004 and 2005 showed that Manakamana-3 (4973 kg/ha), Iquitos 9325 (4321 kg/ha), SZSYNKITH/SZSYNECU573 (4062 kg/ha) and ZM 421 (4006 kg/ha) produced significantly higher grain yield than local check (2849 kg/ha) and they were not different each other (Table 2). Iquitos 9325 and SZSYNKITH/SZSYNECU573 performed poorer than Manakamna-3, P501-SRCO-F1/P502-SRCO-F1 and DRACOSYN-F1/DRBCOSYN-F1 whereas Iquitos 9325 and ZM 421 performed same as Manakamana-3 (ARSP 2005).

TABLE 1. OBSERVED TRAITS OF COMMON MAIZE GENOTYPES IN CVTS CONDUCTED IN 2003 AND 2004

Genotype	Grain yield, kg/ha			Ear height, cm	Plant height, cm	Silking days (50%)	Plant aspect	Shoot lodging plant/ha, n	Harvest plant/ha, n
	2003	2004	Mean						
P501--SRCO-F1/P502- SRCO-F1	6422	4464	5443	124	243	92	2.65	5741	46667
DRACOSYN-F1/DRB-COSYN-F1	5522	3923	4723	113	228	91	2.65	7778	46296
ZM 421	3956	3365	3661	119	221	93	3.25	3333	43148
Manakamana-3	3526	4083	3804	151	266	93	3.05	9444	44074
Farmers' variety	3947	3458	3703	165	278	88	3.3	7593	44074
Mean	4675	3859	4267	135	247	91		6778	44852
F-test Entry	**	*	*	**	*	**		**	**
Year			**	*	**	*		ns	ns
Year x Entry			ns	*	**	**		ns	ns
LSD Entry	1301	1324	1005	16	20	4		7552	6388
Year			636	10	13	2			
Year x Entry				23	29	5			
CV, %	14.5	21	19	10	7	3		91	11

,, Significant at 0.05 and 0.01 probability level respectively. ns, Non significant.*

Over year variance analysis of six genotypes common in IYT 2004 and CVT 2005 showed significant ($P < 0.05$) differences among entries. Across 9942/Across 9942 (5976 kg/ha) and Across 00502 (5509 kg/ha) were evaluated alike Manakamana-3 in grain yield but their yields were found significantly higher than Coxtla 59627 (4876 kg/ha), Across 9942 (4537 kg/ha) and local variety

(3384 kg/ha) (Table 3). Mankamana-3 (5599 kg/ha) also gave significantly higher grain yield than Across 9942 and local check. Across 9942/Across 9942 and Across 00502 were recorded high yielding genotypes alike Manakamana 3 in a study conducted at Pakhribas (ARSP 2006). Across 9942/Across 9942 was also recognized the highest grain yielder genotype across locations as reported in NMRP (2006).

Table 2. Different data of common entries recorded in CVT normal maize 2004 and 2005

Genotype	Grain yield, kg/ha			Ear height, cm	Plant height, cm	Silking days	Root lodging plant/ha, n	Shoot lodging plant/ha, n	Ear aspect (1-5 scale)	Plant aspect (1-5 scale)	Husk cover (1-5 scale)
	2004	2005	Mean								
Iquitos 9325	3596	5047	4321	141	247	85	4255	11111	2.67	2.75	1.83
Szsynkith/Szsynecu 573	3101	5022	4062	149	265	85	4811	5889	2.92	2.9	2.83
ZM 421	3365	4647	4006	112	206	81	3333	4078	2.83	3.5	3.17
Manakamana-3	4083	5863	4973	144	251	86	6666	10367	2.42	2.5	1.33
Farmers' variety	3458	2239	2849	171	284	80	12967	16667	3.5	1.16	0.5
Mean	3642	4688	4042	140	251	84	6411	9633	2.87	3	1.93
F-test: Entry	*	**	**	**	**	**	**	**			
Year			**	*	**	ns	ns	**			
Year x Entry			*	ns	ns	ns	*	**			
LSD: Entry	1324	1232	1064	25	13	3.8	4666	5378			
Year			673	16	8	2.46	2888	3400			
Year x Entry			1505	36	19	505	6555	7600			
CV, %	21	15.5	21.7	15	4.3	3.8	60	46			

,, Significant at 0.05 and 0.01 probability level respectively. ns, Non significant.*

EAR AND PLANT HEIGHT

Five common entries of all three sets showed significant differences for plant and ear height. Farmers' variety possessed taller ear and plant height in each set. P501-SRCO-F1/P502-SRCO-F1 and DRACOSYN-F_1/DRBCOSYN-F1 were recorded shorter both in ear and plant height than Manakamana-3 (Table 1). Plant and ear heights of Iquitos 9325 were found at par with Manakamana-3. SZSYNKH/S2SYNECU573 was measured taller than Manakamana-3 in plant height and found same as Manakamana-3 in ear height. ZM 421 was the shortest among all both in ear and plant heights (Table 2). Across 00502 was found alike Manakamana-3 both in ear and plant heights. Across 9942/Across 9944 and Across 9942 were shorter than Manakamana-3 and Across 00502 in ear height and same as Manakamana-3 in plant height whereas Coxtla was found shorter than Manakamana-3 both in ear and plant heights (Table 3). P501-SRCO-F1/P502-SRCO-F1 and DRACOSYN-F_1/DRBCOSYN-F1 produced ears almost in the mid portion of the plant and ZM 421 produced ears above middle parts of plant. Across 9942, Coxtla 59627 and Across 00502 had the ears almost in mid portion whereas Across 9942/Across 9944, Iquitos 9325 and SZSYNKH/S2SYNECV573 had ears above mid portion of plants. Higher ear placed plants tend to lodge where as lower ear placement is more prone to damage by wild animals like Dumsi, monkey and Dheru particularly in the Eastern hilly areas surrounded by Jungles.

Table 3. Observed traits of common entries in IYT 2004 and CVT 2005 normal maize

Entry name	Grain yield, kg/ha			Ear height, cm	Plant height, cm	Silking days	Ear aspect (1-5)	Plant aspect (1-5)	Husk cover (1-5)	Root lodging plant/ha, n
	IYT 2004	CVT 2005	Mean							
Across 9942	5582	3492	4537	114	227	76	2.83	2.5	1.8	2966
Coxtla 59627	5011	4741	4876	112	220	81	2.17	2	1.8	1300
Across 00502	5761	6053	5909	131	236	88	2.05	2	2	2777
Across 9942/Across 9944	6265	5688	5976	117	221	80	1.83	1.5	1.16	922
Manakamana-3	5336	5863	5599	133	244	83	2	1.8	1.16	4077
Farmers' variety	4528	2239	3384	162	262	80	3	3.16	1	9078
Mean	5115	4679	4897	128	235	82				3522
F-test: Entry	**	**	*	**	**	**				**
Year			ns	**	**	**				**
Year x Entry			ns	*	ns	*				*
LSD Entry	1666	1232	1615	13	20	2.2				4255
Year				8	11	1.3				2455
Year x Entry				18	27	3.2				6030
CV, %	22.5	15.5	27	8.5	7	2.3				101

,, Significant at 0.05 and 0.01 probability level respectively. ns, Non significant.*

Silking days

Tested genotypes differed significantly ($P < 0.01$) in 50% silking days in all three sets. DRACOSYN F1/DRBCOSYN-F1 (91 days) and P501-SRCO-F1/P502-SRCO-F1 (92 days) were not different

from Manakamana-3 (93 days) and farmers' variety (88 days). ZM 421 (93 days) was also evaluated statistically at par with Manakmana-3 and farmers' variety in silking days in combined analysis of CVT 2003 and CVT 2004 (Table 1) and found significantly earlier than Manakamana-3 in combined result of CVT 2004 and CVT 2005 (Table 2), however, its yield potentiality was recorded poorer than others. Across 00502 was recorded very late (88 days) and Across 9942 was the earliest (76 days) in silking than all other tested genotypes in combined analysis of IYT 2004 and CVT 2005 where as silking days of Across 9942/Across 9944 (80 days) was alike with local variety but earlier than Manakamana-3 (Table 3). Similarly, Iquitos 9325 (85 days), SZSYNKH/S2SYNECU573 (85 days) and Manakamana-3 (86 days) were evaluated same in silking days (Table 2).

Ear aspect, plant aspect and husk cover

DRACOSYN-F1/DRBCOSYN-F1 and P501-SRCO-F1/P502-SRCO-F1 were scored better with regard to ear and plant aspects. P501-SRCO-F1/ P502-SRCO-F1 were evaluated fair in husk cover whereas DRACOSYN F1/DRBCSYN-F1 was found poor in husk cover. Across 9942/Across 9944, Across 00502 and Manakamana-3 were evaluated better with regard to plant aspect, ear aspect and husk cover. Iquitos 9325 was better in husk cover and fair in ear and plant aspects. SZSYNKH/S2SYNECV573 was fair in ear aspect, plant aspects and husk cover scores.

DRACOSYN-F1/DRBCOSYN-F1 and P501-SRCO-F1 / P502-SRCO-F1 were of good yield potential (4464 kg/ha) and same as Manakamana-3 in maturity. Both genotypes were recorded shorter in ear height and plant height than Manakamana-3, however, their ear placement was almost in middle part of plant (Table 1). P501-SRCO-F1/P502-SRCO-F1 was found promising considering recorded traits and is promoted for further verification. Iquitos 9325 and SZSYNKH/S2SYNECU573 possessed yield potentiality and silking days same as Manakamna-3 but Iquitos 9325 and Manakamna-3 were evaluated better with regard to ear aspect, plant aspect and husk cover than SZSYNKH/S2SYNECU573. ZM 421 was found earlier in silking, shorter both in ear and plant height and fair in plant aspect, ear aspect and husk cover. Its earliness trait could be used to make early population. Across 9942/Across 9944 and Across 00502 were high grain yielder varieties but their grain yield was not significantly different from Manakamana-3. Across 9942/Across 9944 was found earlier in silking, shorter in both ear and plant height and better with regard to ear aspect, plant aspect and root lodging. Therefore, this variety is recommended to promote for further test on the farmers' field. Across 00502 was found high yielder and good with respect to ear aspect, plant aspect and husk cover, however it was late in silking days like Deuti. Coxtla is same as Across 00502 in many characters but earlier in silking. So this genotype should also be considered for on farm verification.

ACKNOWLEDGEMENTS

Authors are happy to acknowledge SDC, Nepal for providing fund and HMRP leader Dr GO Ferrara and Agronomist Dr TP Tiwari for their technical guidance and necessary support to carry out research activities. We are grateful to the chief of ARS, Pakhribas and coordinator of NMRP for their technical and managerial support for trial accomplishment. We are also thankful to the technicians KB Thapa, A Jha and FB Bruwal for their efforts and supports to trial, data collection and data entry. We also like to thank ASR Bajracharya and Dr TP Brakoti for their support in paper preparation.

REFERENCES

Adhikari BN, S Bajracharya, B Khanal, S Thapa and SK Budathoki. 2004. Maize varietal investigation at Kabre, Dolkha in 2003. **In:** *Proceedings of the 24th National Summer Crops Workshop on Maize Research Production in Nepal* (DP Sherchan, K Adhikari, BK Basta, D Sharma, eds), 28-30 June 2004, Nepal Agriculture Research Institute, NARC, Khumaltar, Lalitpur, Nepal. P. 121.

ARSP. 2004. Maize varietal research. **In:** *Annual report-2004*. Agriculture Research Station, Pakhribas (ARSP), Nepal Agricultural Research Council, Dhankuta, Nepal. Pp.14-15.

ARSP. 2005. Maize varietal research. **In:** *Annual report-2005*. Agriculture Research Station, Pakhribas (ARSP), Nepal Agricultural Research Council, Dhankuta, Nepal. Pp.15-18.

ARSP. 2006. Annual report-2006. Agriculture Research Station, Pakhribas (ARSP), Nepal Agricultural Research Council, Dhankuta, Nepal. Pp.18-21.

Baniya BK, A Priyadarshi and D Dahal. 2002. Maize OPV research works at Khumaltar. **In:** *Proceedings of 23rd National Crop Workshop on Maize Research and Production* (K Adhikari, SR Upadhaya and RR Mahato, eds), NARC, Khumaltar, Lalitpur, Nepal. Pp.119-137.

CBS. 2005. *Statistical Year Book of Nepal 2005*. National Planning Commission, His Majesty Government of Nepal.

NMRP. 2006. Full season maize varietal improvement for hills of Nepal, 2005/2006. **In:** *Annual report-2005/2006*. National Maize Research Program (NMRP), Nepal Agricultural Research Council, Rampur, Nepal. Pp. 8-13.

Sah SN, MN Paudyal and PK Maharjan. 2004. Study on performance of open pollinated maize varieties in the Eastern mid hill of Nepal. **In:** *Proceeding of the 24th National Summer Crops Workshop on Maize Research production in Nepal* (DP Sherchan, K Adhikari, BK Basta, D. Sharma, eds), 28-30 June 2004, Nepal Agriculture Research Institute, NARC, Khumaltar, Lalitpur, Nepal. P. 104.

Sharma D, SR Upadhaya, DC Paudel, KB.Koirala, BK Baniya, BB Pokharel, BN Adhikari, BB Maharjan, NB Dhami and RB Katuwal. 2004. Evaluation of full season open pollinated maize varieties (OPVs) for improving food security in the hill of Nepal. **In:** *Proceedings of the 24th National Summer Crops Workshop on Maize Research Production in Nepal* (DP Sherchan, K Adhikari, BK Basta, D Sharma, eds), 28-30 June 2004, Nepal Agriculture Research Institute, NARC, Khumaltar, Lalitpur, Nepal. P. 49

Sherchan DP. 2004. Maize research highlights (2002-2004): Contribution to food security and improving the livelihood of the Nepalese People. **In:** *Proceeding of the 24th National Summer Crops Workshop on Maize Research Production in Nepal* (DP Sherchan, K Adhikari, BK Basta, D Sharma, eds), 28-30 June 2004, Nepal Agriculture Research Institute, NARC, Khumaltar, Lalitpur, Nepal. Pp. 1-14.

On-Farm Variation and Household Diversity of Pigeon Pea Landraces in Kachorwa, Nepal

Bal K. Joshi[1*], Pitamber Shrestha[2], Madhusudan P. Upadhyay[1], Bedananda Chaudhary[3], Ashok Mudwari[1], Bimal K. Baniya[1] and Hari B. KC[1]

[1] Nepal Agricultural Research Council, Khumaltar, PO Box 1135 Kathmandu, Nepal
[2] LIBIRD, PO Box 324 Pokhara, Nepal
[3] NRRP, NARC, Hardinath, Janakpur, Nepal

* Corresponding author: joshibalak@rediffmail.com

ABSTRACT

Farmers value the diversity because of diverse environments both in production and consumption. On-farm morphological variation on pigeon peas (2n = 2x= 22 or 4x, 6x?) was studied at Kachorwa, Bara, Nepal to assess the household categories that have maintained diversity. Household diversity Index (HDI) of 10 different farmer categories was estimated based on Shanon-Weaver method. Eighteen quantitative and qualitative (quant-qualitative) traits were used for on-farm variation and HDI studies. On farm ANOVA was generated on eight quantitative traits. Highest diversity (HDI, 0.265) was maintained by farmer of medium wealth category who grows pigeon pea in upland bund. Pigeon pea grown in *khet* (low land) bund with Pajawa landrace expressed least diversity (HDI, 0.079). Pigeon pea growing in monoculture was more diverse (HDI, 0.224) for 18 traits than in other production environments. Maximum variation was observed in growth habit followed by seed color pattern. The highest grain yield among the Chanki growers was produced by the farmer of medium wealth category growing pigeon pea in upland bund. Significance variation among farmers in quantitative traits indicates the intra varietal diversity in pigeon pea. Diversity varied with respect to wealth category and production environments. Farmer who has maximum diversity on pigeon pea could able to receive the higher grain yield. Result related to where and who maintain the diversity may be useful for development of on-farm conservation strategy. Possibility of developing good varieties exits using Pajawa and Chanki landraces.

Key words: Household diversity index, on-farm variation, pigeon pea, quant-qualitative traits

INTRODUCTION

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is an important summer crop of farmers living in Tarai and Inner Tarai (< 600 m) of Nepal. It is a multipurpose crop grown as sole crop or an intercrop in many farming systems. It is also grown by small farmers on marginal lands where other crops are not suitable. Many landraces of pigeon pea exist in Nepal (Neupane 1995), which are suitable to diverse environments. Area coverage under pigeon pea is 25460 ha with productivity of 0.78 t/ha in Nepal (NARC 1998). Dehulled seeds of pigeon pea are used as dhal, seed husk are fed to animals, dry stems are used for firewood and to make huts and baskets. Root nodules of this crop fix N_2 thus increasing the soil fertility and the deep roots of pigeon pea take up phosphorus, which is thus available to other crops (Johansen 1990).

Kachorwa is one of selected ecosites to study on-farm agrobiodiversity in Nepal. This site lies in Tarai belt possessing low to moderate level of diversity with high degree of intervention (Upadhyay and Subedi 2000). Pigeon pea is one of the grain legumes growing in larger area in Bara (Sherchand et al 1998). Nepal Agricultural Research Council has released two pigeon pea varieties suitable for low altitude. Even though these improved varieties are not found in this site. Participatory rural appraisal and diversity fair indicated the existence of high landrace richness of pigeon pea in Kachorwa (Khatiwada et al 2000). Among these landraces Chanki is most common. Farmers cultivate pigeon pea under low external input without farmyard manure,

chemicals and agro chemicals (Rana et al 2000). Pigeon pea grains in the market fetches highest price amongst the pulses (Rana et al 2000). Pigeon pea is often self-pollinated and biannual crop. Knowledge on population structure and breeding system could be useful for the maintenance of diversity on-farm. Farmers also use small area to plant pigeon pea. Population genetic structure in such a small population if there is diversity could help in policy formulation for on-farm management of agricultural biodiversity.

On-farm variation is important to farmers, breeders and *in situ* conservationists. Variation in production environments and food value or farmers' need create diversity and help to maintain different forms of crop plants. For genetic resources conservation *in situ* method is treated as complement of *ex situ* conservation. Information on amount and distribution of genetic diversity maintained by farmer and farmers who maintain diversity on-farm is prerequisite for effective implementation of *in situ* conservation activities. Therefore this study was designed to have information about amount and distribution of pigeon pea diversity over household. Additionally population structure of pigeon pea landraces and on-farm characterization and evaluation of these landraces were studied.

MATERIALS AND METHODS

We reviewed baseline report 1998-99 of Kachorwa ecosite to identify/select landraces and farmers. There are 12 different landraces in Bara (Sherchand et al 1998) and 5 landraces in Kachorwa site (Rana et al 2000). But only two landraces are being cultivated now in Kachorwa. Two landraces Chanki and Pajawa were selected in 2003 growing season. Ten farmers were selected based on pigeon pea landraces, cultivation environments and wealth category. Farmers' name and their category are given in Table 1.

Table 1. Farmers' name and their category

SN	Farmer		Category
	Full name	Short form	
1	Shiva Sah	S Sah	Rich growing pigeon pea in upland bund
2	Janga Bahadur Raya Yadav	JBR Rana	Rich growing pigeon pea in lowland bund
3	Rup Narayan P Yadav	RNP Yadav	Medium growing pigeon pea in upland bund
4	Mahanarayan P Raya Yadav	MPR Yadav	Pajawa grower
5	Ram Lal Sah	RL Sah	Medium growing pigeon pea in monoculture
6	Madandas Tatma	M Tatma	Poor growing pigeon pea in lowland bund
7	Shovi Raya Yadav	SR Yadav	Medium growing pigeon pea in lowland bund
8	Narayan Mahato Kahar	NM Kahar	Poor growing pigeon pea in monoculture
9	Rajendra Raya Yadav	RR Yadav	Rich growing pigeon pea in monoculture
10	Mahendra Mahato Kahar	MM Kahar	Poor growing pigeon pea in upland bund

After discussing with farmers, they were categorized rich, medium and poor based on the wealth status, Three types of production environments are common in Kachorwa. These are upland bund, lowland bund and monoculture. MPR Yadav cultivates Pajawa and all others cultivate Chanki landrace.

From each category of farmer, 10 plants were randomly selected from different pigeon pea growing areas of similar production environment of respective farmer. Number of parcels ranged from 1 to 3. We tried to include plant from all parcels in diversity study. Variation within and between parcels of each farmer was captured.

A total of 18 quant-qualitative characters were measured based on descriptors for pigeon pea (IBPGR and ICRISAT 1993). These traits were recorded on individual plant of each farmer's category. For household diversity index (HDI), 14 traits (Table 2) were used. Four quantitative traits, plant height, branch number, raceme number and yield were converted in qualitative traits. Individual plant was defined as tall (> 200 cm) and dwarf (≤ 200 cm) based on plant height. Classes based on total number of branches were high (> 30), medium (16-30) and low (< 16). Similarly classes were high (> 150), medium (51-150) and low (< 50) based on raceme number per plant and high (> 20g), medium (11-20 g) and low (< 11g) based on grain yield per plant. HDI was estimated using the formula of Shanon-Weaver index. This index was used previously for studies of variability in crop plants by Tolbert et al (1979) in barley, Holcomb et al (1977) in rice, and Cruz et al (1997) and Pandey et al (2003) in sponge gourd. The index is calculated as

$$HDI = -\sum_{i=1}^{n} pi \log_2 pi$$

where pi is the proportion of entries in the i^{th} class and n is the number of phenotypic classes for a trait. Mean and standard error (SE) were also estimated for HDI. A hierarchical analysis of variance for testing the significance among farmers and between traits within farmers in HDI was applied.

On farm variation between farmers was tested in quantitative traits. Least significance difference at 5% level was estimated for each trait. Student's t was used to test the yield of two landraces. Population structure of two landraces was studied. Mean, range and standard deviation (SD) were estimated in nine traits for Chanki and Pajawa landraces. Correlation coefficients were estimated among these characters. Characters relation would help in selection process of pigeon pea on-farm. Minitab and Excel computer software were used for analysis.

RESULTS AND DISCUSSION

Phenotypic proportion of classes of each trait with respect to farmers is given in Table 2. Distribution of phenotypic classes was observed in growth habit, plant height, branches, raceme number, seed color pattern, seed eye width, seed shape and grain yield. Distribution pattern of these classes was similar among the farmers. Probably this is due to the low number of observations taken per farmer and these farmers were taken from the same community. Most of the plant showed semi spreading type of growth habit. Trailing growth habit was rare, only observed in Pajawa landrace. Erect and compact was not also common. Such type of plant is important for inter crop planting. Pigeon pea is bund-adopted crop, therefore farmers try to minimize the shading effect of this crop to neighbor crops. All plants have green stem with lanceolate leaflet and smooth leaf surface. Mix pod color with flat shape was common to all populations of these landraces. Two types of seed color pattern were observed. Most of seed were oval and elongate shape was rare. There was variation on plant height, branching type, grain color, seed color and fruiting, which are used by farmers to distinguish landraces (Paudel et al 1999). This means that selection based on farmer's traits could be resulted in positive direction on these traits.

Household diversity index was zero for all farmers for leaf hairiness, leaflet shape, pod color, pod form, pod hairiness and stem color (Table 3). But variation was reported for these characters by Bajracharya et al (1999). This may be due to the inclusion of more landraces in her study. Though low diversity index was not necessarily associated with small sample size (Tolbert et al 1979). The highest HDI (1.00) was observed in grain yield of S Sah, JBR Yadav and R Sah. The lowest HDI (0.095) was recorded on seed shape of RR Yadav. Variation in number of landraces was not found at household (HH) level (Rana et al 2000), but variation exists within population grown by HH. HDI was the highest in pigeon pea grown in monoculture (0.224) followed by upland bund (0.219). Pigeon pea grown in lowland bund expressed the lowest HDI (0.166). Averaging HDI over traits, RNP Yadav expressed highest the HDI and MPR Yadav who grow Pajawa, the lowest. RNP Yadav is a medium wealth status farmer growing pigeon pea in upland bund. Among landraces Chanki gave the highest HDI (0.217). Within landraces medium wealth category farmer has the highest diversity but Rana et al (2000) reported that poor has the highest landrace diversity. Medium wealth category also grows pigeon pea in larger area (Rana et al 2000). Most of the farmers' HDI fall within from 0.18 to 0.26. HDI indicates the farmers that have maintained diversity on-farm. Highly significant difference was found for diversity indices between farmers and between traits within farmer (Table 4).

Table 2. Phenotypic proportions based on percentage of plant numbers (N = 10) for each farmer categoy of pigeon pea for 14 characters

SN	Trait	Classes	S Sah	JBR Yadav	RNP Yadav	MPR Yadav	RL Sah	M Tatma	SR Yadav	NM Kahar	RR Yadav	MM Kahar
1	Growth habit	Erect and compact	0	0	0	0	20	20	0	0	0	0
		Semi spreading	100	100	60	0	80	80	100	90	100	100
		Spreading	0	0	30	100	0	0	0	10	0	0
		Trailing	0	0	10	0	0	0	0	0	0	0
2	Plant height	Tall	20	80	10	0	80	90	90	70	90	100
		Dwarf	80	20	90	100	20	10	10	30	10	0
3	Branches	High	80	70	80	100	90	80	30	60	0	40
		Medium	20	30	20	0	10	20	60	30	80	50
		Low	0	0	0	0	0	0	10	10	20	10
4	Stem color	Green	100	100	100	100	100	100	100	100	100	100
		Sun red	0	0	0	0	0	0	0	0	0	0
		Purple	0	0	0	0	0	0	0	0	0	0
		Dark purple	0	0	0	0	0	0	0	0	0	0
5	Leaflet shape	Lanceolate	100	100	100	100	100	100	100	100	100	100
		Narrow elliptic	0	0	0	0	0	0	0	0	0	0
		Broad elliptic	0	0	0	0	0	0	0	0	0	0
		Obcordate	0	0	0	0	0	0	0	0	0	0

SN	Trait	Classes	S Sah	JBR Yadav	RNP Yadav	MPR Yadav	RL Sah	M Tatma	SR Yadav	NM Kahar	RR Yadav	MM Kahar
6	Leaf hairiness	Glabrous	100	100	100	100	100	100	100	100	100	100
		Pubescent	0	0	0	0	0	0	0	0	0	0
7	Raceme number	High	40	20	60	100	60	60	20	40	0	0
		Medium	60	70	40	0	40	40	50	20	70	80
		Low	0	10	0	0	0	0	30	40	30	20
8	Pod color	Green	0	0	0	0	0	0	0	0	0	0
		Purple	0	0	0	0	0	0	0	0	0	0
		Mixed (green + purple)	100	100	100	100	100	100	100	100	100	100
		Dark purple	0	0	0	0	0	0	0	0	0	0
9	Pod form	Flat	100	100	100	100	100	100	100	100	100	100
		Cylindrical	0	0	0	0	0	0	0	0	0	0
10	Pod hairiness	Glabrous	0	0	0	0	0	0	0	0	0	0
		Pubescent	100	100	100	100	100	100	100	100	100	100
11	Seed color pattern	Plain	100	40	60	20	100	100	100	90	100	100
		Mottled	0	60	40	80	0	0	0	10	0	0
		Speckled	0	0	0	0	0	0	0	0	0	0
		Mottled and speckeled	0	0	0	0	0	0	0	0	0	0
		Ringed	0	0	0	0	0	0	0	0	0	0
12	Seed eye width	Narrow	0	0	0	0	0	0	0	0	40	0
		Medium	100	100	100	100	100	100	100	100	60	100
		Wide	0	0	0	0	0	0	0	0	0	0
13	Seed shape	Oval	80	100	100	100	70	100	90	100	90	80
		Globular	0	0	0	0	0	0	0	0	0	0
		Square (angular)	0	0	0	0	0	0	0	0	1	0
		Elongate	20	0	0	0	30	0	10	0	0	20
14	Grain yield	High	50	20	80	70	30	80	10	50	0	60
		Medium	30	30	10	0	20	20	50	10	60	30
		Low	20	50	10	30	50	0	40	40	40	10

Table 3. Household diversity indices (HDI) for farmers and characters and mean diversity and its standard error

SN	Trait	S Sah	JBR Yadav	RNP Yadav	MPR Yadav	RL Sah	M Tatma	SR Yadav	NM Kahar	RR Yadav	MM Kahar	Mean	SE
1	Branches	0.500	0.611	0.500	0.000	0.325	0.500	0.668	0.668	0.179	0.713	0.466	0.073
2	Grain yield	1.000	1.000	0.639	0.611	1.000	0.500	0.943	0.943	0.673	0.898	0.830	0.064
3	Growth habit	0.000	0.000	0.898	0.000	0.500	0.500	0.000	0.325	0.000	0.000	0.222	0.101
4	Leaf hairiness	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0
5	Leaflet shape	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	Plant height	0.500	0.500	0.325	0.000	0.500	0.325	0.325	0.611	0.325	0.000	0.341	0.065
7	Pod color	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8	Pod form	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0
9	Pod hairiness	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10	Raceme number	0.673	0.572	0.673	0.000	0.673	0.673	0.668	0.688	0.250	0.179	0.505	0.081
11	Seed color pattern	0.000	0.673	0.673	0.500	0.000	0.000	0.000	0.325	0.000	0.000	0.217	0.093
12	Seed eye width	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.673	0.000	0.067	0.067
13	Seed shape	0.500	0.000	0.000	0.000	0.611	0.000	0.325	0.000	0.095	0.500	0.203	0.08
14	Stem color	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0
	Mean	0.229	0.242	0.265	0.079	0.260	0.179	0.209	0.254	0.157	0.164		
	SE	0.092	0.095	0.091	0.054	0.092	0.069	0.087	0.090	0.065	0.082		

On-farm variation on eight different quantitative traits exists among these farmers. Variation was observed between and within landraces. Significance yield variation (P = 0.006) was found between Chanki and Pajawa landraces. Pajawa produced grain yield three times more than Chanki. Due to the unwanted traits of Pajawa, farmers prefer Chanki even it produces less grains. Intra landraces diversity was also reported by Bajracharya et al (1999) and Khatiwada et al (2000). Plant height, stem thickness and seed characters were mentioned as important traits for measuring variability in pigeon pea (Bajracharya et al 1999). Our study also supports the finding of Bajracharya et al (1999).

Table 4. Analysis of variance for diversity indices between and within farmers for 14 characters

Source	df	MS	%	P
Between farmers	9	0.04898	3.4	< 0.01
Between traits within farmer	130	0.09598	92	< 0.01

Significance variation was found in seven traits among farmers (Table 5). MPR Yadav has pigeon pea with the highest tertiary branches, raceme number per plant and the highest grain yield. The tallest plant was found in SR Yadav's field. Pod was the longest in pigeon pea of S Sah but the highest seed number per pod was in MM

Kahar. Among the Chanki growers NM Kahar produced highest yield. He is a poor farmer growing pigeon pea in monoculture. Rich farmer growing pigeon pea in low land received the lowest grain among Chanki growers. Much variation in the yield level of pigeon pea was already reported at Kachorwa, which ranged from 0.1 to 1.26 t/ha (Rana et al 2000). Medium wealth category had produced the highest yield than rich and poor (Rana et al 2000).

Two populations of Pajawa and Chanki were different for 10 studied traits (Table 6). Chanki landrace showed relatively more variation than Pajawa in plant height, branches, pod number, seed number and 100-seed weight. More variation was observed in Pajawa than Chanki for raceme number, pod length and grain yield. Number of sample for each landrace was different which might have some effect on capturing variability. Due to the variation within and between populations, these landraces are able to cope different biotic and abiotic stresses.

Table 5. Response of different traits with respect to farmers

SN	Farmer	Plant height, cm	Branches, n			Raceme /plant, n	Pod/ raceme, n	Seed/ pod, n	Pod length, cm	Grain yield/plant, g
			Primary	Secondary	Tertiary					
1	S Sah	186.70	1.300	18.100	28.50	130.90	2.6800	2.930	4.5900	23.50
2	JBR Yadav	215.90	1.400	21.000	21.00	115.50	3.2700	3.120	4.0800	11.70
3	RNP Yadav	188.80	1.100	14.800	42.80	186.80	3.0500	3.510	4.5500	41.30
4	MPR Yadav	183.40	1.200	17.700	63.40	365.60	3.0000	2.970	4.1900	57.50
5	RL Sah	220.20	1.300	19.000	31.90	149.70	3.0100	2.980	4.3700	15.30
6	M Tatma	231.40	2.800	13.200	34.70	206.00	2.7900	3.000	4.0200	30.50
7	SR Yadav	235.40	1.600	13.300	14.00	105.10	2.6800	2.960	4.0500	14.30
8	NM Kahar	219.90	2.800	33.800	16.70	141.90	3.0800	3.020	4.4100	33.20
9	RR Yadav	224.70	1.100	15.900	5.30	69.20	2.8600	3.030	4.1400	12.50
10	MM Kahar	272.60	1.200	16.100	10.60	79.80	2.9500	3.190	4.5000	28.90
	P	0.00	0.000	0.001	0.000	0.000	0.315	0.001	0.002	0.000
	LSD (5%)	21.12	0.937	8.72	15.51	70.12	0.001	0.262	0.343	19.66

Table 6. Population structure of Chanki and Pajawa pigeon pea landraces in Kachorwa

Variable	N		Mean		SD		Minimum		Maximum	
	Chanki	Pajawa	Chanki	Pajawa	Chanki	Pajawa	Chanki	Pajawa	Chanki	Pajawa
Plant height, cm	90	10	221.7	183.4	33.95	10.08	145	166	316	200
Primary branches, n	90	10	1.622	1.2	1.241	0.422	1	1	8	2
Secondary branches, n	90	10	18.36	17.7	11.4	6.53	2	9	76	29
Tertiary branches, n	90	10	22.83	63.4	20.23	19.62	0	36	74	100
Raceme/plant, n	90	10	131.7	365.6	85.75	91.6	22	240	438	476
Pod/raceme, n	90	10	2.93	3	0.553	0.508	1.4	2.3	4.3	3.7
Seed/pod, n	90	10	3.082	2.97	0.336	0.221	2	2.7	4	3.3
Pod length, cm	90	10	4.301	4.19	0.418	0.489	3.5	3.3	5.7	4.7
100 seed weight, g	87	8	6.126	8.375	1.054	0.744	4	7	8	9
Grain yield/plant, g	90	10	23.47	57.5	19.2	47.8	1	1	95	133

SD, Standard deviation.

Table 7. Correlation coefficients among nine characters based on 10 farmers' field observations

	Plant height	2	3	4	5	6	7	8
2. Primary branches	0.075							
3. Secondary branches	0.018	0.508**						
4. Tertiary branches	-0.268**	0.056	-0.075					
5. Raceme/plant	-0.186	0.277**	0.183	0.846**				
6. Pod/raceme	0.083	0.114	0.162	0.328**	0.259**			
7. Seed/pod	0.160	0.028	0.136	0.159	0.163	0.378**		
8. Pod length	0.108	-0.008	0.313**	-0.020	-0.016	-0.014	0.341**	
9. Grain yield	-0.110	0.212*	0.260**	0.500**	0.647**	0.212*	0.247*	0.115

**, **, Significantly different from zero at 5 and 1 % level.*

Traits relationship was given in Table 7. There is highly significance correlation between secondary branches and yield, tertiary branches and yield, and raceme number and yield. Coefficients indicate that for yield increment, branches (secondary and tertiary) and raceme number should be given priority during selection. Second important traits are pod number, pod length and seed number for yield improvement. Farmers commonly consider branches and raceme number during selection of pigeon pea plants.

Existence of on-farm variation for these traits indicates the possibility of improvement of these landraces. Branches and raceme number are the primary traits, which would response positively to selection. Rana et al (2000) documented the preferred and unpreferred traits of Chanki and Pajawa landraces. Chanki has more preferred traits but Pajawa produced more grains yield. Major traits considered by farmers are branching, yield and quality. Stem and branches are important for fencing and cooking purpose in Tarai areas. There was diversity at population level among these landraces. Improving Chanki landrace on branching and yield using Pajawa might be a better strategy to conserve landraces and meet the farmers' need. Medium wealth category growing pigeon pea in upland has the greatest diversity. Farmer having the highest diversity could able to produce the highest grain yield. Because of on-farm variation especially in farmer's traits, selection response is expected in both Chanki and Pajawa landraces.

ACKNOWLEDGEMENTS

Farmers of Kachorwa ecosite, FK Chaudhary, SS BK, L Karna and K Shrestha are acknowledged for implementing this research and IPGRI for financial support.

REFERENCES

Bajracharya J, DK Rijal, SP Khatiwada, CL Poudel, MP Upadhyay, YR Pandey, PR Tiwari, RB Yadav and P Chaudhary. 1999. Farmer selection of germplasm using agro morphological and isozyme characteristics. **In:** *A scientific basis of in situ conservation of agrobiodiversity on-farm: Nepal's contribution to the global project* (B Sthapit, M Upadhyay and A Subedi, eds). NP Working Paper No 1/99. Pp. 49-66.

Cruz VMV, MIS Tolentino, NC Altoveros, MLH Villaricercio, LB Siopongeo, AC Dela Vina and RP Laude. 1997. Correlations among accessions of Southeast Asian Luffa genetic resources and variability estimated by morphological and biochemical methods. *Philipp J Crop Sci* 22:131-140.

Holcomb J, DM Tolbert and SK Jain. 1977. A diversity analysis of genetic resources in rice. *Euphytica* 26:441-450.

IBPGR and ICRISAT. 1993. *Descriptors for pigeon pea (Cajanus cajan* (L.) Millsp). International Board for Plant Genetic Resources, Rome, International Crops Research Institute for the Semi-Arid Tropics, India.

Johansen C. 1990. Pigeon pea: Mineral nutrition. **In:** *The pigeon pea* (YL Nene, SD Hall and VK Sheil, eds), CAB International, ICRISAT. Pp. 209-231.

Khatiwada SP, BK Baniya, DK Rijal, CL Panday, RB Rana, P Chaudhary, PR Tiwari, MP Upadhyay, YR Panday and A Mudwari. 2000. Nepal. **In:** *Conserving agricultural biodiversity in situ: A scientific basis for sustainable agriculture* (D Jarvis, B Sthapit and L Sears, eds). IPGRI, Rome. Pp. 134-138.

NARC. 1998. *NARC Annual Report 1996/97 (FY 2053/54)*. Nepal Agricultural Research Council, Khumaltar Kathamndu.

Neupane RK. 1995. Status of grain legumes genetic resources in Nepal. **In:** *Plant genetic resources: Nepalese perspective* (MP Upadhyay, HK Saiju, BK Baniya and MS Bista, eds). Proceedings of National Workshop 28 Nov - 1 Dec 1994. NARI, IPGRI, Kathmandu. Pp. 83-87.

Pandey YR, D Rijal, MP Upadhyay, B Sthapit and BK Joshi. 2003. In-situ characterization of sponge gourd at Begnas, Kaski, Nepal. **In:** *On farm management of agricultural biodiversity in Nepal* (BR Sthapit, MP Upadhyay, BK Baniya, A Subedi, BK Joshi, eds). Proceedings of the National Workshop, 24-26 April 2001, Lumle Nepal. NARC, LIBIRD and IPGRI, Kathmandu. Pp. 63-70.

Paudel CL, P Chaudhary, DK Rijal, SN Vaidya, PR Tiwari, RB Rana, D Gauchan, SP Khatiwada, SR Gupta and BR Sthapit. 1999. Agro ecosystem factors for in situ conservation of agrobiodiversity in different eco-zones of Nepal. In: A scientific basis of in situ conservation of agrobiodiversity on-farm: Nepal's contribution to the global project (B Sthapit, M Upadhyay and A Subedi, eds). NP Working Paper No 1/99. NARC, Nepal. Pp. 9-23.

Rana RB, P Chaudhary, D Gauchan, SP Khatiwada, BR Sthapit, A Subedi, MP Upadhyay and DI Jarvis. 2000. *In situ crop conservation: Findings of agro-ecological, crop diversity and socio-economic baseline survey of Kachorwa ecosite Bara, Nepal*. NP Working Paper No. 1/2000. NARC/LIBIRD/IPGRI.

Sherchand KK, NP Adhakari, SP Khatiwada, AC Shrivastav, J Bajracharya, KD Joshi, KB Kadayat, M Chaudhary, P Vhaudhary, SS Vishwakarma and S Yadav. 1998. *Strengthening the scientific basis for in situ conservation of agrobiodiversity: Findings of site selection in Bara, Nepal*. NP working Paper No 2/98. NARC/LIBIRD/IPGRI.

Tolbert DM, CD Qualset, SK Jain and JC Craddock. 1979. A diversity analysis of a world collection of barley. *Crop Sci.* 19: 789-794.

Upadhyay MP and A Subedi. 2000. Nepal. **In:** *Conserving agricultural biodiversity in situ: A scientific basis for sustainable agriculture* (D Jarvis, B Sthapit and L Sears, eds). IPGRI, Rome. Pp. 15-18.

Effect of River Salinity on Crop Diversity: A Case Study of South West Coastal Region of Bangladesh

Animesh K. Gain[1], Kul P. Aryal[2], Pritish Sana[3] and Md Nazim Uddin[3]

[1] Institute of Water and Flood Management, BUET, Dhaka, Bangladesh <animesh.gain@gmail.com>
[2] Agricultural Economist, NARC, Nepal, <kparyal@narc.org.np>
[3] Environmental Science Discipline, Khulna University, Bangladesh

ABSTRACT

Saline water intrusion is a major problem and conflicting issue in south-west coastal region of Bangladesh. The increased salinity has negative impact on agricultural diversity in this region. The present study provides an assessment of perception of local farmers about changes of agricultural diversity mainly diversity of vegetable species (both summer and winter) and standing plants with the changes of salinity level in the nearby river. This study was carried out in January to August 2005 through semi-structured questionnaire in selected villages of different salinity prone areas such as high saline zone and moderate saline zone, namely Paikgacha and Rampal, respectively. The study has revealed that in Paikgacha, the salinity varies approximately within the range from 20,000 to 45,000 micro-mhos and in Rampal it is from 10,000 to 30,000 micro-mhos. Due to increased salinity, the summer vegetable species in Paikgacha and Rampal have been reduced from 16 to 2 and 15 to 9 respectively during the period 1975-2005. For winter vegetable species, this figure was reduced from 13 to 9 in Paikgacha but in Rampal this number remained unchanged. Standing plant species in Paikgacha and Rampal have been reduced from 31 to 14 and 35 to 21 respectively during that period. Agricultural diversity is reducing in substantial rate both spatially and temporally.

Key words: Biodiversity, salinity, trend analysis

INTRODUCTION

Saline water in southwest coastal region of Bangladesh has created severe problems in agricultural diversity. Changes in tide and fresh-water flow retreat of the salinity limit of the area and productivity of soil and crop both reducing continuously. Under this process, during the wet season, local rainfall associated with flood flows from upland regions keeps the salinity limit near the coastline. Again, salinity starts increasing and introducing upland from the beginning of November with the cessation of the rains and consequent reduction of river flows (Pramanik 1986). The upland fresh water flowing from the Ganges through the Gorai-Madhumati channel governs the state of the salinity of the southwestern region. But the Ganges outflow during the lean period has been reducing since the commissioning of the Farraka barrage in 1975 (Rahman et al 2000). As a result of the reduced flow of water to the southwest region, the intrusion of saline water progressively upstream has made the region vulnerable to increasing salinity. Inundation of surface soil in inland during high tide is common phenomena, the salinity state in river water controls the soil salinity of the region (Chowdhury 1993).

Salinity intrusion has developed a significant change in soil fertility and immunity, increases disease and insect infestation in field crops, restricts germination and normal growth of plants there by reducing the crop yield (Mannan et al 2004). Salinity is the single most important factor for limiting the improvement of crop intensity (Karim et al 1990). Gupta (1990) reported that salinity affect crop diversity in terms of plant growth and yields of plants by general osmotic effect and specific ion effect. Crop diversity is in fact correlated with the stability and resilience of an ecosystem which would have positive relationship with the well being of the existing species structure including the humans (Sengupta 2001). Farmers are leaving low productive crops and only concentrated high yielding crop which is encouraging monocropping. Therefore, reduced crop diversity is a serious

threat to the environment. With time these impacts will be severe if no measure is taken to manage it. So, salinity is now recognized as a serious issue in south-west coastal region of Bangladesh and has a direct impact on ecological sustainability. This study was focused on the changes of crop diversity due to change in river water salinity.

METHODOLOGY

The study area was selected randomly considering the factor that the area should have high or moderate saline zone. Considering the fact, Paikgacha under Khulna district (highly saline zone) and Rampal under Bagerhat district (moderately saline zone) were selected. Two unions were selected from the two sub-districts and from each union two villages were selected because two salinity measuring stations of Bangladesh Water Development Board (BWDB) for Shibsa river of Paikgacha and Passur river of Rampal are very close to the study area. Salinity data of these rivers represents the salinity state of the selected regions. Yearly maximum salinity level series of 27 years (1975-2004; 3 yrs missing) of these two rivers was collected from hydrology department of BWDB. The statistical trend analysis was carried whether the time series data of salinity has statistically significant positive trend or not in both two regions. The procedure of testing linear trends is described herein:

Assume that y_t, $t = 1, \ldots, N$ is an annual time series and N is sample size. Simple linear trend can be written as:

$$y_t = D + Mt$$

where D and M are the parameters of the regression model. Rejection of hypothesis $M = 0$ can be considered as a detection of a linear trend. The hypothesis that $M = 0$ is rejected if

$$T_c = \left| \frac{R\sqrt{(N-2)}}{\sqrt{(1-R^2)}} \right| > T_{1-\alpha/2,v}$$

in which R is the cross-correlation coefficient between the sequences $y_1, \ldots, y_N$ and $1, \ldots, N$ and $T_{1-\alpha/2,v}$ is the $1-\alpha/2$ quantile of the student t distribution with $v = N - 2$ degrees of freedom. Then, trend line was drawn by using MS Excel which represented the changed level of salinity graphically from the year 1975 to 2004.

To detect the change of soil salinity state with the change of river salinity, secondary data were collected from Soil Research Development Institute (SRDI). To collect the crop diversity related data, a semi-structured questionnaire through purposive sampling was prepared. A household was selected as the sampling unit considering the factors that at least one member of each household was related with farming whose age was not less than 60 years during data collection period and permanent resident of the study area. The sample size was determined following the principle of Berensen and Levine (1992) and this was 35 and 45 for Paikgacha and Rampal respectively. The age of the respondents was not less than 60 years because the questionnaire was used to obtain information on the situation of the study area in the past from the year 1975 to 2005 from the local residents. The statistics of the respondent's age profile was 67.67 yrs (0.30 SE) and 66.38 yrs (0.15 SE) of Paikgacha and Rampal respectively.

The year 1975 was selected as the base year as it reflected the year of lowest salinity level in the study area. Data of three years (1975, 1990, and 2005) with an interval of 15 years were undertaken for the analysis of the situation. The interval was relatively high so that local farmers could assess the changes clearly with this long year variation. As it was impossible to collect quantitative data about amount of crop species for the previous years; numeric values 0 to 10 were assigned against the qualitative data collected from respondents. Here rank 0 represents non-availability of a species and 10 represents highly available for the species. Rank 0 to 10 was used to detect the subtle change

of qualitative amount. Based on the quantification of qualitative data, diversity calculation was accomplished.

Shannon Diversity Index (H^-), Species richness index (d) and Species evenness index (e), Index of dominance (c) following the methods described in Odum (1969), Shannon and Weaver (1963) and Simpson (1949) are mostly used in biodiversity calculation and these are the appropriate calculation of biodiversity. An actual number of individuals per species is obligatory to use the biodiversity index. As crop related previous data for the study area was no longer available to calculate diversity even in the agriculture department, the above indices were not used. So, local farmers' perception was used to assess the change of crop diversity. Moreover, farmers were primarily involved with farming, supplying crops in local market for a long period, so they were the reliable source for collecting related data. That is why local farmers' perception was used for better approximation of the changes of crop diversity. The farmers whom were interviewed were the residents of the villages of Charbanda and Golbunia of Paikgacha; Gonabelai and Durgapur of Rampal. The respondents and their predecessors were related with farming since long years ago.

Finally, to detect the actual factors, which are responsible for biodiversity degeneration eight resource persons were interviewed. Among them, there were four agriculture scientists who were working on Paikgacha and Rampal, two soil scientists and two university teachers of agrotechnology disciplines of Khulna University.

RESULTS AND DISCUSSION

Assessment of changes of salinity

The yearly maximum salinity level series of 27 years in Shibsa river of Paikgacha and in Passur river of Rampal have been considered for a comparison of the two study areas (Figure 1). It is shown that salinity level in Shibsa of Paikgacha is always higher than the salinity level in Passur of Rampal because Passur gets more upstream discharge through Nabaganga river from the Gorai channel. In Paikgacha, it ranges approximate from 20,000 to 45,000 micro-mhos and in Rampal it is from 10,000 to 30,000 micro-mhos. Therefore, it is said that Paikgacha is under High Saline Zone (HSZ) and Rampal is in Moderate Saline Zone (MSZ) because it falls under the defined ranges (FAP 1993). In the study, the regression test for linear trend was carried out for the annual maximum salinity level series of salinity measuring station of BWDB at Shibsa and Passur river from the year 1975 to 2004. The result (Table 1) shows that linear trend could be detected in the annual sediment load at the 5% significant level. So, the time series data of salinity has the statistically significant positive trend in both two regions which eventually indicate the increased level of salinity from the year 1975 to 2004. The trend line is also shown graphically in Figure 1.

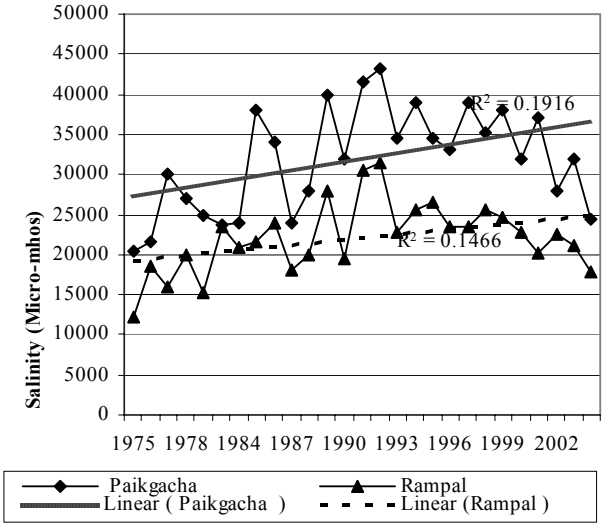


Figure 1. Comparison of highest salinity level between Sibsa of Paikgacha and Passur of Rampal in different years showing trend line.

Table 1. Result of trend analysis of salinity level series of Shibsa and Passur river

Name of the River	Correlation coefficient, R	Trend: test statistics, T_c	Critical value at 5% significance level, $T_{1-\alpha/2,\ v}$ (t distribution)	Existence of trend
Shibsa (Paikgacha)	0.438	2.195	2.06	Trend exist
Passur (Rampal)	0.383	2.073	2.06	Trend exist

With the increase of river water salinity, soil salinity is also increased in the study area. SRDI (2001) made a comparison on soil salinity status in the study area between the year 1973 and 2000. The result of comparison is shown in Table 2 and it was found that over three decades, total saline affected area was increased by 21% in Khulna and 15.9% in Bagerhat. Among different salinity classes, the affected area of higher order classes (S_3 and S_4) was increased significantly.

TABLE 2. DISTRIBUTION AND EXTENT OF DIFFERENT CATEGORIES OF SOIL SALINITY IN KHULNA AND BAGERHAT

District	Total Salt affected area ('000 hectare)		Salt affected area in different salinity class								% increase of total saline affected area over the period (1973-2000)
			S1		S2		S3		S4		
	1973	2000	1973	2000	1973	2000	1973	2000	1973	2000	
Khulna	120.04	145.25	3.90	28.83	92.54	33.32	13.00	59.49	9.80	19.61	21.00
Bagerhat	107.98	125.13	8.30	35.66	77.08	41.50	2.60	41.23	0.00	6.74	15.90

Source: SRDI, 2001.

Soil salinity class and effect of each class on plant is shown in Table 3. Brammer (1996) demonstrated that soil weathering process and saline water intrusion by surface inundation are the main source for soil salinization but surface inundation is dominant in the south west coastal Bangladesh. So, river water salinity is responsible for soil salinization.

Table 3. Soil salinity class on the basis of electrical conductivity (EC) and its effect on plant

Salinity Class	Salinity level (micro-mhos)	Plant growth condition
Non saline (S_o)	< 2000	Salinity affects mostly negligible
Slightly saline (S_1)	2000-4000	Yields of very sensitive crops may be restricted
Moderately saline (S_2)	4000-8000	Yields of many crops may be restricted
Saline (S_3)	8000-16000	Only tolerant crops yield satisfactory
Highly saline (S_4)	> 16000	Only very tolerant crops yield satisfactory

Source: Karim et al 1990.

Assessment of changes in agricultural diversity due to salinity

To assess the changes of diversity of vegetables (both summer and winter) and standing plants, perception of local farmers in Paikgacha and Rampal was used. About hundred percent respondents in both two regions thought that number of crop species are gradually decreasing. When they were asked how much a particular crop they produced in 1975, 1990, 2005; they responded qualitatively. Through the qualitative data it was difficult to assess the changes. Then, they were asked to put the value in between 0 and 10 against their qualitative amount per species; they chose the comparative number through their inherent capacity. Based on assigned ranking, statistical analysis (Mean & Standard) for Paikgacha (HSZ) and Rampal (MSZ) was carried out. The result of the analysis for summer vegetables in different years is shown in Table 4. Statistical result of winter vegetables and standing crops is also shown in Table 5 and Table 6. The name of the species is shown in Table 7 against each species ID which is used in Table 4, Table 5 and Table 6 for summer vegetables, winter vegetables and standing crops respectively.

The mean value represents the average rank among the respondents and standard deviation represents the variation of their perception. The lower value of standard deviation in the study indicates accuracy of the approximation of amount of species. If we ignore the decimal places of mean value, Table 4 indicates that in 1975, 16 summer vegetable species was present in Paikgacha (HSZ) and in 1990, no species was extinct but availability of vegetables were reduced substantially. But in 2005, substantial change occurred and only 2 summer vegetable species was present. In Rampal (MSZ), 15 species was present in 1975, but in 2005 this figure was reduced to 11. Similarly, Table 6 indicates that in Paikgacha (HSZ) 13 winter-vegetable species was present, but in 2005 this was reduced to 9 and in Rampal (MSZ) number of winter-vegetables species remained unchanged but their amount was reduced. This is because in 1975, salinity level in river as well as in soil was so

small that it had insignificant impact on plant growth condition. In 1990, the level was increased and created impact on yield of each species. But in 2005, salinity level was so severe that most of the species were extinct and only few salinity tolerant species were available.

Similarly, in Table 6, it is found that standing plants in Paikgacha (HSZ) was reduced from 31 species in 1975 (as species No 19, 27, 32 and 34 are absent in HSZ) to 14 species in 2005. In the case of Rampal this figure was reduced from 35 species in 1975 to 21 in 2005. Only salinity tolerant species played dominant role in the study area.

Table 4. Statistics of assigned rank against qualitative amount of summer vegetables produced by farmers in Paikgacha (HSZ) and Rampal (MSZ) in 1975, 1990 and 2005

Species ID	Paikgacha (HSZ)						Rampal (MSZ)					
	1975		1990		2005		1975		1990		2005	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sp1	8.87	0.81	5.83	0.51	2.06	0.31	8.01	0.79	5.83	0.51	2.11	0.21
Sp 2	6.24	0.92	5.00	0.43	0.03	0.21	6.13	0.91	2.13	0.49	0.03	0.25
Sp 3	6.12	0.59	5.01	0.41	0.06	0.23	8.13	0.51	5.12	0.45	1.96	0.31
Sp4	6.01	0.58	2.63	0.60	0.03	0.20	8.29	0.60	7.68	0.52	5.61	0.41
Sp 5	7.01	0.60	2.98	0.31	0.03	0.23	0.00	0.58	0.00	0.30	0.00	0.64
Sp 6	8.19	0.52	4.98	0.30	0.03	0.02	9.01	0.41	4.31	0.35	2.13	0.26
Sp 7	7.59	0.52	4.95	0.29	0.09	0.31	6.13	0.31	4.13	0.21	2.30	0.28
Sp 8	8.13	0.51	4.86	0.30	0.03	0.20	5.16	0.61	5.10	0.31	1.96	0.17
Sp 9	8.97	0.49	5.32	0.27	0.03	0.30	8.10	0.53	7.13	0.51	5.12	0.17
Sp 10	6.25	0.36	2.12	0.30	0.07	0.31	8.10	0.39	5.10	0.30	2.10	0.38
Sp 11	6.13	0.46	2.78	0.31	0.03	0.31	7.86	0.47	6.13	0.35	2.10	0.31
Sp 12	9.13	0.42	4.13	0.42	1.10	0.26	8.10	0.21	4.62	0.45	2.96	0.52
Sp 13	6.12	0.42	2.11	0.31	0.03	0.23	4.36	0.21	2.11	0.35	0.01	0.41
Sp 14	5.23	0.43	1.98	0.41	0.03	0.19	5.02	0.23	2.33	0.32	0.02	0.71
Sp 15	7.12	0.30	2.06	0.31	0.06	0.33	6.13	0.51	5.03	0.45	0.06	0.31
Sp 16	7.12	0.26	1.98	0.39	0.07	0.21	7.12	0.21	4.85	0.18	2.15	0.21

Source: Field Survey, 2005.

Table 5. Statistics of assigned rank against qualitative amount of winter vegetables produced by farmers in Paikgacha (HSZ) and Rampal (MSZ) in 1975, 1990 and 2005

Species ID	Paikgacha (HSZ)						Rampal (MSZ)					
	1975		1990		2005		1975		1990		2005	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sp1	7.98	0.65	5.98	0.54	2.13	0.35	6.13	0.61	5.98	0.16	3.12	0.23
Sp 2	6.94	0.76	6.01	0.45	2.35	0.25	8.15	0.39	4.96	0.29	2.13	0.29
Sp 3	6.89	0.49	5.69	0.39	0.04	0.26	0.00	0.59	0.00	0.36	0.00	0.32
Sp4	9.13	0.61	7.03	0.59	2.13	0.38	8.32	0.69	7.13	0.35	4.96	0.43
Sp 5	8.13	0.51	6.15	0.39	1.98	0.39	7.13	0.39	4.13	0.45	2.15	0.59
Sp 6	9.11	0.59	6.65	0.29	1.98	0.61	6.12	0.45	4.98	0.49	2.36	0.28
Sp 7	9.16	0.49	7.15	0.39	2.10	0.54	7.89	0.35	6.94	0.48	4.69	0.27
Sp 8	6.14	0.53	2.21	0.35	0.04	0.39	0.00	0.64	0.00	0.53	0.00	0.17
Sp 9	9.14	0.49	5.32	0.35	2.13	0.36	9.12	0.39	7.16	0.59	4.97	0.19
Sp 10	8.94	0.35	4.12	0.36	2.36	0.39	9.13	0.46	6.98	0.34	4.99	0.35
Sp 11	9.10	0.54	6.13	0.35	4.12	0.37	8.98	0.37	7.13	0.29	5.12	0.34
Sp 12	8.96	0.45	2.13	0.45	0.35	0.35	8.12	0.26	7.96	0.41	4.13	0.32
Sp 13	7.13	0.41	2.13	0.54	0.39	0.29	7.84	0.29	6.15	0.31	4.11	0.39

Source: Field Survey, 2005.

To detect the changing pattern for every species in Paikgacha and Rampal, graphical presentation is carried out. In Figure 2a, each line represents the changing pattern of every summer vegetable in Paikgacha whereas in Figure 2b, each line represents the status of every species in Rampal. From Figure 2a, it is found that summer vegetables in HSZ could not bear the extent of salinity in 2005 and substantial reduction occurred. Figure 2 (a) and (b) also indicate the spatial and temporal variation of summer vegetable species. In HSZ, about total species was extinct; only two species

remained but in MSZ reduction was lower compared to HSZ. Because in summer due to high temperature, evaporation of fresh water occur which eventually increases salinization. As salinity level in river and soil is high in HSZ compared to MSZ, this figure become high in high saline zone. Similarly, Figure 3 (a) and (b) indicate the spatial and temporal variation of winter vegetable species. From all comparative analysis it is concluded that agricultural diversity was changed both in Paikgacha and Rampal but it is noticeable in Paikgacha rather than Rampal due to high degree of salinity. The reduction of species in winter season is in smaller quantity rather than the summer season because of lower evaporation rate in winter compared to summer.

Table 6. Statistics of assigned rank against qualitative amount of standing plants in Paikgacha (HSZ) and Rampal (MSZ) in 1975, 1990 and 2005

Species ID	Paikgacha (HSZ)						Rampal (MSZ)					
	1975		1990		2005		1975		1990		2005	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sp1	8.02	0.55	2.41	0.41	0.02	0.31	8.11	0.52	3.12	0.49	2.11	0.49
Sp 2	6.12	0.69	1.10	0.37	0.02	0.19	7.98	0.31	2.56	0.39	1.98	0.54
Sp 3	5.98	0.37	0.06	0.35	0.31	0.35	8.13	0.45	0.06	0.36	0.32	0.59
Sp4	5.43	0.29	0.13	0.31	0.07	0.49	6.98	0.31	2.31	0.32	1.31	0.31
Sp 5	6.13	0.37	5.14	0.36	2.02	0.29	6.23	0.35	5.14	0.37	2.02	0.29
Sp 6	7.16	0.25	2.91	0.35	0.07	0.47	7.69	0.29	2.91	0.45	0.07	0.47
Sp 7	8.13	0.39	1.93	0.49	0.04	0.24	8.05	0.40	2.31	0.39	2.10	0.24
Sp 8	5.16	0.38	0.09	0.47	0.02	0.31	6.91	0.39	0.10	0.45	0.03	0.31
Sp 9	5.14	0.45	0.10	0.42	0.07	0.16	7.15	0.37	2.13	0.41	0.13	0.51
Sp 10	7.02	0.49	0.99	0.36	0.03	0.34	9.11	0.39	1.15	0.39	0.03	0.54
Sp 11	6.19	0.59	1.10	0.35	0.07	0.45	8.11	0.59	1.16	0.34	0.07	0.56
Sp 12	8.02	0.61	3.10	0.48	0.03	0.15	7.91	0.61	2.10	0.49	0.29	0.26
Sp 13	7.98	0.24	7.90	0.39	7.80	0.33	6.56	0.24	7.15	0.42	8.13	0.29
Sp 14	6.91	0.26	3.15	0.31	2.04	0.31	7.12	0.26	4.65	0.31	2.11	0.80
Sp 15	7.64	0.21	7.59	0.39	7.10	0.35	7.12	0.26	7.40	0.35	7.98	0.31
Sp 16	7.69	0.15	4.95	0.28	2.10	0.26	6.91	0.21	4.16	0.29	3.54	0.45
Sp 17	8.59	0.21	4.31	0.31	1.98	0.33	7.12	0.21	2.13	0.35	1.98	0.35
Sp 18	7.15	0.21	0.41	0.37	0.07	0.33	6.19	0.29	2.31	0.39	2.01	0.56
Sp 19	0.00	0.26	0.00	0.45	0.00	0.34	7.16	0.28	4.56	0.45	2.31	0.31
Sp 20	2.13	0.26	2.00	0.49	0.04	0.21	4.65	0.27	2.31	0.39	2.14	0.49
Sp 21	6.98	0.26	4.31	0.49	4.13	0.34	7.13	0.29	6.12	0.37	4.32	0.42
Sp 22	8.15	0.21	7.07	0.47	6.93	0.33	8.20	0.35	8.10	0.47	8.00	0.39
Sp 23	5.12	0.26	2.91	0.45	2.16	0.37	5.20	0.38	2.11	0.45	1.97	0.37
Sp 24	2.13	0.37	0.07	0.38	0.06	0.33	4.13	0.37	2.15	0.38	0.06	0.34
Sp 25	4.61	0.39	6.13	0.34	7.98	0.49	4.61	0.49	6.15	0.35	8.10	0.49
Sp 26	2.13	0.26	0.91	0.42	0.02	0.47	4.25	0.41	2.11	0.47	0.02	0.47
Sp 27	0.00	0.40	0.00	0.35	0.00	0.37	7.12	0.38	2.16	0.49	0.03	0.37
Sp 28	4.31	0.26	4.10	0.29	1.21	0.21	6.98	0.37	4.21	0.31	2.11	0.21
Sp 29	6.91	0.34	4.15	0.37	3.13	0.36	8.13	0.28	4.31	0.38	2.11	0.31
Sp 30	8.12	0.32	9.12	0.45	9.12	0.16	6.54	0.31	7.12	0.48	7.50	0.35
Sp 31	8.16	0.22	8.50	0.29	8.95	0.49	5.65	0.29	6.65	0.35	8.65	0.49
Sp 32	0.00	0.29	0.00	0.28	0.00	0.47	4.65	0.31	2.10	0.31	0.61	0.48
Sp 33	2.10	0.34	1.00	0.27	0.03	0.31	4.13	0.34	2.16	0.32	0.04	0.31
Sp 34	0.00	0.39	0.00	0.29	0.00	0.16	4.13	0.41	2.41	0.46	0.03	0.39
Sp 35	4.10	0.38	0.31	0.28	0.03	0.48	7.13	0.39	0.02	0.48	0.04	0.49

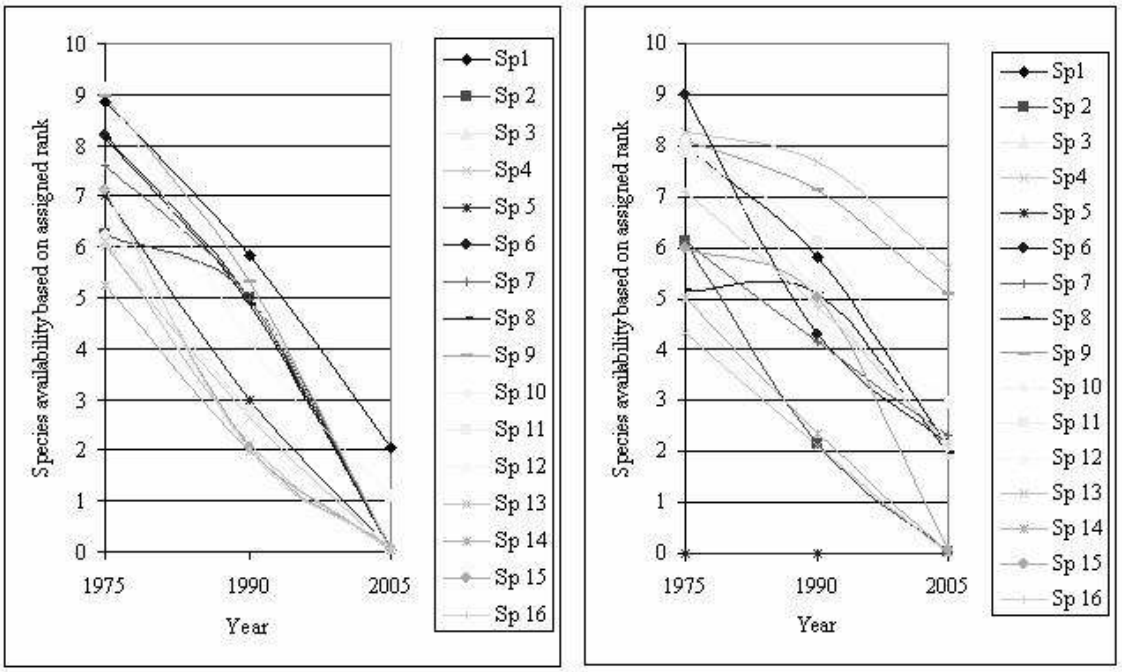
Source: Field Survey, 2005.

Through an open ended question in the questionnaire, the factors responsible for such changes of species diversity were captured. About cent percent local farmers assumed that salinity is the only factor which is responsible for such changes. As the farming habit and other instruments, which they used in farming were remained unchanged and salinity is increasing significantly, their perception is reliable.

Table 7. Scientific name of summer vegetables, winter vegetables and standing plants

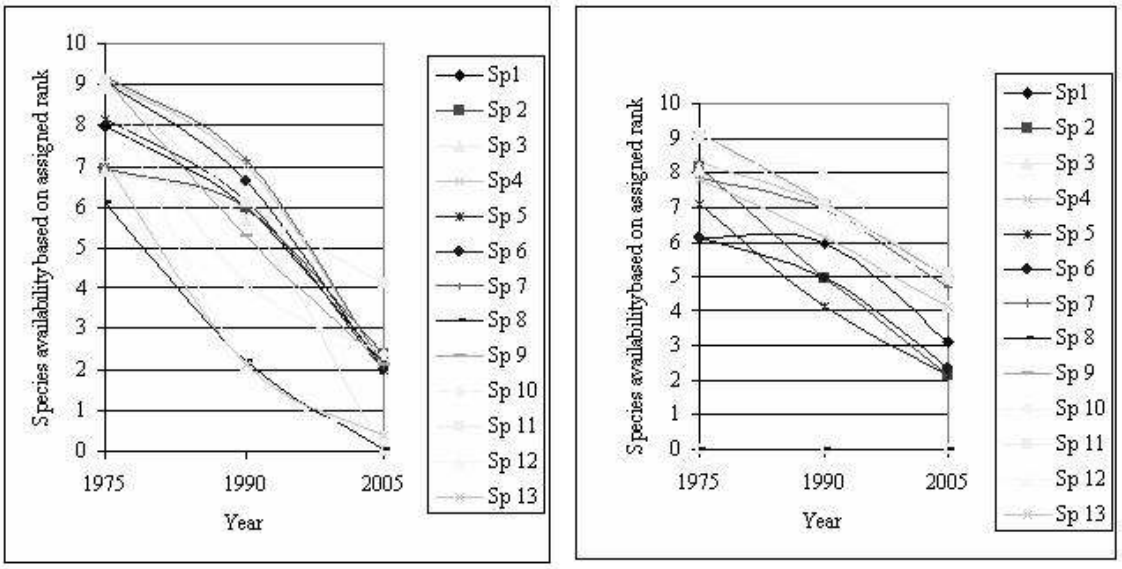
Species ID	Summer vegetable	Winter vegetables	Standing plants
Sp 1	*Solanum melongena*	*Brassica oleracea var. capitata*	*Mangifera indica*
Sp 2	*Benincasa hispida*	*Vigna unguiculata var.*	*Spondias mangifera*
Sp 3	*Trichosanthes anguina*	*Brassica campestris var. chinensis*	*Aegle marmelos*
Sp 4	*Luffa cylindrica*	*Amaranthus oleraceus*	*Punica granatum*
Sp 5	*Momordica dioica*	*Abelmoschus esculentus*	*Diospyros peregrina*
Sp 6	*Ipomoea aquatica*	*Brassica oleracea var. botrytis*	*Ficus carica*
Sp 7	*Momordica charantia*	*Amaranthus tricolor*	*Syzygium cumini*
Sp 8	*Cucurbita moschata*	*Ipomoea batatas*	*Syzygium samarangense*
Sp 9	*Lagenaria siceraria*	*Raphanus sativus*	*Citrus grandis*
Sp 10	*Alocasia indica*	*Beta vulgaris var. bengalensis*	*Muca cvs.*
Sp 11	*Dioscorea alata*	*Basella alba*	*Citrus aurantifolia*
Sp 12	*Colocasia esculenta*	*Lycopersicon esculentum*	*Artocarpus heterophyllus*
Sp 13	*Amorphophallus campanulatus*	*Lablab niger var.*	*Phoenix dactylifera*
Sp 14	*Corchorus oletorius*		*Zizyphus mauritiana*
Sp 15	*Cucumis sativus*		*Cocos nucifera*
Sp 16	*Luffa acutangula*		*Carica papaya*
Sp 17			*Psidium guajava*
Sp 18			*Moringa oleifera*
Sp 19			*Areca catechu*
Sp 20			*Borassus flabellifer*
Sp 21			*Tamarindus indica*
Sp 22			*Manilkara achras*
Sp 23			*Prunus amygdalus*
Sp 24			*Terminalia arjuna*
Sp 25			*Acacia arabica*
Sp 26			*Ficus benghalensis*
Sp 27			*Anthocephalus cadamba*
Sp 28			*Albizzia sp.*
Sp 29			*Delonix regia*
Sp 30			*Azadirachta indica*
Sp 31			*Excoecaria agallocha*
Sp 32			*Bambusa sp.*
Sp 33			*Bombax ceiba*
Sp 34			*Terminalia belerica*
Sp 35			*Terminalia chebula*

There are several factors like salinity, acidity, nutrients and minerals of soil, climatic conditions, application of fertilizer and insecticides etc which are responsible for crop diversity degeneration. To detect which factors are responsible in our study area, eight resource persons were interviewed who were working with agriculture and soil of the study area. All of them mentioned that climatic conditions and application of fertilizer and insecticides remained more or less unchanged. Other factors were not changed substantially which are able to create biodiversity degeneration. They remarked that salinity level was changed noticeably which eventually replaced minerals and reduces nutrient status of the study area. So salinity is a stressed condition, which changes the agricultural diversity in study area.



(a) **(b)**

Figure 2. Changing pattern of amount of summer vegetables for every species in (a) Paikgacha (HSZ) and (b) Rampal (MSZ) based on assigned rank.



(a) **(b)**

Figure 3. Changing pattern of amount of winter vegetables for every species in (a) Paikgacha (HSZ) and (b) Rampal (MSZ) based on assigned rank.

CONCLUSION

The study findings show that agricultural diversity of the study area is reducing with the increase of salinity. The reduced agricultural diversity eventually decreases the agricultural production and creates extinction of several species. Thus, desertification is going to be created in the study area.

These consequences eventually create instability in the socio-economic sector of the study area in terms of increased poverty of local farmers. So, salinity is now a terrible problem in south west coastal region of Bangladesh. Immediately, Government should take step for reducing salinity level of the study area. The reduction of salinity level in south west region of Bangladesh can be accomplished by increasing upland freshwater flow from the Ganges through the Gorai-Madhumati channel.

ACKNOWLEDGEMENT

The authors are grateful to the inhabitants of Paikgacha and Bagerhat for their help and cooperation during data collection there. Authors included in the reference are greatly acknowledged for their contributions to this subject matter.

REFERENCES

Berensen ML and DM Levine. 1992. *Basic business statistics: Concepts and application*. 5th Edition. Pentin Hall, Inc., USA, 1992.

Brammer H. 1996. *The geography of the soils of Bangladesh*. University Press Limited, Dhaka. 287 Pp.

Chowdhury MSU. 1993. *Importance of natural resource management for sustainable agricultural development: Bangladesh context*. BARC, Dhaka. 38 pp.

FAP. 1993. Southwest area water resources management project: Land resources, agriculture and fisheries. **In:** *Final Report-1993,* Volume 6. Flood Action Plan (FAP), Dhaka.

Gupta IC. 1990. *Use of saline water in agriculture: a case study of arid and semi-arid zones of India*. Oxford and IBH Pub. Co. Pvt. Ltd., New Delhi. 398 pp.

Karim Z, SG Hussain and M Ahmed. 1990. Salinity problems and crop intensification in the coastal regions of Bangladesh. *BARC Soil Publication No. 33*, Dhaka. 63 pp.

Mannan MA, MY Miah, MAM Mahamud and T Baida. 2004. Salinity on cultivable land and its effects on crops. *Pakistan Journal of Biological Sciences.* 7 (8):1322-1326.

Odum EP. 1969. *Fundamentals of Ecology*. 3rd Edition. Saunders College Publishing, Philadelphia. 574 pp.

Paramanik MAH. 1986. *The dynamics of coastal processes and land use changes*. Bangladesh Space Research and Remote Sensing Organization (SPARRSO), Dhaka, Bangladesh.

Rahman MM, MQ Hassan, MS Islam and SZKM Shamsad. 2000. Environmental impact assessment on water quality deterioration caused by the decreased Ganges outflow and saline water intrusion in south-western Bangladesh. *Environmental Geology* 40:31-40.

Sengupta R. 2001. *Ecology and economy: An approach to sustainable development*. Oxford University Press, New Delhi. 264 pp.

Shannon CE and W Weaver. 1963. *The mathematical theory of communication*. University of Illinois Press, Urbana. 117 pp.

Simpson EH. 1949. Measurement of Diversity. *Nature* 163:688.

SRDI. 2001. *Soil salinity in Bangladesh*. Soil Research Development Institute (SRDI), Dhaka.

Yield Loss Assessment and Management of Violet Wood Sorrel Weed on Maize

Govind KC

Ginger Research Programme, Kapurkot, Salyan

Current Address: National Maize Research Programme, Rampur, Chitawan

ABSTRACT

A field experiment was conducted during rainy season of 2003 and 2004 in farmers' field of Dang district (650 masl) to assess the yield loss caused by violet wood sorrel (*Oxalis corymbosa* Dc) for maize (*Zea mays*) and to work out effective measure to control this weed. Violet wood sorrel (Chari Amilo) infestation was observed in maize, ginger, and Toria fields of Dang and Salyan districts as well as various mid hilly areas of Nepal. The weed once introduced in uplands, spreads in large scale within few years. Six treatments, comprising 2,4-D Na salt 2 kg/ha, Glyphosate 2 l/ha, Soil digging, turning and expose to sun two months prior to maize sowing were compared with weed free, weed free except *Oxalis* and weedy check plots. The presence of violet wood sorrel (VWS) in maize field ranging from 38 to 352.3 per square meter in 2003 and 18.6 to 195.5 per square meter in 2004 one month after intercultural operation does not cause significant effect on maize grain yield, 4297 to 4694 kg/ha in 2003 and 3751.4 to 4473.6 kg/ha in 2004 respectively. Thus significant loss in maize grain yield was not found due to higher VWS weed population (352.3 and 195.5/m^2) resulted in weedy check treatment and lower weed population (38 and 18.6/m^2) in herbicide applied treatment. Repeated application of herbicides at 17 and 37 days after maize sowing (DAMS) both 2,4-D and Glyphosate reduced the weed population in maize field but could not eradicate as it appears in next year but the population was significantly low (59.5 and 65.3/m^2) in herbicide applied plots compared to weedy check plot (322.6/m^2) at 26 DAMS. It was revealed that presence of violet wood sorrel weed in maize field does not affect the grain yield in farmer's practice of weeding i.e. intercultural operation at 20 to 30 DAMS.

Key words: Chari Amilo, herbicide, *Oxalis*, violet wood sorrel, *Zea mays*

INTRODUCTION

Maize, the second important cereal, is the major staple food crop of the hilly regions in Nepal. Maize occupies 849,892 ha of land in Nepal with the productivity of 2.019 mt/ha (MoAC 2005). The national yield level of maize has been found lower as compared to its potentiality (Rampur Composite, 4.4 t/ha). Despite of various high yielding varieties of maize available, poor adoption of integrated production technology by farmers has been existing situation, which might be responsible for lower productivity of maize in Nepalese context. There is ample opportunity to increase the production and productivity of maize in the country.

Maize is most sensitive to weed competition during its early growth period of 2-6 weeks after sowing due to profuse weed germination and rapid growth of weeds resulted by wider row spacing of maize.

The loss caused by weed in maize ranges 40-70% (Mandal 2000) and yield loss depend on type of weed flora and its severity. At ARS Pakhribas (eastern mid hill of Nepal) experimental result showed weedy environment resulted yield reduction up to 70% in maize (Mishra 2004). Maize - Toria + Lentil is the major cropping system in upland in Dang. The farmers of different area in Dang reported the violet wood sorrel (*Oxalis corymbosa* Dc) weed problem in maize based cropping system since two decades ago. That noxious weed is perennial in nature and locally called Chari Amilo. This weed and its related species *O. europea, O. latifolia* and *O.corniculata* comes under oxaladaceae family infest different upland crops like maize, ginger, turmeric, toria, lentil etc. Infestation of this weed was observed in different up land crops in mid hills of eastern, western and mid western Nepal. But, survey and statistics for such particular weed is not available at present context. The weed once introduced in field, it spreads and cover whole field within 3-4 years due to its nature of spreading through bulb and bulbils as one bulb contains 70 to 120 bulbils (Rao 2000). All the crops in rainfed upland have been found infested and infestation takes place in early stage of crops as it germinated in June-July and Oct-Nov when it receives adequate moisture in soil. Farmers reported that fodder grass to livestock from maize field was decreased due to this weed and the effect of this weed was found negative on lentil crop amongst Toria + lentil mixed cropping system. This weed causes uneasy to field work too. Rao (2000) reported that this weed can be eradicate with repeated spray of Paraquat, Glyphosate is very effective and 2,4 D is partially effective. Loss assessment of this weed has not been found studied yet. The objective of the study was to assess the grain yield loss caused by violet wood sorrel on maize crop. This also aims to find out effective measure to control this weed in maize field under up land Bari condition.

MATERIALS AND METHODS

The experiment was carried out during rainy season of 2003 and 2004 at farmer's field of Dang valley (650 masl). The violet wood sorrel (VWS) infested field was selected for experimentation that was acidic in nature (pH 5.0). The experiment was laid out in randomized complete block design with 3 replications and plot size was 11.25 m^2. Six treatments were selected for the present study (Table 1) and treatments were post-emergence application of Glyphosate and 2,4-D Na salt, soil digging followed by turning and exposed to sun 2 months prior to maize sowing, weed free up to 45 DAMS, weed free except Violet wood sorrel and weedy check in both years. Crop was fertilized with 80:40:40 kg/ha N:P_2O_5:K_2O through DAP, Urea and Murate of Potash. Half of the N was top dressed at knee-high stage. Manakamana–1 was sown in rows 75 cm apart and 25cm plant spacing. Planting was done in last week of May in 2003. Herbicides were applied at 17 days of maize sowing to those plots, which were with herbicides treatments and intercultural operation was done on 4th day of spraying to all plots as per farmers' practice. Herbicides were reapplied at 37 and 45 days after sowing. Directed spray was done in inter row space of maize using plastic hood fitted at the nozzle of the sprayer. Soil digging followed by turning and exposed to sun two months before sowing was performed for soil turning treatment and for weed free up to 45 DAMS weeding of all weeds was done at 17, 37 and 45 DAMS while violet wood sorrel was not weeded and other weeds were weeded for weed free except *Oxalis* treatment. Intercultural operation was done for all treatments on 4th day of herbicide application as per local practice. Plant population of VWS at 55 DAMS was observed for each treatment and effect in yield loss and variation was considered due to weed population difference.

In 2004 season, experiment was conducted in same field and plots with standard package of practices. Maize was sown in first week of May. One superimposed treatment, application of Agricultural lime 2 t/ha was included in second year of study. Lime was applied during maize sowing because it could not applied earlier due to moisture unavailability in soil. First weed count was done at 26 DAMS and herbicide application was done at 26 and 45 DAMS as per treatments. Second observation on weed population was made after intercultural operation and re-germination of weeds at 65 DAMS.

RESULTS AND DISCUSSION

In 2003, post emergence application of Glyphosate 2 l/ha reduced the violet wood sorrel (VWS) weed infestation with weed control efficiency over check plot 89.2 % followed by 2, 4 D Na salt 2 kg/ha application (82.2%). VWS weed population at 55 DAMS was 192.3/m2 in that plot which was treated as soil digging, turning and exposed to sun while VWS weed population was found 62.5 and 38.0 per square meter in 2,4 D and Glyphosate applied plot respectively. Presence of VWS population was very high (352.3/m^2 at 55 DAMS) in weedy check plot. Being a perennial weed and propagated through bulb and bulbils this weed germinated repeatedly. Other weed flora found infesting the experimental field was dominated by VWS. However, *Ageratum conyzoids*, *Digitaria* spp. were present in small number. All the VWS weeds in experimental field were killed and found in process of decomposing during 67 DAMS.

Effect of different treatments included in study was found non significant for maize grain yield variation in 2003 (Table 2). Oxalis weed infestation ranging from 38 to 352.2 per square meter was recorded in various treatments and there was no significant decrease in grain yield (4332 kg/ha) due to higher VWS weed present (352.3/m^2 at 55 DAMS) in weedy check plot compared to VWS infestation (38/m^2 at 55 DAMS) in Glyphosate 2 l/ha applied treatment which yielded 4468 kg/ha maize grain. Post emergence application of both herbicides does not affect the maize plants as all the treatments gave similar result of barren plants in percentage (6.3 to 8.3).

In 2004, after maize sowing, drought was occurred and maize growth was affected and slowed as well as weed was also affected and germinated late. First weed count and herbicide application were accomplished in 26 DAMS and after hoeing *Oxalis* population was observed at 65 DAMS. Like in first year, other weeds in the field were dominated by *Oxalis*. At 26 DAMS, weed population was significantly lower (59.3 and 65.3/m^2) in herbicide-applied plots in previous year compared to weed free except *Oxalis* and weedy check (307.1 and 322.6/m^2). This revealed that VWS infestation could be controlled by application of 2,4-D or Glyphosate. However, it could not be eradicate. Again *oxalis* weed population was significantly decreased (81.6 to 88.2%) over check in herbicide-applied plots compared to weedy check at 65 DAMS (Table 1). Soil digging, turning prior to sowing (130.6/m^2) and lime application (195.5/m^2) were significantly inefficient to control VWS weed population compared to herbicide application (18.6 and 29.8 /m^2) at 65 DAMS.

Table 1. Effect of herbicides and weeding on violet wood sorrel (*Oxalis corymbosa)* weed population (pop.) in maize field, 2003-2004

SN	Treatment	Oxalis Pop. /m^2, 2003		Oxalis Pop./m^2, 2004		
		55 DAMS	Decrease over check, %	26 DA MS	65 DA MS	Decrease over check, %
1	Soil digging, turning and expose to sun 60/30 days before maize sowing	192.3	45.3	184.4	130.6	17.4
2	2, 4 D 80WP Na salt 2 kg/ha (Post Emer)	62.5	82.2	59.5	18.6	88.2
3	Glyphosate 41% LS, 2 l/ha (Post Emer.)	38.0	89.2	65.3	29.8	81.6
4	Weed free except Oxalis up to 45 DAMS	191.3	45.4	307.1	122.6	22.5
5	Weed free up to 45 DAMS	101.0	71.3	93.7	52.9	66.5
6	Weedy check (Farmer's practice)	352.3	-	322.6	158.2	-
7	Agric. Lime, 2 t/ha	-		356.8	195.5	-23.5
	CV, %	10.4		16.6	11.0	
	LSD at 0.05	29.53		58.7	19.8	

Table 2. Maize grain yield as affected by different weed control regimes against violet wood sorrel weed, 2003-2004

SN	Treatment	Maize, 2003			Maize, 2004
		Grain yield, kg/ha	Barren plants, %	Plant pop/m^2	Grain yield, kg/ha
1	Soil digging, turning and expose to sun 60/30 days before maize sowing	4297	6.3	4.0	4054.2
2	2, 4 D 80WP Na salt 2 kg/ha (Post Emergence)	4457	7.3	3.55	4342.0
3	Glyphosate 41% LS, 2 l/ha (Post Emergence.)	4468	6.4	4.19	4473.6
4	Weed free except *Oxalis* up to 45 DAMS	4694	6.8	3.93	3751.4
5	Weed free up to 45 DAMS	4349	7.3	4.12	4208.4
6	Weedy check (Farmer's practice)	4332	8.3	3.49	4001.7
7	Agric. Lime, 2 t/ha			4.19	3984.6
	CV, %	8.1		6.9	13.2
	LSD at 0.05	-		0.54	-

In 2004, significant variation was found in maize plant population and therefore covariate analysis was performed for grain yield. All the treatments gave statistically comparable maize grain yield ranged from 3751.4 to 4473.6 kg/ha. However, highest adjusted grain yield (4473.6 kg/ha) was obtained with Glyphosate 2 l/ha application followed by 2,4-D application (4342 kg/ha) and weed free up to 45 DAMS (4208.4 kg/ha). Lowest grain yield (4001.7 kg/ha) was obtained with weedy check treatment.

This experiment result showed that the weed had no significant effect on maize grain yield variation, though VWS weed population ranged significantly. From this, it was revealed that, presence of VWS in higher number (352.3/m^2 and 195.5/m^2) and lower number (38.0/m^2 and 18.6/m^2) resulted in comparable maize grain yield indicate that this VWS weed does not cause significant yield loss of maize grain under one intercultural operation practice at 20 to 30 DAMS.

CONCLUSION

Result of two year experiment indicate that the presence of weed Violet wood sorrel (Chari Amilo) in maize field ranging from 38 to 352.3 in 2003 and 18.6 to 195.5 per square meter in 2004 under one intercultural operation practice does not cause significant effect on maize grain yield (4297 to 4694 kg/ha in 2003 and 3751.4 to 4473.6 in 2004) respectively. Repeated application of both herbicides Glyphosate 2 l/ha and 2,4 -D 2 kg/ha reduced the VWS weed population in maize field and for its eradication more repeated application of herbicides has been required. Further study of yield loss assessment on other upland crops like toria, lentil, potato, ginger etc. caused by VWS (*Oxalis corymbosa*) will be helpful to know its harmful effect.

ACKNOWLEDGEMENT

The author is grateful to the NARC/NMRP/HMRP/CIMMYT for approving the Small Grant Project (SGP) and providing financial and technical support. Technical advice and suggestion of Dr JD Ranjit at Agronomy Division Khumaltar is highly appreciated. A special thank goes to CM Bhattarai and Durga Bahadur KC of GRP, Salyan for their field works to conduct the research activity.

REFERENCES

Mandal RC. 2000. Weed, weedicides and weed control. Principle and practice. Agrobios, India

Mishra M. 2004. Studies on weeds management in maize crop in the eastern hills of Nepal. ***In:*** *Proceeding of the 24th National Summer Crops Research Workshop, 2004*. NARC. Pp. 180-181.

MoAC. 2005. *Statistical information on Nepalese agriculture 2004/05*. Agri-Business Promotion and Statistics Division, Ministry of Agriculture and Cooperatives, Singha Durbar, Kathmandu, Nepal.

Rao VS. 2000. *Principles of weed science*. Second Edition. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi.

Use of Fertilizers and Lime for Enhancing Productivity of Maize Genotypes in Western Hill of Nepal

Bishnu H. Adhikary[1] and Krishna B. Karki[2]

[1] National Maize Research Program (NMRP)-NARC, Rampur, Chitwan
[2] Soil Science Division-NARC, Khumaltar, Lalitpur Nepal

ABSTRACT

Farmers' field experiment was conducted at Outreach sites in Tanahun and Palpa districts of Western Nepal with the objectives of evaluating the effects of fertilizers and agricultural lime on grain production of the local and improved (Manakamana-1) maize varieties during summer season of year 2003 and 2004. Farmer's practice (without fertilizers) and with fertilizer 60: 30: 30 kg (N: P_2O_5: K_2O) per hectare with or without agricultural lime (4 t ha^{-1}), were the treatments in experimental study that was conducted in factorial RCB design with four farmers as replications. The results revealed that response of fertilizers and agricultural lime was observed significant plant growth and kernels production including varieties in both sites of Palpa and Tanahun districts. The highest grain yield of 5.1 t ha^{-1} was produced by the local variety when the crop was supplied with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} along with 4 t ha^{-1} of agricultural lime in Tanahun. Manakamana-1 variety produced the maximum grain yield (4.45 t ha^{-1}) with the same level of fertilization. The two district mean grain yield result indicated that the highest grain yield of 3.9 t ha^{-1} was produced when the crop was supplied with 60: 30: 30 kg N, P_2O_5 and K_2O ha^{-1} with agri-lime. Hence it is suggested that to increase maize productivity in the hills irrespective of the maize variety application of fertilizers (60: 30: 30) with agricultural lime (4 t ha^{-1}) is needed.

Key words: Agriculture lime, farmer's practice, yield increase, *Zea mays*

INTRODUCTION

Maize (*Zea mays* L.) is a principle staple food crop for over 55% of people in the hills of Nepal. Traditionally, farmers have been applying ample amount of organic manures to sustain maize production and maintain soil fertility. Because of low content of plant nutrients in organic manure the amount of nutrient supplied through FYM/compost is not sufficient to explore the genetic potential of improved maize varieties. Soil analysis data revealed wide variation in fertility status across the hills of Nepal that differed in land type, altitude and soil type (Bennett 1995).

Increased use of high yielding maize varieties in intensive cropping system has led to increased demand of plant nutrients. Depletion of organic matter (OM) has been a key factor in overall decline of soil fertility in hills. Therefore, there is an increasing trend towards reduced nutrient balance in these soils (Ghani and Brown 1997). Much higher response to fertilizer is expected from crops when micronutrients are included in the treatments (Karki et al 2000, Karki 2003).

Soil acidification is one of the major factors contributing soil fertility decline in the hills of Nepal (Karki and Dacayo 1990, Turton et al 1996). Since lime is the cheapest and widely used materials to ameliorate acid soils, Karki (1986), studied lime requirement and recommended 4 to 9 t ha^{-1} of agricultural lime to strong to extreme acidic soils. Similar results were also obtained by Isherwood (1978), Tripathi (2002), Adhikary and Ranabhat (2004). To increase efficiency crop production soil amelioration is not enough. Nitrogen is another limiting nutrient in Nepalese soils. Nitrogen application practiced by farmers' is not responsive as expected. So split application of nitrogen with lime in upland condition has been found more effective (Joshy and Shrestha 1979). These treatments on both local and improved maize varieties responded well (NMRP 2005). Local maize varieties have been found performing well in soil acidity and low nutrients status and improved crop varieties are scarce in remote areas with the objective of promoting local maize variety that could be

comparable to improved one under similar condition and nutrient supply rate in western hills of Nepal this study was carried out.

MATERIALS AND METHODS

Series of experiments on maize genotypes using fertilizers and agricultural lime were conducted during the year 2004 and 2005 in the peri-urban areas of Tansen and Vyash municipalities to evaluate their effect on the grain production of maize (*Zea mays* L.). Four experiments were conducted in each site with local and improved maize varieties. Same local variety was used in the study with the four farmers that were available on the area and improved variety was Manakamana-1. Experimentation was carried out in the summer season (last week of April) on both sites. The experiment was designed in factorial RCB with four replications (farmers as replicates) in each site. Farmer's practice was used as control. Chemical fertilizers were applied with or without agricultural lime. The plot size taken in the experiments were (4-m × 3-m) with the line spacing of 75-cm × 25-cm. Plant growth and yield parameters were recorded and analyzed manually following Gomez and Gomez (1984). The following treatments were used in the experiment.

Treatment

Manakamana-1

T1 (V1F1) = (V1) Farmers practice/FP (F1).
T2 (V1F2) = (V1) 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} (F2).
T3 (V1F3) = (V1) 4 t ha^{-1} of agricultural lime (F3).
T4 (V1F4) = (V1) 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} plus 4 t ha^{-1} agri.-lime.

Local variety

T5 (V2F1) = (V2) Farmers practice/FP (F1).
T6 (V2F2) = (V2) 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} (F2).
T7 (V2F3) = (V2) 4 t ha^{-1} of agricultural lime (F3).
T8 (V2F4) = Local variety (V2) 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} plus 4 t ha^{-1} agri.-lime (F4)

RESULTS AND DISCUSSION

Fertilizer and agricultural lime significantly affected by the plant and ear head growth on both sites in Palpa and Tanahun districts (Table 1 and Table 2). Tallest plant height of 221.0 cm was observed in Tanahun district in Manakamana-1 when crop was fertilized with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1}. Shortest plant height of 189.2 cm was recorded with this variety in farmer's practice (T1 in Table 1). Longest ear height (123 cm) was observed in Manakamana-1 fertilized with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} along with 4 t of agricultural lime application (T4) and shortest (95.7 cm) also with this variety in farmers' practice condition (T1) in Tanahun district (Table 1).

Effect of lime and fertilizer looks positive. Farmers' practice looks inferior to all the treatments in both the varieties as indicated by straw and biomass yield (Figure 1). In both the varieties response of lime is apparent which is obvious and the results resemble the finding of Karki (1986). Fertilizer alone has given positive response and lime with fertilizer in Manakamana-1. Highest result is clearly observed with the T4 in Manakamana-1 and T6 in local varieties. Grain yield local variety did not result better grain yield with fertilizer application but performed well when fertilizers is combined with lime (T8). But the grain yield in T6 is decreased as compared to other treatments.

Table 1. Response of fertilizers and agri-lime on the plant growth and kernel production in Tanahun district

Treatment	Plant height, cm	Ear site height, cm	Ear length, cm	Kernel rows ear^{-1}	Kernels row^{-1}	Stover yield, t ha^{-1}	Grain yield, t ha^{-1}
T1	189.2	95.7	11.3	11.0	23.5	4.29	1.78
T2	221.0	116.7	13.7	11.5	27.7	7.29	4.10
T3	206.2	108.5	11.7	11.7	24.4	6.16	2.83
T4	220.5	123.0	13.4	11.7	26.0	6.91	4.45
T5	200.5	109.7	11.9	12.0	22.5	5.08	2.66
T6	208.0	110.0	14.3	12.5	26.2	6.79	4.55
T7	191.7	102.7	12.3	11.7	23.5	6.08	3.70
T8	213.5	114.7	14.0	12.0	27.3	8.00	5.10
Mean	206.3	110.2	12.8	11.7	25.1	6.32	3.65
CV, %	8.8	9.3	11.9	6.8	10.3	13.93	23.82
F-test	*	*	*	ns	*	**	**
LSD 0.05	26.9	15.1	2.2	-	3.8	1.29.	1.27

**, *Highly significant*. *, *Significant*. n*s, Non significant.*

In this experiment treatments T1 to T4 are similar to T5 to T8. The same treatments were applied to other variety. In Tanahun site, response of the two varieties to fertilizers and lime are compared. There was highly significant difference in the yield response. The local varieties performed significantly better than the improved one (Manakamana-1). In straw yield with T2 (application of mineral fertilizer alone) yielded much higher than T1 (farmers practice) and T3 (lime alone) but lime along with mineral fertilizer yielded highest irrespective of varieties (Figure 1). The longest ear length of 14.3 cm was observed in Tanahun district when the local maize crop was supplied with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} (T6, Table 1) whereas the shortest ear length (11.3 cm) was observed in this district in Mana-1 variety in farmer's practice condition (T1, Table 1). The effect of fertilizers and lime in kernel row production was observed to be non significant in Tanahun but significant in Palpa district. The number of kernel rows were to be highest (12.5 rows) in the local variety fertilized with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} in Tanahun district (T6, Table 1) and lowest (11.0 rows) with the farmer's practice condition in Manakamana-1 variety (T1), however, non significant difference in kernel rows was observed among the treatments in Tanahun district (Table 1). The number of kernel rows were to be highest (12.5 rows) in the local variety fertilized with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} in Tanahun district (T6, Table 1) and lowest (11.0 rows) with the farmer's practice condition in Manakamana-1 variety (T1), however, non significant difference in kernel rows was observed among the treatments in Tanahun district (Table 1).

The highest kernel numbers (27.7 kernels row^{-1}) were produced in Manakamana-1 variety when the crop was fertilized with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} (T2) followed by the local variety fertilized at this level of fertilizers applied along with 4 t ha^{-1} of agri-lime, and the lowest (22.5 kernel/row) in the local variety in farmer's practice condition in Tanahun district (T1, Table 1).

In Palpa district the results was little deviated from the results of Tanahun. Tallest plant height (245.2 cm) was observed in local variety fertilized at the same level of fertilizers whereas shortest plant height (171.2 cm) was observed in Manakamana-1 variety fertilized with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} (T2, Table 2). Longest ear length (138.2 cm) was observed in local variety when the crop was supplied with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} (T6) and the effect were significant. But the cob length (12.3 cm) was longest when lime is applied along with mineral fertilizer (T8). Likewise, the shortest length (9.1 cm) was observed in the same variety (local) when the crop was not supplied with fertilizers (T5), however, the effect of fertilizers and lime was found to be significant. Lime helped amelioration of soil pH and enhanced nutrients uptake (Maskey et al 2004). Since nitrogen is one of the limiting elements lime induced microbial activities and applied nitrogen in organic and mineral form is mineralized and hence biomass is increased with the application of lime and fertilizer in T8, which is similar to the finding of Joshy and Shrestha (1979).

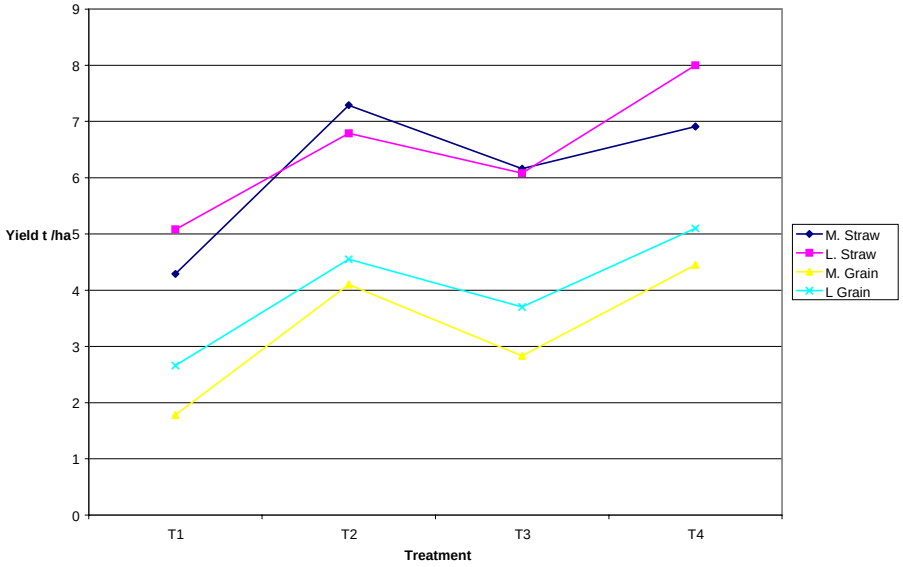


Figure 1. Response of lime and fertilizers on the yield of local and improved maize varieties in Tanahun district.

The numbers of kernels rows^{-1} the cobs were also significant indicating 21 as grand mean. The kernels number varies from 17.3 to 23.2 kernels row^{-1}. Highest numbers (23.2) were found in T2 and T8 both local and Manakamana. Effect of treatments on the kernel rows production was found to be significantly affected (Table 2). The highest kernel rows number (13.0 rows ear^{-1}) was produced by the Manakamana-1 variety when the crop was supplied with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} (T2). Besides these, kernel rows number ear^{-1}, production of kernels in the rows was found to be significantly affected. Similarly, the highest kernel numbers (24.5 kernels row^{-1}) were produced by Manakamana-1 variety when the crop was supplied with 60:30: 30 kg N, P_2O_5 and K_2O and 4 t ha $^{-1}$of agri-lime in Palpa district (T4, Table 2). The lowest kernels (17.3 kernels /row) were produced by the local variety in farmer's practice condition (T5).

The effect of fertilizers and lime on stover production was observed to be highly significant in both districts. The highest stover yield (8.0 t ha^{-1}) was produced by the local variety fertilized with 60:30:30 kg N, P_2O_5 and K_2O and 4 t ha^{-1} of agri-lime in Tanahun district (T8) and lowest stover yield (4.29 t ha^{-1}) with the Mana-1 variety in farmer's practice condition (T1, Table 1). The production of stover yield was almost double (15. 41 t ha^{-1}) in Palpa district when the crop was fertilized with 60:30:30 kg N, P_2O_5 and K2O ha^{-1} and 4 t ha^{-1} of agri-lime (T8, Table 2). The lowest stover yield (6.08 t ha^{-1}) was produced by both varieties, one supplied with agri-lime in farmer's practice condition (T3) and another without lime but farmer's practice (T5). The second highest stover yield (12.41 t ha^{-1}) was produced in Palpa district when the local maize crop was supplied only with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} and 4 t ha^{-1} of agri-lime (T6, Table 2).

Table 2. Response of fertilizers and agri-lime on the plant growth and kernel production in Palpa district

Treatment	Plant height, cm	Ear height, cm	Ear length, cm	Kernel rows ear^{-1}, n	Kernels row^{-1}, n	Stover yield, t ha^{-1}	Grain yield, t ha^{-1}
T1	190.2	89.7	10.2	11.0	19.7	6.43	1.49
T2	171.2	98.0	11.4	13.0	23.2	8.45	2.43
T3	180.2	88.4	9.6	11.5	17.5	6.08	1.63
T4	208.0	119.5	12.2	12.7	24.5	11.12	2.96
T5	206.5	113.7	9.1	9.7	17.3	6.08	1.47
T6	246.2	138.2	11.1	10.8	22.8	12.41	1.97
T7	194.2	117.5	9.2	10.5	19.1	6.62	1.35
T8	267.0	162.5	12.3	11.5	23.2	15.41	2.71
Mean	207.96	115.95	10.62	11.36	20.92	9.08	2.00
CV, %	18.79	19.29	10.54	11.80	13.09	27.03	36.00
F-test	*	*	**	*	**	**	**
LSD 0.05	57.45	32.89	1.64	1.97	4.02	3.61	1.06

***, Highly significant. *, Significant. ns, Non significant.*

Effect of fertilizers and agricultural lime on the maize grain production

A positive response of fertilizer and agri-lime on grain production of both maize genotypes was observed. Highly significant effect of treatments on the grain production was observed in both districts (Table 3). The highest grain yield (5.1 t ha^{-1}) was recorded in Tanahun district when the local variety was fertilized with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} and 4 t ha^{-1} of agri-lime (T8) followed by the same variety fertilized only with 60:30:30 kg N, P_2O_5 and K_2O /ha (T6). Non significant difference was observed in grain production between the Manakamana-1 (4.1 t ha^{-1}) and local variety (4.55 t ha^{-1}) at the same level of fertilization at 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} (T2 and T6, Table 3) in Tanahun. Similarly, non significant difference in grain yield was observed among the Manakamana-1 variety either in farmer's practice condition (1.78 t ha^{-1}) or with farmers practice fertilized with 4 t ha^{-1} of agri-lime (T3) in Tanahun. The response of treatments on grain production in Palpa district was found almost similar to Tanahun district but the quantity of production in an area of land was almost half to that of Tanahun district (Table 3). A maximum of 2.96 t ha^{-1} of grains were recorded by the Mana-1 variety when the crop was supplied with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} and 4 t ha^{-1} of agri-lime in Palpa district (T4, Table 3) followed by the local variety (2.71 t ha^{-1}) at the same level of fertilization (T8); however, non-significant differences in grain production was observed between them. The lowest grain yield of 1.35 t ha^{-1} was observed in local variety in farmer's practice condition when crop was fertilized with 4 t ha^{-1} of agri-lime (T7), which was less than the Mana-1 variety at farmer's practice condition (1.49 t ha^{-1}) in Palpa district.

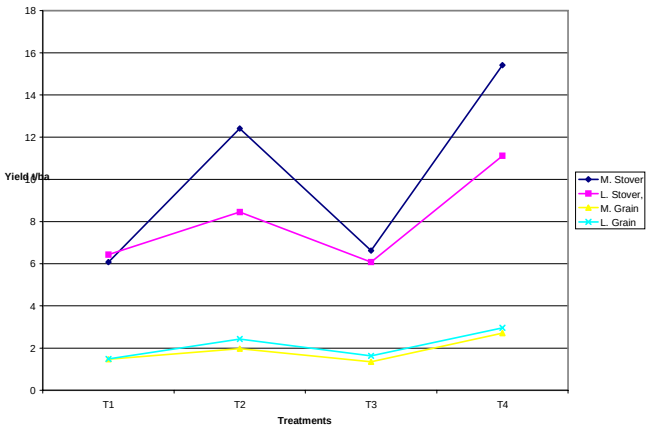


Figure 2. Yield response to local and Manakamana-1 maize varieties in Palpa.

Table 3. Response of fertilizers and agri-lime on the grain production of maize genotypes tested in Palpa and Tanahun districts

Treatment	Grain yield, t ha^{-1}		Two-districts mean, t ha^{-1}	Increment over the check, %
	Tanahun	Palpa		
T1	1.78	1.49	1.63	(-) 20.8
T2	4.10	2.43	3.26	58.2
T3	2.83	1.63	1.96	(-) 4.8
T4	4.45	2.96	3.70	79.6
T5 (Check)	2.66	1.47	2.06	00.0
T6	4.55	1.97	3.26	58.2
T7	3.70	1.35	2.52	22.3
T8	5.10	2.71	3.90	89.3
Mean	3.65	2.00	2.82	
CV, %	23.82	36.00	29.91	
F-test	**	**	**	
LSD 0.05	1.27	1.06	1.16	

**, *Highly significant.*

Table 4. Two-way table of means of grain yield at Palpa and Tanahun districts

Treatment (Fertilizer), F	Palpa district, yield, t ha^{-1}			Tanahun district, yield, t ha^{-1}		
	Variety I, V1	Variety II, V2	Mean, t ha^{-1}	Variety I, V1	Variety II, V2	Mean, t ha^{-1}
F1	1.49	1.47	1.48	1.78	2.66	2.22
F2	2.43	1.97	2.20	4.10	4.55	4.32
F3	1.63	1.35	1.49	2.83	3.70	3.26
F4	2.96	2.71	2.83	4.45	5.10	4.78
V-mean	2.13	1.87		3.29	4.00	
F-test, V		ns			*	
F-test F		**			**	
F-test, VxF		ns			ns	
SEM, V		0.1805			o.2174	
SEM, F		o.2553			0.3074	
SEM, VxF		0.3610			0.4347	
CV, %		36.02			23.82	

***, Highly significant. *, Significant. ns, Non significant.*

Two sites mean response revealed that highest grain yield (3.9t ha^{-1}) was recorded in the local maize variety when fertilized with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} and 4 t ha^{-1} of agri-lime (T8, Table 3). The highest yield of 3.7 t ha^{-1} was produced by the Mana-1 variety at this level of fertilization (T4, Table 3) in Palpa district. Manakamana-1 variety produced less grain yield (1.63 t ha^{-1}) by 20.8% to that of the local check at farmer's practice (T5, Table 3). The crop yield was increased by 89.3% over the local check crop (T5) when fertilized with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} and 4 t ha^{-1} of agri-lime (T8). The second highest increment (79.6%) was recorded in Mana-1 variety at this level of fertilization (T4, Table 3).

The mean grain yield results as shown in the two-way table (Table 4) revealed that higher grain production (2.13 t ha^{-1}) was produced by the Mana-1 variety as compared to that of the local variety. Similarly, nitrogen mean effect on grain production was observed to be highest (2.83 t ha^{-1}) in Palpa district when the crop was supplied with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} and 4 t ha^{-1} of agri-lime (F4, Table 4). The lowest grain yield of 1.48 t ha^{-1} was recorded in the farmer's practice condition in Palpa district (F1, Table 4). The effect of variety, as shown in Table 4, was observed to be non significant, whereas the effect of fertilizer application on grain yield was observed to be highly significant. On the contrary, the effect of variety was observed to be non significant on the grain production in Tanahun district. However, the effect of nitrogen was found to be highly significant. The interaction effect on the grain production was observed to be non significant in both sites (Table 4). The mean grain yield as shown in Table 4, indicated that increased grains (4.78 t ha^{-1}) could be produced when the crop was supplied with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} applied along with 4 t ha^{-1} of lime (F4, Table 4) followed by the crop (2.22 t ha^{-1}) supplied only with fertilizers at 60: 30:30 kg N, P_2O_5 and K_2O ha^{-1} (F1) in Tanahun district. Mean results also indicated that higher grain production was obtained (4.0 t ha^{-1}) from local variety (V2) compared to that of the Manakamana-1 variety (3.29 t/ha) in Tanahun district. This increment of grain yield should be release of nutrients especially P, Ca and Mg due to amelioration of soil pH due to addition of agricultural lime and application of fertilizer. Joshy and Shrestha (1979) and Karki (1986) obtained similar results.

Table 5. **Soil test results before crop planting (BCP) and after crop harvesting (ACH) of the experimental plots in Tanahun and Palpa districts**

Treatment	Tanahun district						Palpa district					
	Soil pH		OM, %		Total N, %		Soil pH		OM, %		Total N, %	
	BCP	ACH	BCP	ACH	BCP	ACH	BCP	ACH	BCP	ACH	BCP	ACH
T1	5.67	5.22	2.22	2.5	0.187	0.822	5.4	5.4	2.2	3.7	0.152	0.822
T2	5.78	6.00	2.35	2.4	0.227	0.647	5.5	6.0	2.6	2.8	0.153	1.015
T3	6.12	6.40	2.42	2.2	0.212	0.400	5.4	5.9	2.5	2.5	0.145	1.067
T4	5.83	6.78	2.37	2.3	0.185	0.770	5.4	6.0	2.8	2.9	0.177	0.717
T5	5.86	5.12	1.84	2.3	0.210	1.225	5.6	5.4	2.3	2.5	0.132	0.770
T6	5.88	5.93	2.38	2.3	0.265	0.690	5.3	5.7	2.5	2.8	0.191	0.700
T7	5.85	6.01	2.15	2.3	0.192	1.025	5.4	6.1	2.2	2.3	0.146	0.757
T8	5.83	6.16	2.03	2.2	0.190	0.762	5.4	6.0	2.6	2.7	0.173	0.735
Mean			2.22		0.208				2.46		0.158	

Soil samples were analyzed at the soil laboratory of National Maize Research Programme (NMRP), Rampur, Chitwan, Nepal during the year 2005.

Effects on the nutrient status of the soil

Soil samples were collected and analyzed for the pH, organic matter (OM) and nitrogen (N) content before the crop planting (BCP) and after the crop harvesting (ACH) in both sites. The results revealed that soil pH was observed to be increased with the application of fertilizers and agricultural lime in both districts (Table 5). Similarly the OM and N content was found increased after crop harvest with the application of fertilizers in the experimental plots in both districts. The mean OM and N content of 2.31% and 0.208% was observed before crop planting in Tanahun district. The N content was increased drastically in all the treated plots in Tanahun (Table 5). The highest N content of soil (1.025%) was observed in the Farmer's practice plot that received probably only farmyard manures (T5). The lowest increment of N content (0.400%) was observed in the plot supplied with 4 t ha^{-1} of agricultural lime in Farmer's practice condition (T3) in Tanahun district. Similarly, the mean OM content of 2.46% was observed before crop planting in Palpa district. The OM content was increased in all the plots after crop harvest (ACH) irrespective of the treatments used in the experiment (Table 5). The mean of total N content of 0.158% was observed before crop planting and its content was also increased after the application of fertilizers and agricultural lime in all the treated plots after crop harvesting (Table 5). Soil analysis results show increment in soil pH, soil organic matter and also nitrogen content of soil after crop harvest. Increment of 1 unit of soil pH after harvest of crop that is two year consecutive experiment on the same plot is not worthy. This increment enhances nutrients availability especially Ca^{++} and Mg^{++} and PO_4^{-}. This result is similar to the finding of Karki (1986).

CONCLUSION

It is noticed that the effect of fertilizers and agricultural lime was found to be significant on the plant growth and grain production on both local and improved (Mana-1) varieties in both districts. The tallest plant height (267.0 cm) was observed in the local maize variety with the application of 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} and 4 t ha^{-1} of agri-lime in Palpa district. The highest number of kernels (27.7 kernels row^{-1}) in a kernel row was observed in Tanahun district when the Mana-1 variety was supplied with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1}. The highest grain yield (5.1 t ha^{-1}) was recorded in Tanahun district in the local variety when fertilized with 60:30:30 kg N, P_2O_5 and K_2O ha^{-1} and 4 t ha^{-1} of agri-lime. Two districts mean grain yield of 3.9 t ha^{-1} was observed in this variety at this level of fertilization. A maximum of 4.45 t ha^{-1} of grains were produced by the manakamana-1 variety when the crop was supplied with 60:30:30 kg N, P_2O_5 and K_2O and 4 t ha^{-1} of agri-lime and was observed to be non significantly different to that of local variety at this level of fertilization. The productivity of maize crop was observed to be less in Palpa (lowest 1.35 t and highest 2.96 t ha^{-1}) as compared to that of Tanahun district (lowest 1.78 t and highest 5.1 t ha^{-1}). Farmers are suggested to grow not only improved maize varieties but also local varieties in acidic and marginal fertility soils in the hills of Nepal. It is recommended to apply 60:30:30 kg N, P_2O_5 and K_2O and 4 t ha^{-1} of agri-lime for increased grain production irrespective of maize genotypes.

ACKNOWLEDGEMENT

This study was conducted under National Maize Research Program. Therefore, we would like to thank Mr. K. Adhikari, then Maize Coordinator for his support. During the course of study number of staffs of NMRP Rampur who have directly or indirectly helped during the experiment and this study deserve our sincere thanks. Our special recognition goes to Mr BR Pandey, Mr DD Neupane, NMRP. It would be injustice if we did not remembered the farmers who allowed us to conduct this study on their farms. All of the farmers and their family deserve our cordial appreciation.

REFERENCES

Adhikary BH and DB Ranabhat. 2004. An economic perspective of manures and fertilizers application on maize production in the acidic soils of Pokhara. **In:** *Proceedings of the 24th National Summer Crops Research Workshop on Maize Research and Production in Nepal* on 28-30 June 2004, Kathmandu, Nepal. National Maize Research Programme (NMRP), NARC. Pp. 287-290.

Bennett JG. 1995. *An analysis of soil fertility systems in the hills of Nepal*. A Joint project between Lumle Agriculture Research Center and the Natural Resources Institute, Kent, UK. Pp. 87.

Ghani A and MW Brown. 1997. *Improvement of soil fertility in Nepal through balanced application of fertilizer*. AgResearch. Hamilton, New Zealand. A study report for Ministry of Foreign Affairs and Trade, Govt. of New Zealand. Pp. 197.

Gomez KA and AA Gomez. 1984. *Statistical procedures for agricultural research*. Second Edition. Wiley Interscience Publication, John Wiley and Sons, IRRI, Philippines. Pp. 680.

Isherwood RJ. 1978. Fertilizer work at PAC. *Technical Paper No-23*. Pakhribas Agriculture Center (PAC), British Gorkha Ex-Servicemen Re-integration Training Scheme, Dhankuta, Nepal. Pp. 17.

Joshy D and SK Shrestha. 1979. Effect of time of N application at varying levels on yield of maize. **In:** *Proceedings of the 5th National Maize Development Workshop,* 15-19 Jan 1979, Naktajhij, Dhanusha, Nepal. Pp. 80-83.

Karki KB and JB Dacayo. 1990. Assessment of and degradation in Southern Lalitpur of Nepal. **In:** *Proc. 14th Congress of International Society of Soil Science*, 12-19 August 1991, Kyoto, Japan.

Karki KB, A Mentler and WEH Blum. 2000. Food security and crop productivity situation in Kathmandu valley (Extended abstract). **In:** *Proceeding, International Seminar and Workshop on Food Security in the Urban and Peri-urban Areas of Developing Countries*, Vienna, Austria. Pp.28-31.

Karki KB. 1986. Assessment of land degradation and formulation of productivity improvement strategies in Southern Lalitpur of Nepal. *MS Thesis*. CLSU, The Philippines. Pp. 115 (Unpublished).

Karki KB. 2003. Estimation of Plant Nutrients contents in biogas slurry and its losses during handling (Abstract). **In:** *Proc. 2nd National Convention of Society of Agriculture Scientists, Nepal*. ASA 2:31.

Maskey SL, S Bhattarai and KB Karki. 2004. Long–term effect of different sources of organic manures on wheat-soybean rotation. *SAARC J. of Agric.2:* 243-256

NMRP. 2005. Increased productivity of maize landraces through the use of inorganic fertilizers and agricultural lime in the hills of Nepal. **In:** *Annual Report for the Year 2004/05*. National Maize Research Programme (NMRP), Rampur, Chitwan, Nepal. Pp. 91.

Tripathi BP, RC Munankarmy and PR Shakya. 2002. Enhancement of soil fertility and crop productivity in acidic upland soil in the hills of Nepal. **In:** *Proceedings of the 23rd National Summer Crops Workshop on Maize Research and Production*, 2-4 July 2002, NARC, Khumaltar, Lalitpur, Nepal. Pp. 289-304.

Turton CN, A Vaidya, JK Tuladhar and KD Joshi. 1996. *Towards sustainable soil fertility management in the hills of Nepal*. A joint publication by Lumle ARC, Lumle and Natural Resources Institute, Center Avenue Chatham Maritime, Kent, UK. Pp. 57.

Participatory Evaluation of Some Tomato Genotypes for Resistance to Bacterial Wilt

Ram D. Timila* and Sharada Joshi

Plant Pathology Division, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal
<rdevi04@yahoo.com>

ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum* E.F. Smith is one of the destructive diseases of tomato. Experiments were conducted to confirm the resistance and to evaluate varieties received from Asian Vegetable Research and Development Center against bacterial wilt disease in the farmers' bacterial wilt sick fields at Thaiba and at Panchkhal during 1999 and 2000-2001 respectively. A total of five varieties such as CLN 2026 C, CLN 2026 D, CLN 1466 J, CLN 1466 P and susceptible check Lapsigede or L 390 were included in the experiments. Randomized complete block design with 4 replications was commenced at both the locations. In terms of disease incidence and yield parameters as well as consumers' preference on the fruit size, CLN 2026 C was found the best variety followed by CLN 2026 D. Wilt incidence was significantly lower in those varieties showing resistant reaction than in susceptible check. However, the marketable fruit yields were significantly higher with low wilt incidences in CLN 1466 P and CLN 1466 J also compared to susceptible check. But the large fruit size of those varieties was not acceptable to the consumers and the growers. Thus, CLN 2026 C and CLN 2026 D could be used as resistant variety to minimize crop losses from bacterial wilt disease.

Key words: Bacterial wilt, *Ralstonia solanacearum,* resistant variety, Tomato

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* E.F. Smith (RS) is widely distributed disease in the world. It is one of the limiting factors to the successful cultivation of solnaceous vegetables throughout the world, where warm and humid climate is prevalent (Kelman 1953, Vawdrey and Gounder 1993). In Nepal, Tomato (*Lycopersicon esculentum* L.) is one of the fruit vegetables grown extensively in terai, foothills and valleys of Nepal. After late blight, bacterial wilt is considered to be the second most important disease in tomato. In Nepal, it was first recorded in 1978 in tomato crop (Shrestha 1990) and proved to be one of the limiting factors for successful cultivation in lowlands, foothills and valleys. Its incidence was reported as high as 80% causing considerable yield losses in tomato growing areas of Nepal (Timila et al 1997). The primary source of inoculum for the occurrence and spread of the pathogen is considered to be infested soil, plant debris and weeds (Kelman 1953). Bacterial wilt is difficult to control, once established in the field because of soil borne nature of the pathogen, and having wide host range, wide spread distribution and vast genetic variability of the pathogen (Hayward 1991). In recent years, RS is reported to be capable of infecting more than 50 botanical families with more than 200 plant species (Marco et al 2005). Due to the limited efficacy of current integrated management strategies of bacterial wilt, this disease is continued to be economically important (Pradhananga et al 2003).

Wide use of pesticides has favored the development of pathogen resistance towards pesticides. Concerns for human health and the environment have pushed the crop production to reduced use of pesticides. Host resistance plays an important role in the development of novel strategies to control bacterial wilt disease. Planting resistant varieties is the most effective and simplest methods for controlling bacterial wilt disease and also environmentally sound. Virulence differences among pathogen strains and biotic and abiotic factors can cause resistance instability in host (Hayward 1991). The least aggressive strain affects least on the resistant cultivars. Instability in host resistance appeared to be a major problem in using resistant lines (Wang et al 1997). However, effective and

long-term disease control could be achieved with resistant or tolerant varieties as a part of integrated disease management strategies (Saddler 2005). So, the experiments were conducted in the farmers' field to evaluate the varieties and to verify their resistance to bacterial wilt that was previously reported to be resistant at Asian Vegetable Research and Development Center (AVRDC).

MATERIALS AND METHODS

Seeds of the five tomato varieties (CLN 2026 D, CLN 2026 C, CLN 1466 P, CLN 1466 J supposed to be resistant and susceptible check, L 390 were received from AVRDC, Taiwan under South Asian Vegetable Research Network (SAVERNET). In the year 1999, the experiment was conducted at Thaiba, Lalitpur (1350 masl) in naturally infested farmers field. Five varieties from AVRDC and one of the popular hybrid variety (NS 815) in that locatons in addition to susceptible check from AVRDC were included in the trial. In the following consecutive years (2000 and 2001), experiments were conducted at Panchkhal of Kavrepalanchowk (850 masl) district in naturally infested farmers field. In both the locations, the experimental fields were chosen with uniform distribution of bacterial wilt disease in previous season. The basis for determining uniform distribution of bacterial wilt disease was uniform distribution of wilted tomato plants (which showed bacterial ooze in water from the cut surface) during previous season in the fields. Above five genotypes including local Lapsigede (susceptible check) were included in the experiment. Seedlings were raised under screenhouse conditions at Plant Pathology Division using steam sterilized soil to make sure of seedlings free from disease. The experimental design was randomized complete block with four replications. The number of plants per replication of each variety was 20. Plant to plant and row to row spacing were 30 and 60 respectively. Chemical fertilizer applied was 85:20:50 kg NPK/ha in addition to 10mt/h of compost. One month old seedlings were transplanted during first and third week of August at Thaiba and Panchkhal respectively. Agronomical practices were given as per need. Wilt incidence was recorded based on the survival of the plants every 7 days interval until disease became stable. Ripened fruits were harvested by hand plucking and calculated in terms of t/h. Marketable and unmarketable fruit yield due to late blight were separated. For determining the size of the fruit, average of 10 fruits were taken. Three sprays of 0.15% Krilaxyl (metalaxyl 8% plus mancozeb 64%) were given to save plants from Late Blight disease. Area under disease progress curve (AUDPC) for promising varieties at Panchkhal were calculated according to Shanner and Finney (1977) using following formula.

$$\text{AUDPC} = \sum_{i=1}^{n} [(y_{i+1} + y_i)/2] [x_{i+1} - x_i]$$

where, yi = disease index or incidence at the i^{th} observation, xi = time at the i^{th} observation, and n = total number of observations.

For assessing resistance level of the genotypes categorization was followed according to Mew and Ho (1976) as given below. The data were analyzed using MSTATC after transformation whenever necessary.

Resistant = less than 20% wilt incidence
Moderately resistant = 21-40% wilt incidence
Moderately susceptible = 41-60% wilt incidence
Susceptible = 61-100% wilt incidence.

RESULTS

At Thaiba, the varieties CLN 2026 C and CLN 1466 J were resistant with wilt incidences, 12.8% and 24, 3% respectively. Varieties, CLN 2026 D and CLN 1466 P were moderately resistant with

wilt incidences, 21% and 30.6% respectively. Those all varieties were significantly better and are at par in response to bacterial wilt (Figure 1). Yield could not be taken due to severe infection of late blight inspite of Krilaxyl spray. However the farmers at the location were highly impressed by the higher percentage of survival (less wilt incidence) of the plants compared to susceptible check variety, L 390 (90%) or Pusa Ruby (65%) and popular commercial variety, NS 815 (57%) of that location.

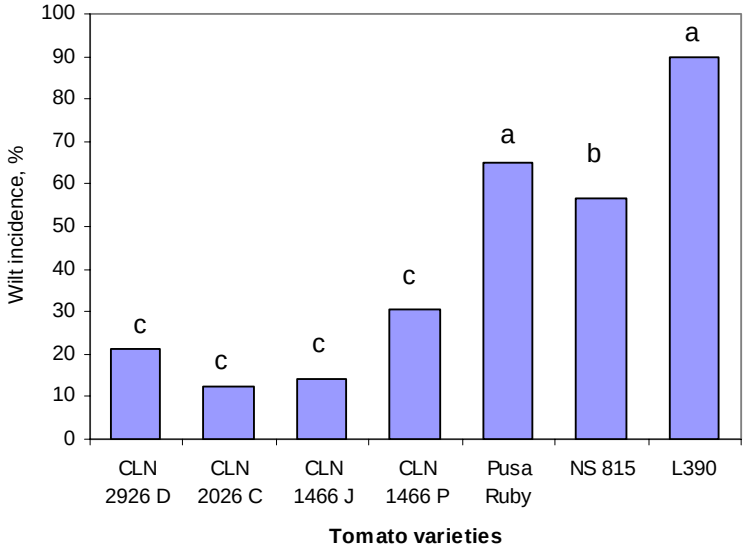


Figure 1. Onfarm evaluation of tomato varieties against bacterial wilt in the farmers' field at Thaiba, 1999. (CV = 26.6%. Column with same letter are not significantly different at P ≤ 0.05 using DMRT)

Wilting of the tomato plants were started two weeks after transplanting in susceptible check, local Lapsigede variety in both the years at Panchkhal. In the year 2000, disease incidence was found least in CLN 2026 C followed by CLN 1466 J, CLN 1466 P and CLN 2026 D based on terminal disease incidence. The latest variety was statistically different from CLN 2026 C (Table 1). Hundred percent wilt incidence was found in local susceptible check variety, Lapsigede before fruiting stage. Regarding yield parameter, highest marketable fruit yield was found in the variety CLN 1466 P in 2000. Other three varieties CLN 2026 C, CLN 2026 D, and CLN 1466 J were not statistically different in marketable fruit yield. In fruit size, CLN 2026 C and CLN 2026 D, and CLN 1466 P and CLN 1466 J were at par. Lesser unmarketable fruit yield due to late bight infection was found in CLN 1466 J. Similarly in the year 2001, same trend of disease incidences were found in the test varieties. All test varieties from AVRDC were statistically similar but different from the local susceptible check in wilt reaction (wilt incidence). The disease incidence was higher in 2000 than in the year 2001, it could be due to slightly higher temperature in 2000 (more than 30^{o}C). Similarly in the year 2001, highest fruit yield was found in CLN 2026 C and it was statistically different from other rest of the tested varieties. Size of the fruit was found better in the varieties CLN 2026 C and CLN 2026 D with medium sizes, which were closer to local Lapsigede variety (Table 1).

Table 1. Response of some promising tomato genotypes to bacterial wilt disease and yield parameters at Panchkhal during 2000 and 2001

SN	Varieties	2000				2001			
		Wilt incidence, %	Yield, t/h			Wilt incidence, %	Yield, t/h		
			Marketable	Unmarketable	Fruit size, g/fruit		Marketable	Unmarketable	Fruit size, g/fruit
1	CLN 2026 D	47.73 b	7.25 b	3.86	73.08 b	27.13 b	10.03 b	2.23	40.6 b
2	CLN 2026 C	19.44 c	13,25 b	2.36	71.94 b	18.17 b	23.00 a	3.6	46.25 b
3	CLN 1466 J	38.18 bc	12.25 b	1.50	132.96 a	22.65 b	8.92 b	1.7	87.28 a
4	CLN 1466 P	40.72 bc	21.00 a	2.99	132.41 a	23.59 b	12.35 b	1.9	80.4 a
5	Lapsigede susceptible local check	100.00 a	-	-	-	69.08 a	8.18 b	1.0	20.75 c
	CV, %	17.6	37.51	43.22	23.35	51.76	40.34	71.85	20.19

Mean in the column with same letter are not significantly different at P ≤ 0.05 using DMRT.

Disease progress was much faster in local susceptible check than in other varieties. Disease incidence reached nearly 90% within 4 weeks where as, the incidence was less than 25% in CLN 2026 C during that period (Figure 2). The disease progress much slower in other varieties compared to local susceptible check. Same trend of disease progress was found in the year 2001 also, however, disease progress curves were not drawn for that year. ANOVA showed that there was no significant difference among those varieties from AVRDC in AUDPC values. Those varieties were significantly different from the local susceptible check in terms of wilt incidence. The AUDPC values for CLN 2026 C was very low and it was very high in local Lapsigede. CLN 2026 C was at par with CLN 1466 P and J but different from CLN 2026 D in their AUDPC values (Figure 3).

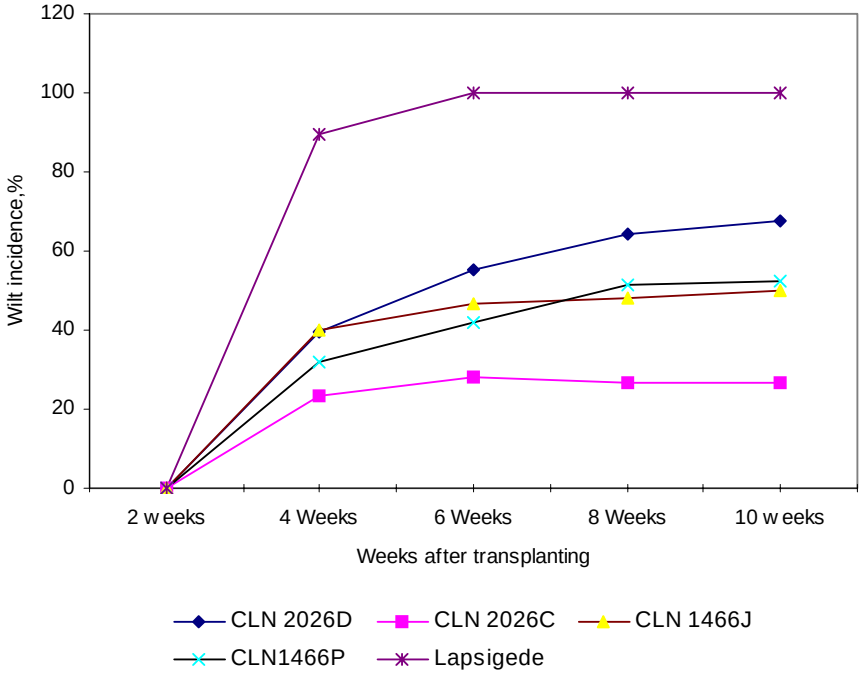


Figure 2. Disease progress curves of bacterial wilt of different promising tomato varieties at Panchkhal, Kavrepalanchowk, 2000.

DISCUSSION

Tomato is number one fruit vegetable grown in terai, foothills and the valleys of Nepal. The crop is attacked by *Ralstonia solanacearum* causing wilting of the plants in warm and humid conditions. The farmers experienced considerable economic losses due to this disease. Because of soil borne nature, location specific strain, wide host range with wide spread distribution and vast genetic variability (Hayward 1991), bacterial wilt disease is difficult to control once established in the field. Use of resistant variety is the most effective and simplest methods of controlling bacterial wilt (Monma and Sakata 1983), however, the genetic basis of resistance breaks down due to the changes in the host or pathogen under condition of high temperature (Mew and Ho 1977).

Four promising varieties with resistance to bacterial wilt from AVRDC were tested at Panchkhal of Kavrepalanchowk, which is one of the most bacterial wilt prone areas and at Thaiba of Lalipur where outbreak of bacterial wilt occurred in recent years. Our results showed that the varieties CLN 2026 C and CLN 2026 D were better than other varieties in terms of disease and yield parameters. These varieties were preferred by the farmers and the consumers as well. However, CLN 1466 J also showed better performance. But the limitation with this variety was bigger fruit size, which do not preferred by the consumers. Concerning fruit characteristics same type of observation was found by

Budhathoki and Ahamad (2001). However, the yield and fruit size of Lapsigede could not be compared with the test varieties due to 100% wilt before fruiting during 2000. Better fruit yield in CLN 2026 C and CLN 2026 D were also reported by Budhathoki et al (2004) under plastic house conditions, thus suitable for off season cultivation. In Nepal previously, the variety CL 1131 (now NCL 1) was resistance to bacterial wilt but its resistance was found to be broken at Panchkhal area (Timila and Shrestha 2001).

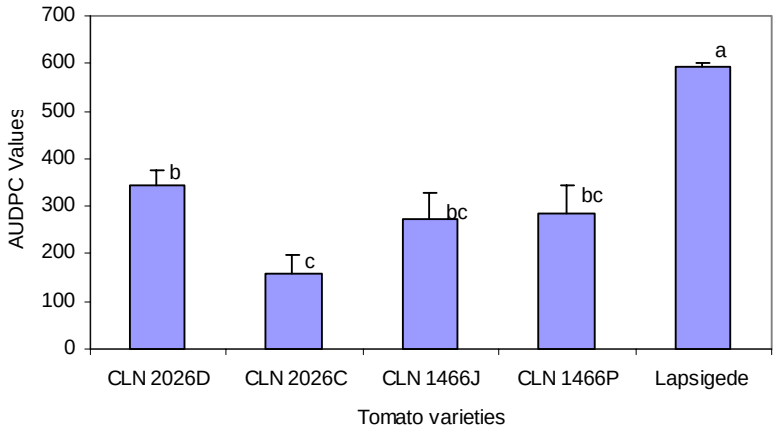


Figure 3. Area under disease progress curve (AUDPC) values for different tomato varieties at Panchkhal, 2000 (Bar indicates the standard error of the mean). CV = 24.9% Mean in the column with same letter are not significantly different at $P \leq 0.05$ using DMRT.

The variety CLN 2026 C and CLN 2026 D observed to be promising in all respect, however the fruit test may not replace the local variety, such as Lapsigede which is highly preferred by the consumers. Local variety get higher price than the bigger sized fruits. The later get good price only when the supply of local tomato is less in the market. But in absence of other resistant varieties these could be cultivated as an alternative to other susceptible varieties in disease prone locations to over come the losses caused by bacterial wilt disease.

ACKNOWLEDGEMENTS

We thank Dr SK Shrestha, Former Chief Plant Pathologist, Plant Pathology Division, NARC for valuable suggestions during the coarse of experiments. Sincere thank goes to Dr JF Wang, Associate Professor, AVRDC for supplying seed and the technical suggestions. Financial supports from SAVERNET program of AVRDC is also highly acknowledged. We also express our thanks to the farmers, Mr Damodar Adhikari of Panchkhal and Mr Madhav Khadka of Thaiba for their participation, helps and availing fields for the experiments.

REFERENCES

Budhathoki K and S Ahamad. 2001. Evaluation of tomato cultivars for normal and off-season cultivation for the hills and plain conditions of Nepal. Nepal. **In:** Proceedings of the South Asia Vegetable Research Network (SAVERNET II) Final Workshop, 3-8 June 2001, Bangkok, Thailand. Pp. 1-10.

Budhathoki K, NG Pradhan HN Regmi and PR Bhurtyal. 2004. Evaluation of tomato cultivars for off-season production under polyhouse and open field conditions. **In:** *Proceedings of the fourth National Workshop on Horticulture*. NARI and HRD, NARC, Kathmandu. Pp. 413-418.

Hayward AC. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annul. Rev. Phytopatho*. 29:65-67.

Kelman A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum.* Technical Bulletin 99. North Carolina Agricultural Experiment Station. 194 Pp.

Marco Y, A Trigalet, J Vasse, J Olivier, DX Feng and L Deslandes. 2005. Host resistance to *Ralstonia solanacearum*. **In:** Bacterial wilt disease and the *Ralstonia solanacearum* species complex (C Allen, P prior and AC Hayward, eds). The American Phytopathological Society. Pp. 275-283.

Mew TW and WC Ho. 1976. Varietal resistance to bacterial wilt in tomato. *Plant Disease Reporter* 60(3):204-208.

Mew TW and WC Ho. 1977. Effect of soil temperature on resistance of tomato cultivars to bacterial wilt. *Phytopathology* 67:909-911.

Monma S and Y Sakata. 1983. Inheritance to bacterial wilt in tomato. **In:** *Bacterial wilt ACIAR Proceedings. No. 45*. 145-153

Pradhanang PM, MT Mamol, SM Olson and JB Jones. 2003. Effects of plant essential oils to *Ralstonia solanacearum* population density and bacterial wilt incidence in tomato. *Plant Dis*. 87:423-427.

Saddler GS. 2005. Management of bacterial wilt disease.. **In:** Bacterial wilt disease and the *Ralstonia solanacearum* species complex (C Allen, P Prior and AC Hayward, eds). The American Phytopathological Society. Pp. 121-132.

Shanner G and RE Finney. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. *Phytopathology* 67:1051-1056.

Shrestha K. 1990. *Major diseases of vegetable crops in Nepal*. Nepali book. Vegetable Development Division, HMG/Fresh Vegetable and Vegetable Seed Production Project, FAO, Khumaltar, Lalitpur, Nepal. 111 pp.

Timila RD and K Shrestha. 2001. Sources of resistance to bacterial wilt in tomato and its management approach. **In:** *Proceedings of the First SAS/N Convention*, 29-31 March 1999, Kathmandu. Society of Agricultural Scientists (SAS), Nepal. Pp. 161-167.

Timila RD, K Shrestha and S Joshi. 1997. Bacterial wilt of tomato and eggplant in Nepal. **In:** *Proceedings of the Phase I Final Workshop of the SAVERNET*. AVRDC Pubication No. 97-458. Pp. 156-167.

Vawdrey LL and Gounder RK. 1993. A glasshouse seedling test for bacterial wilt resistance in tomato. *Bacterial Wilt Newsletter* No. 9:3.

Wang JF, P Hanson and JA Barnes. 1997. Worldwide evaluation of an international set of resistance sources to bacterial wilt in tomato. **In:** *Bacterial wilt disease: Molecular and ecological aspects* (P Prior, C Allen and J Elphinstone, eds). Springer-Verlag, Berlin, Germany. Pp. 269-275.

Participatory Black Scurf Disease Management on Potato in Nepal

Buddhi P. Sharma[1] and Ram B. KC[2]

[1] Senior Scientist, National Potato Research Programme, NARC, Khumaltar

<bpsharma@narc.gov.np>

[2] Senior Scientist, Regional Agricultural Research Station, NRC, Nepalganj

ABSTRACT

A farmer's participatory field experiment was accomplished during three consecutive years (2003-2005) for the sustainable management of black scurf disease of potato at Mainapokhar, Bardiya, Nepal. A heavily infested field with *Rhizoctonia solani* was selected prior to experimentation. Experiment was laid out in randomized complete block design with three replications. The efficacy of two concentrations (2% and 3%) of boric acid and 0.15% of Thiophanate Methyl were tested for disease control through seed treatment. An antagonistic fungus *Trichoderma harzianum* was used as seed and soil treatment. As per treatment plan some plots were sterilized with formaldehyde 1% prior to planting. The plots having healthy seed (pre-basic seed) planted in sterilized plots showed minimum disease (6.8%) followed by 3% boric acid treated seed planted in sterilized plots (10.8%) and 2% boric acid without soil sterilization (13.1%) and seed and soil treated plot with *T. harzianum* (17.1%). Farmers did not accept treatments connected with soil sterilization by Formalin. After analyzing the overall results of three years experiment farmers choose seed treatment with 2% boric acid prior to planting and identified application of *Trichoderma harzianum* for black scurf management as second option.

Key words: Boric acid, fungicides, potato, *Rhizoctonia solani, Trichoderma harzianum*

INTRODUCTION

Potato (*Solanum tuberosum*) is one of the important food crops after rice, wheat and maize which occupy total area of 1,50,864 hectares with the productivity of 13.09 t/ha (ABPSD 2006). Present productivity of potato in Nepal seems to be very low as compared to European countries (40-45 t/ha). Diseases are one of the yield reducing factors, which causes low yield of potato. Black scurf caused by *Rhizoctonia solani* is one of the most important diseases of potato. Black scurf disease symptoms can be found on all underground parts of the plant at different times during the growing season. Disease is pronounced when black sclerotia cover the tuber surface. Under minimum disease severity it just lower down the market price but may not reduce the yield. Under severe conditions, when sclerotia cover more than 50% area hinders germination of tubers, if get germinated there will be poor plant growth and leads to low yield. Although yield losses associated with this disease are not well documented in Nepal, however, considerable losses due to this disease have been frequently reported.

In Nepal yield losses due to this disease has been estimated ranging 5-60 percent depending on the prevailing weather during the crop period and crop rotation followed. Mid western region particularly Mainapokhar Bardiya is one of the black scurf affected areas, where highest yield losses observed in 2003. Some recommendations have been made for disease control through seed tuber

treatment with Sodium hypo-chloride (NaOCl). The seed treatment with mixture of Acetic acid 1% + Zinc sulphate 0.05% solution for 15 min dip gave 100% kill of sclerotia (Dutta and Gupta 1982, NPRP 1994).

The fungus survives from season to season primarily as black structures called sclerotia, which are present in soil and on potato seed tubers. After harvest of crop, sclerotia that can be seen on potato seed tubers do no wash off with water. The fungus maintained its life cycle and transmitted by sclerotia on the tubers and within the soil or plant residues. With optimal temperature (18^{o}C) and soil moisture, the sclerotia germinate and penetrate stems, roots and stolons. Development of sclerotia on tubers is pronounced in poorly drained soils.

The hyphae of *Rhizoctonia,* attacks developing sprouts, when the soil is cool (50-59^{o}F) and moist. Symptoms on developing sprouts appear as reddish-brown, discolored areas, and the growing point of severely infected sprouts is often killed (DPP 2000). It was observed that the differences in the incidence of stem canker, stolon canker and black scurf were dominated by the effect of inoculums on seed tubers at planting (Simons and Gilligan 1997).

It was also suggested in the previous report that, tuber treatment of either 2 min dip in 2% formaldehyde, spray with Peneyethorn, Iprodione or a spore suspension of 106 spores/ml *Verticillium biguttatum* were found most effective if planted in fumigated soil with 500 L/ha Metham (Wicks et al 1996) but they have not described effects on environment.

Despite of these recommendations made by the different researchers, almost none of the farmers of Nepal applied these chemicals against this disease because either of low severity of disease in the past or high cost of treatment. Mainapokhar is one of the main potato seed production centers in the mid western development region of the country, where potato being grown continuously for more than a decade. Keeping these points in view farmer's participatory research was under taken at Mainapokhar, for empowering farmers to identify low cost, environment friendly and sustainable technology for black scurf management in collaboration with Regional Agriculture Research Station Nepalganj.

MATERIALS AND METHODS

A heavily infested field with *Rhizoctonia solani* was selected prior to experimentation. Treatments were fixed on consultation with participant farmers. Experiment was laid out in randomized complete block design with seven treatments and three replications. Black scurf infected farmers' own potato variety 'Cardinal' was used in the field experiment. Experiment was planted on second week of November in all the experimental years (2003- 2005). Plot size was 2.4- × 3.0-m. Sprouted seed tubers were planted in 25- × 60-cm spacing at a normal depth. Recommended dose of Manures (10 t/ha) and fertilizers 150:100:60 kg N, P_2O_5 and K_2O Kg/ha respectively were applied. Nitrogen was applied as three split doses. Half amount of Nitrogen, during planting as basal and remaining half was applied during two intercultural operations as split doses. As per the treatments soil was sterilized with Formaldehyde 1.0% ai concentration through commercial Formalin 39%. Trichoderma in the form of NIPROT was applied as seed and soil treatment and drenching after germination. As per manufacture's instructions *Trichoderma* was multiplied in well-decomposed FYM @ 10 g NIPROT/kg FYM. Commercial brand of *Trichoderma* 'NIPROT' was well mixed in FYM and kept for 10 days under polyethylene cover to maintain the conducive temperature. Seeds were treated with boric acid of two concentrations (2% and 3%) prior to panting.

Crop was harvested at 105-110 days after planting. Total yield per plot was recorded. Finally, black scurf severity was recorded on percentage comparing the corresponding pictorial severity scale developed (key for black scurf scoring). Black scurf severity index was computed as reported by Tanil et al 1982.

$$\text{Disease severity index} = \frac{\sum (n \times r) \times 100}{5 \times N}$$

Where, n = number of samples in each rating, r = each rating number, 5 = highest rating, N = Total number of samples examined.

On completion of two-season experiment, a village level field workshop was organized involving 20 farmers, two extension staff of concern district. Farmers experience on disease symptoms, experimental procedures and research outputs were shared with other potato seed producing farmers.

In vitro* efficacy test of antagonist (*T. harzianum*) against *R. solani

Trichoderma harzianum was isolated from the commercial product "NIPROT" manufactured by Bio-Control Research Laboratories, Bangalore and cultured on Potato Dextrose Agar (PDA). Causal pathogen, Rhizoctonia solani was also isolated from infected tubers collected from experimental plot and cultured on PDA medium.

For competitive fungal growth studies 10 mm size culture disk was inoculated onto the freshly made PDA. *Trichoderma* culture was placed in the center and *R. solani* culture was placed four sides around the antagonistic fungus. Five such inoculated plates were incubated for 7 days at 22^{o}C for their colony development.

RESULTS AND DISCUSSION

Disease index

There were significant differences in the disease index observed due to the treatments. The plots having healthy seed (PBS) planted in sterilized plots showed minimum disease index by 6.8% followed by 3% boric acid treated seed planted in sterilized plots (10.8%) and in seed and soil treated with *T. harzianum* (17.1%). Seed treatment with Thiophanate methyl could not found effective in controlling the black scurf under field conditions (Table 1).

Black scurf disease severity in 2003 was 27% whereas in 2004 severity reached up to 29%. Disease severity in 2004 was two percent higher than the previous experimental year. The mean disease index over the three experimental years is presented in Figure 1. Disease severity over the experimental years was significantly low as compared to adjoining farmer's field where Cardinal variety was grown. The reason behind it could be the timely irrigation and shallow planting which enhanced the early germination and reduced the infection.

Regarding with the efficacy of fungicides tested against black scurf (*Rhizoctonia solani*) in other countries, Fludioxonil (Maxim) consistently provided superior protection (Bains et al 2002). Boric acid (17% ai Boron) a potential alternative after Benlate was tested against *Eutypa lata* of grapevines. Five % boric acid (18.75 mg/ml water) significantly reduced the Rhizoctonia disease *in vitro* and in field trials (Rolshausen and Gubler 2005).

Table 1. Black scurf disease index on potato tuber at harvest under field conditions

SN	TREATMENT	DISEASE INDEX			
		2003/04	2004/05	2005/06	MEAN
T1	SEED AND SOIL TREATMENT WITH *T. HARZIANUM*	17.7	18.7	15.1	17.1
T2	BORIC ACID (3%) TREATED SEED IN STERILIZED SOIL	14.7	7.6	10.0	10.8
T3	BORIC ACID TREATED (2%) SEED IN INFECTED SOIL	18.3	8.4	12.6	13.1
T4	INFECTED SEED IN STERILIZED SOIL	24.0	22.2	17.0	21.1
T5	TREATED SEED WITH ROKO FUNGICIDE IN INFECTED SOIL	25.0	28.0	15.8	22.9
T6	HEALTHY SEED IN STERILIZED SOIL	9.7	3.1	7.6	6.8
T7	INFECTED SEED IN INFECTED SOIL (FARMER'S PRACTICE)	27.0	29.3	18.7	25.0
	F-TEST	**	**	*	
	CV, %	13.4	22.2	10.4	
	LSD 0.05	4.6	2.4	2.05	

There was no significant differences between the disease control in the plots of 3% boric acid treated seed and of boric acid 2% concentration, in the first two consecutive experimental years, where as in third year disease index was found significantly low in boric acid 3% treated plots (Table 1). Despite of low mean disease index of three years due to seed treatment of boric acid 3% planted in sterilized soil (10.8) farmers choose seed treatment with boric acid 2% planted in unsterilised soil simply because of low cost on seed treatment and no need of formaldehyde for soil sterilization (Figure 1). Over all results of three years showed that planting healthy seed in sterilized soil was the best option for black scurf management. On the other hand, farmers hesitate to use chemicals like formaldehyde for soil sterilization, which increases the cost of production. Evaluating the pros and cons of all treatments farmers choose seed treatment with boric acid 2 percent. Application of antagonistic fungus (*T. harzianum*) to tuber treatment, soil treatment and drenching was found comparable with infected seeds planted in sterilized soil with formaldehyde (Table 1). Overall black scurf disease severity in the check plot was highest during 2004 crop season, whereas in 2005 disease severity was minimum. The reason behind it could be the cumulative rainfall during the tuber maturity period was high as compared to other years. In 2005/06, there was no rainfall in whole potato crop season (Figure 3).

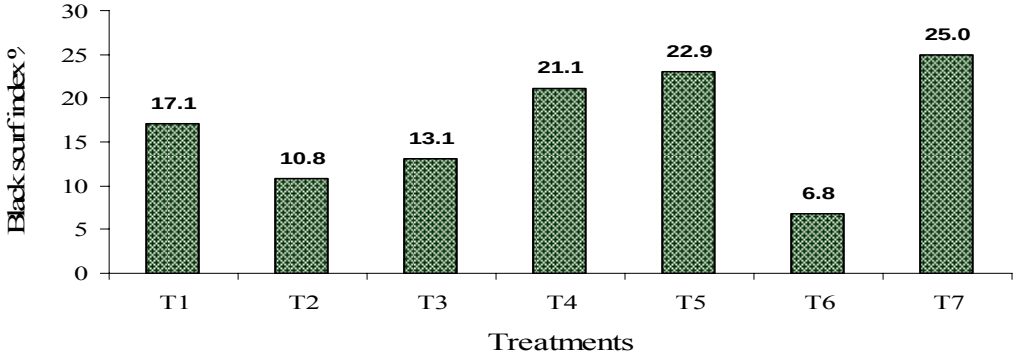


Figure 1. Mean effects of treatments over the three years on black scurf disease index.

In-vitro studies also showed that growth of *R. solani* was completely covered and suppressed by the profuse growth of *T. harzianum*, which was isolated from "NIPROT" under the temperatures of 22^{o}C. *In-vitro* results showed that use of *Trichoderma harzianum* can check the *R. solani* significantly if the population of antagonist sufficiently exists in the soils.

Results of similar studies have been reported by Martin and Robert in 2005. They found that out of twenty eight potential bio control organisms tested for efficacy against *Rhizoctonia solani* on potato in series of greenhouse trial, one combination of bio-control organisms *Bacillus subtilis* and *Trichoderma virens* demonstrated some what better control of stem canker than each organism alone and has been suggested that this approach may provide improved bio-control efficacy. In 2001 Tsror et al had also reported the efficacy of *Trichoderma harzianum* on pathogenic *Rhizoctonia* in reducing the incidence of black scurf on daughter tubers using naturally infested soil and contaminated seed tubers.

Disease control

Treatments were assessed for impact on black scurf disease control based on the severity disease index as shown in Table 1. On an average disease control was achieved up to 70.9% where disease free tubers planted in sterilized soil (Table 2). Efficacy of *T. harzianum* showed effective control ranging 19.0 to 36.4%. Mean disease control was 30.0% over the three crop seasons (Table 2). Three percent boric acid treated seed plots showed significant control of black scurf (55.4%)

followed by boric acid 2% (41.7%). Efficacy of these boric acid concentrations were found comparable regarding with disease control. Soil sterilization with Formaldehyde 1% had a role of controlling 14.8%. Role of infected seed tubers on developing disease severity was found higher than the *Rhizoctonia* infested soil.

Soil sterilization with Formalin is common practice in glass house crop cultivation. Formaldehyde at 0.8% has been used for substrate (soil) sterilization in glasshouse by covering soils for 7 days for the control of soil borne diseases (NPRP 2005). It has been also reported that when formalin is used for soil fumigation, it does not harm to antagonistic fungus *Trichoderma*. In rhizosphere soil of potato field of CPRI Shimla eighteen pathogenic and non pathogenic fungi had been recovered. When soil was treated with Formalin at 400 ppm there was no effects on none pathogenic fungi like *Trichoderma*, *Penicillium, Aspergillus* and *Gliocladium* whereas *Rhizoctonia* and *Fusarium* spp were controlled in the treated plots (Rai 1984)

TABLE 2. BLACK SCURF DISEASE CONTROL % ON POTATO TUBER AT HARVEST UNDER FIELD CONDITIONS

SN	Treatment	Disease control, %			
		03/04	04/05	05/06	MEAN
T1	Seed and Soil treatment with *T. harzianum*	34.6	36.4	19.0	30.0
T2	Boric acid (3%) treated seed in sterilized soil	45.7	74.2	46.2	55.4
T3	Boric acid treated (2%) seed in infected soil	32.1	71.2	21.9	41.7
T4	Infected seed in sterilized soil	11.1	24.2	9.0	14.8
T5	Treated seed with ROKO fungicide	7.4	4.5	15.2	9.1
T6	Healthy seed in sterilized soil	64.2	89.4	59.0	70.9
T7	Infected seed in infected soil (Check)	0.0	0.0	0.0	0.0

EFFECTS ON YIELD

Tuber yield from the plot of pre basic seed (PBS) planted in sterilized plot was highest (29.73 t/ha) followed by seed treatment with boric acid 3% planted in sterilized plot (27.7 t/ha) and *Trichoderma harzianum* applied plot (24.88 t/ha). This yield was comparable with the yield of boric acid 2% treated plot (26.04 t/ha). Among the results of three years, yield differences between the two treatments boric acid 2% and 3% in the last two experimental years were found at par. Significantly higher tuber yield was produced in *T. harzianum* treated plot (25.12 t/ha) as compared to farmers practice (Table 3). Results indicate that biological control through *T. harzianum* would be one of the new options for long-term black scurf management under mid western terai conditions of Nepal. When population of *T. harzianum* gets well established in the soil, disease could be controlled significantly in the succeeding years.

Abada in 1994, reported that *Trichoderma harzianum* caused a great reduction in the infection level of damping off and root rot disease of sugar beet and resulted increased root weight both in pot and in field experiment.

Table 3. Effects of treatment on potato tuber yield (t/ha) under field conditions

SN	Treatment	Tuber yield, t/ha				Farmers Acceptance score (1-5)*
		03/04	04/05	05/06	Mean	
T1	Seed and Soil treatment with *T. harzianum*	27.41	25.12	22.11	24.88	2
T2	Boric acid (3%) treated seed in sterilized soil	30.32	28.76	24.00	27.70	4
T3	Boric acid treated (2%) seed in infected soil	27.08	27.66	23.38	26.04	1
T4	Infected seed in sterilized soil	23.80	26.50	22.08	24.13	5
T5	Treated seed with ROKO fungicide	24.81	22.89	21.71	23.14	5
T6	Healthy seed in sterilized soil	32.87	30.50	25.81	29.73	3
T7	Infected seed in infected soil (Check)	23.61	22.49	21.39	22.50	
	F-test	**	**	*		
	CV, %	4.54	4.47	7.15		
	LSD 0.05	1.58	2.7	2.89		

**** 1= HIGHLY ACCEPTED. 5 = POORLY ACCEPTED.***

Regarding with the tuber yield of three years it was found in decreasing trend. The factors behind it could be fertile soil and better irrigation in the first year. On second and third year experimental plot was changed as per the farmers' crop rotation scheme. In addition, there was no rainfall during the crop period of third year (Figure 3).

FARMER'S ACCEPTANCE

The participants did not accept sterilization of soil with Formalin in a larger potato growing area. All the treatments connected with soil sterilization were poorly accepted (rejected) even yield was significantly higher (Table 3). Tuber yield in seed treatment with 2% boric acid planted in un-sterilized soil was comparable with boric acid 3% along with soil sterilization. Therefore, farmers selected seed treatment with lower dose of boric acid. Similarly, Application of *T. harzianum* produced the significantly higher yield than the check plot. These two treatments were considered as effective and easy to use under farmers' conditions.

During the experimental period, black scurf symptoms as whitish-gray mycelia covering over the base of stem near the soil surface, (Figure 2a), aerial tubers on the leaf axils (Figure 2b) and sclerotia were observed on tuber (Figure 2c)

Figure 2a. Sclerotia on tuber

Figure 2b. Aerial tubers on leaf axils base of stem

Figure 2c. White mycelia on

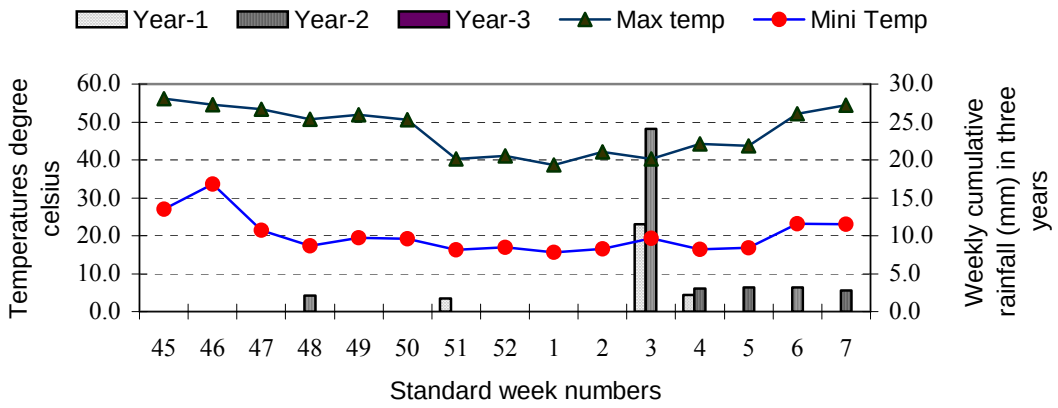


Figure 3. Weekly minimum and maximum temperatures (mean of three years) and weekly cumulative rainfall during the crop period of experimental years (2003-2005).

CONCLUSION

Potato seed growers empowered for the sustainable management of black scurf of potato and became able to diagnose the disease at different crop growth stages. Farmers selected the option of seed treatment dipping seed tubers in 2% boric acid concentration for 30 minutes prior to planting to manage the black scurf without soil sterilization with Formalin. However, best results were obtained with healthy seed planted in sterilized soil followed by seed treatment with 3% boric acid along with

soil sterilization. Farmers were found reluctant to soil fumigation with formaldehyde because it requires better technical skill otherwise it may be hazardous to user's health**.** Farmers selected biological control of black scurf through *T. harzianum* as second option. Results obtained from the experiment under naturally infected field conditions of Mainapokhar Bardiya, antagonistic fungus *T. harzianum* could be the environment friendly option for black scurf disease management. Effectiveness of such antagonistic fungus may be increased in the succeeding crops because of its multiplication in the soil.

ACKNOWLEDGEMENT

Member farmers of 'Kalika Potato Seed Production Group Mainapokhar' who participated in an on-spot black scurf disease management research programme are highly acknowledged for their active participation and collaboration. Authors are thankful to the chairperson of farmers group Mr Kamal Giri, for his effective coordination among the member farmers for conducting this study. Technicians Mr Hari Bahadur KC and Kesab Bahadur Chand are highly acknowledged for experiment management and data collection.

REFERENCES

Abada KA. 1994. Fungi causing damping off of sugar-beet and their biological control with *Trichoderma harzianum*. *Agriculture, Ecosystem & Environment* 5(3):333-337.

ABPSD. 2006. *Statistical information on Nepalese agriculture 2004/05 (2061/62)*. Agri-Business Promotion and Statistics Division, Ministry of agriculture and cooperatives, Singha Durbar, Kathmandu, Nepal. Pp….

Bains PS, HS Bennypaul, DR Lynch, LM Kawchuk and CA Schaupmeyer. 2002. Rhizoctonia disease of potatoes (*Rhizoctonia solani*): Fungicidal efficacy and cultivar susceptibility. *American Journal of Potato Research* 79(2): 99-106.

DPP. 2000. Rhizoctonia disease of potato. **In:** *Vegetable Disease Information Note No. 26*. Department of Plant Pathology, North Carolina State University.

Dutta BL and SC Gupta.1982. Efficacy of treatment of tubers in the control of potato black scurf. Potato in developing countries. **In:** Proceedings of International Seminar, 20-30 Nov. 1978. Pp. 333-338.

MARTIN TB AND PL ROBERT. 2005. EFFICACY OF SEVERAL POTENTIAL BIO-CONTROL ORGANISMS AGAINST *RHIZOCTONIA SOLANI* ON POTATO. *CROP PROTECTION* 21:939-950.

NPRP. 1994. The effects of different chemicals for the control of black scurf (*Rhizoctonia solani*) disease of potato. **In:** *Annual Report 2050/51 (1993/94)*. National Potato Research Programme,.NARC, Khumaltar, Lalitpur, Nepal. Pp. 36-37.

NPRP. 2005. Glasshouse activities for pre basic seed production. *Annual Report 2003/04 (2060/61)*. National Potato Research Programme, NARC, Khumaltar, Lalitpur, Nepal. Pp. 70.

Rai RP. 1984. Effect of formalin on soil-borne pathogens of potato. *Indian Phytopathology* 37:694-696.

Rolshausen PE and WD Gubler. 2005. Use of boron for the control of Eutypadiaback of grapevines. *Plant Disease* 89(7). Pp. 734-738.

Simons SA and CA Gilligan. 1997. Relationship between stem canker, stolon canker and black scurf (*Rhizoctonia solani* and yield of potato, *Solanum tuberosum*) under different agronomic conditions. *Plant Pathology* 46(5). Pp. 651-658.

Tanil AJ, Akai and T Aota. 1982. Chemical control of several diseases transmitted through seed tubers. Potato in developing countries. **In:** *Proceedings of International Seminar*, 20-30 Nov. 1978. Pp. 271-278.

Tsror L, R Barak and B Synch. 2001. Biological control of black scurf on potato under organic management. *Crop Protection* 20: 145-150.

Wicks TJ, B Morgan and B Hall. 1996. Influence of soil fumigation and seed tuber treatment on the control of *Rhizoctonia solani* on potatoes. *Australian Journal of Experimental Agriculture* 36(3):339-344.

Management of Leaf Blight of Garlic with Fungicides in Central Tarai of Nepal

Prem C.P. Chaurasia[1], Jang B. Prasad[2] and Aanandi Mandal[2]

[1] Sugarcane Research Programme, Jeetpur, Bara
[2] Reasonal Agrilcultural Research Station, Parwanipur, Bara, Nepal

ABSTRACT

Management of purple blotch in garlic was carried out in 2005/06 and 2006/07 at horticultural farm of Regional Agricultural Research Station, Parwanipur in Complete Randomized Block Design with three replications. First year's results indicated insignificant effect of fungicides on Percentage Disease Intensity (PDI) of purple blotch disease of garlic as number of sprays used seemed to be inadequate. Second year's result revealed that two sprays of Bavistin @ 0.2% had lowest PDI value but at par to other different number of sprays of different fungicides except no spray of fungicide. Bulb yield was highest in two sprays of Dithane M-45 @ 0.3% followed by two sprays of Krinoxyl @ 0.15%, three sprays of Blitox-50 @ 0.3%. Plant height was insignificant and highest bulb weight was found with three sprays of Blitox-50 followed by two sprays of Bavistin. Two year's combined results indicated that two sprays of Bavistin @ 0.2% had lowest PDI value and all others PDI values were at par except control. The highest bulb yield was given by two sprays of Dithane M-45 followed by three sprays of Bavistin. Plant height and bulb weight were insignificant. It can be recommended that purple blotch disease can be managed by spraying Bavistin @ 0.2% thrice at 15 days interval or any other tested fungicides to have less disease, higher bulb yield and more economic return.

Key words: *Alternaria porri*, disease, percentage disease intensity, purple blotch

INTRODUCTION

Garlic (*Allium sativum* L.) is the most important spice crop of Nepal. It is extensively grown by every household in their kitchen gardens on small scale. It is used as spice in every vegetable dishes by Nepalese communities. It has medicinal value also. Most of the diseases reported on onion (*Allium cepa L*) are common on garlic (Miller and Lacy 1996). Garlic is heavily attacked by purple blotch disease, which is caused by *Alternaria porri* (Ell.) Neergard. Purple blotch is characterized by appearance of whitish sunken lesions on succulent leaves. The lesions appear on leaf tip and spread later to lower parts. With time they enlarge, several of them coalesce to cover large patches and girdle the succulent leaf. Pandey et al (2002) tested many fungicides and plant extracts against purple blotch which is a common disease of onion and garlic and reported that all fungicides and leaf extracts significantly controlled disease but Indofil M-45 (Mancozeb) was found the best in respect to disease control. In vitro screening of fungicides revealed them to be highly fungitoxic to leaf blight (purple blotch) of onion. Mancozeb was the effective fungicide against purple blotch disease (Quadri et al 1982). Vijay and Rahman (2004) reported that four sprays of Mancozeb @ 0.3% with Monocrotophos @ 0.05% was the best treatment and recorded the least disease incidence and highest yield. This study was undertaken to find the most effective fungicide in managing purple blotch disease of garlic.

MATERIALS AND METHODS

The trial was carried out in Randomized Complete Block Design with three replications in 2005/06 and 2006/07. The plot size was 3.0- × 2.4-m. Row to row spacing was 15 cm and bulb to bulb spacing was 10 cm. Fertilizer were applied at the rate of 80:40:50 kg NPK/ha. Half of the nitrogen and all P and K were applied as basal at the time of planting and remaining half of nitrogen was

applied in two equal parts. First part of nitrogen was given on first irrigation just 30 days after planting and second part was given one month later. In both year the trial was planted on 13 November 2005 and 2006 and harvested on 30 March 2006 and 28 March 2007. Fungicides were applied on 24 Jan and 8 Feb 2006 in first year and 2 Dec 25, Dec 2006 and 4 Jan 2007 in second year respectively. In first year one and two sprays of each fungicide were applied. However in second year two and three sprays of each fungicide were given. Fungicides used were Dithane M-45 (Mancozeb) @ 0.3%, Bavistin (carbendazim) @ 0.2%, Blitox-50 (copper oxychloride) @ 0.3% and Krinoxyl (Metalaxyl + Mancozeb) @ 0.15%. The fungicides were started to apply when leaf tip started to become yellow. The fungicides were sprayed at 15 days interval. Disease was scored once just after a month of last spray of fungicides. A half meter quadrate was thrown in the centre of plot and garlic plants enclosed inside the quadrate were graded into different grades and Percentage Disease Intensity (PDI) was calculated using the scale and formula as under:

Scale:
1 = No disease symptoms on leaves
2 = 1-5% leaf area covered with lesions
3 = 6-20% leaf area covered with disease lesions
4 = 21-50% leaf area covered with disease lesions
5 = more than 50% leaf area covered with disease lesions

$$\text{Percentage Disease Intensity (PDI)} = \frac{100 \times \text{Grade for each plant} \times \text{Number of plants}}{\text{Maximum grade} \times \text{Number of plants observed}}$$

PDI values were transferred to Arcsine values according to Gomez and Gomez (1983).

RESULTS AND DISCUSSION

First year results did not indicate significant effect of any fungicides used on management of purple blotch of garlic as number of sprays of fungicides were inadequate to bring significant effects (Table 1). However, highest bulb yield was obtained with one spray of Dithane M-45 @ 0.3% and least PDI with two sprays of Blitox -50 @ 0.3%.

Table 1. Effect of sprays of different fungicides on PDI of purple blotch, average bulb weight and bulb yield in 2005/06

SN	Fungicide	Plant ht, cm	Average bulb weight, g	Bulb yield kg/ha	Normal value
1	Dithane M -45 @0.3% one spray	44.3	22.7	2210	19.0
2	Dithane M -45 @0.3% two sprays	47.8	21.2	1650	15.5
3	Bavistin @ 0.2% one spray	48.9	30.4	1958	14.3
4	Bavistin @ 0.2% two sprays	48.0	19.8	2034	15.7
5	Blitox-50 @ 0.3% one spray	47.7	22.2	1692	15.2
6	Blitox-50 @ 0.3% two sprays	51.0	24.9	1983	14.2
7	Krinoxyl @ 0.15% one spray	46.3	25.0	1983	17.2
8	Krinoxyl @ 0.15% two sprays	48. 6	25.5	1983	14.7
9	Control (no spray0	50.0	25.4	1388	22.8

The control had highest plant height and lowest bulb yield and average bulb weight was highest in case of one spray of Bavistin @ 0.2%. Vijay and Rahman (2004) found that four sprays of Mancozeb @ 0.3% was the most effective in controlling purple blotch of onion. In he current study, use of one and two sprays of different fungicides' results was inconclusive.

From second year's results, it was evident that two and three sprays of different fungicides had significant effect on PDI of purple blotch, bulb weight (kg/ha), average bulb weight and arcsine value of PDI (Table 2). The lowest PDI value was recorded in two sprays of Bavistin @ 0.2% followed by three sprays of Krinoxyl @ 0.15%, two sprays of Blitox-50 @ 0.3%, three sprays of

Bavistin, three sprays of Blitox-50 @ 0.3% and two sprays of Krinoxyl @ 0.15% and two spray of Dithane M-45 @ 0.3% respectively. Control had significantly higher PDI value. Plant heights were insignificant and highest average bulb weight was found in case of three sprays of Blitox-50 @ 0.3% followed by two sprays of Bavistin @ 0.2%. The highest bulb yield was found in two sprays of Mancozeb (Dithane M-45) followed by two sprays of Krinoxyl and three sprays of Blitox -50.

Table 2. Effect of two and three sprays of different fungicides on PDI, Arc sine value of PDI and other agronomic parameters 2006/07

SN	Fungicides	Plant ht, cm	Average bulb weight, g	Bulb yield, kg/ha	Percentage Disease Intensity (PDI)	
				kg/ha	Normal value	Arc sine value
1	Dithane M-45 two sprays @ 0.3%	40.9	27.2b	2328.7a	11.6 bc	19.8 bc
2	Dithane M-45 three sprays @ 0.3%	42.0	22.7e	1254.6fe	12.1 b	20.4 b
3	Bavistin two sprays @ 0.2%	47.3	30.4a	1505.3d	9.8 c	18.3c
4	Bavistin three sprays @ 0.2%	40.4	20.6f	1745.4cb	11.2 bc	19.4bc
5	Blitox -50 two sprays @ 0.3%	42.7	24.3d	1486.1d	11.1 bc	18.4 bc
6	Blitox-50 three sprays @ 0.3%	47.3	31.6a	1893.5b	11.4 bc	19.7 bc
7	Krinoxyl two sprays @ 0.15%	41.8	27.9b	1898.1b	11.4 bc	19.7 bc
8	Krinoxyl three sprays @ 0.15%	44.5	27.9b	1824.1b	10.7 bc	19.1 bc
9	No spray, control	46.3	26.6c	1388.6e	20.6 a	26.9 a
	CV, %		13.9	14.4	8.5 3	4.72

Figures marked with same letters are not significantly different at P = 0.05% as judged by DMRT.

Two year's pooled results indicated that three sprays of Bavistin @ 0.2%, three sprays of Blitox-50 @ 0.3% and three sprays of Krinoxyl @ 0.15% gave better control and were at par in respect to PDI followed by three sprays of Dithane M-45 @ 0.3%, two sprays of Bavistin @ 0.2% (Table 3). Highest bulb yield was obtained with two sprays of Dithane M-45 @ 0.3% and lowest bulb yield was given by control (no spray of fungicide).

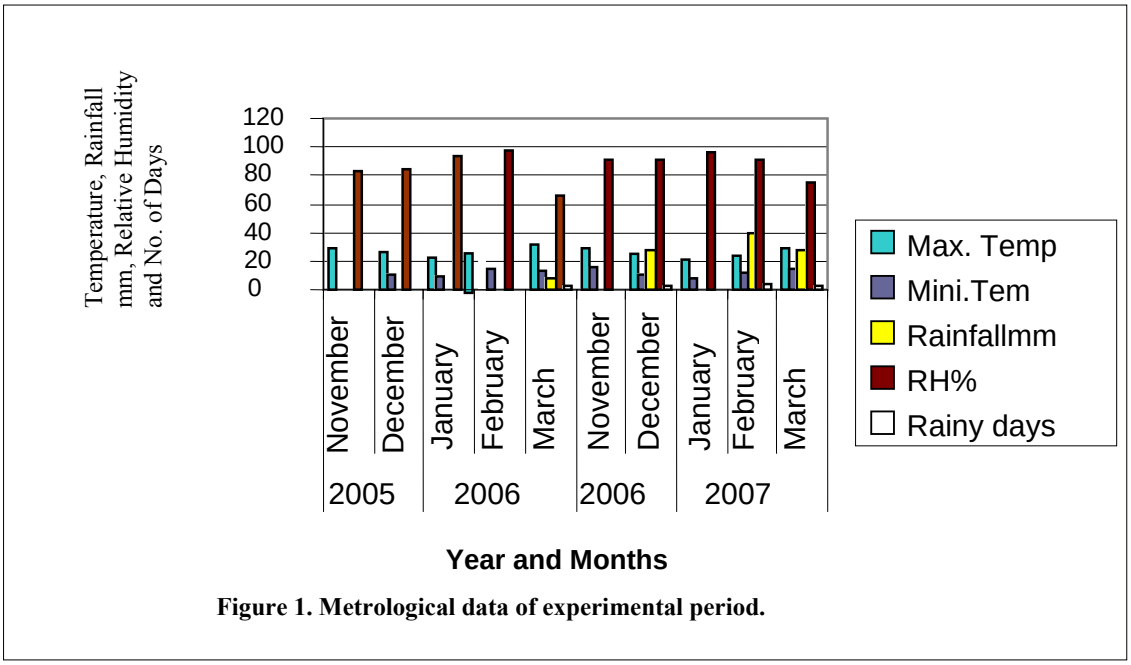


Figure 1. Metrological data of experimental period.

This contracdict with others' findings who had reported that four sprays of Dithane M-45 @ 0.3% was the most effective in controlling purple blotch of onion (Vijay and Rahman 2004, Panday et al 2002). However, we have not tested four sprays of any fungicides so effects of four sprays of fungicides were unknown. From above discussion, it is clear that purple blotch of garlic can be effectively managed by applying three sprays of either Bavistin @ 0.2% or Blitox-50 @ 0.3%, Krinoxyl @ 0.15% or Dithane M-45 @ 0.3%. Maximum, minimum temperature and relative

humidity were favorable for disease development in both the years (Figure 1) during experimental periods.

Table 3. Two years pooled effect of different number of sprays of different fungicides on PDI, Arc sine PDI value and agronomic parameters

SN	Fungicides	Plant ht, cm	Average bulb weight, g	Bulb yield, kg/ha	Percentage Disease Intensity (PDI)	
					Normal value	Arc sine value
1	Dithane M-45 two sprays	44.3	22.7	2210 a	19.0 b	25.4 b
2	Dithane M-45 three sprays	47.8	21.2	1650 f	15.5 c d	23.1 c d
3	Bavistin two sprays	48.9	30.4	1958 d	14.1d	21.8 d
4	Bavistin three sprays	48.0	19.8	2034 b	15.7 cd	23.0 c d
5	Blitox two sprays	47.7	22.2	1692 e	15.2 d	22.8 d
6	Blitox three sprays	51.0	24.9	1983 c	14.2 d	22.0 d
7	Krinoxyl two sprays	46.3	25.0	1990 c	17.2 c	24.2 b c
8	Krinoxyl three sprays	48.6	25.0	1986 c	14.7 d	22.1d
9	Control	50.0	25.0	1388 g	22.8 a	28.1 a
	CV, %		21.27	21.27	27.14	3.68

Figures marked with same letters are not significantly different at P = 0.05% as judged by DMRT.

REFERENCES

Gomez KA and AA Gomez. 1983. *Statistical procedures for agricultural research*. 2nd Edition. John Wiley and Sons, New York.

Miller ME and ML Lacy. 1996. Purple blotch. **In:** *Compendium of onion and garlic diseases* (HF Schvartz and SK Mohan, eds). American Phytopathological Society, St. Paul, Minnosta,55121, USA. Pp. 23-24.

Pandey PK, RS Rajput, R Lekh and RS Rathore. 2002. Management of purple blotch of onion through the foliar application of fungicides and plant extracts (Abs.). *J. Mycol. Pl. Pathol*. 32 (2):277.

Quadri SMH, KJ Srivastav, SR Bhonden, VB Pandey and PM Bhagchandani. 1982. *Pesticides* 16:11-16.

Vijay, M and MA Rahman, 2004. Efficacy of fungicides in the control of leaf blight disease of onion (*Allium cepa*). *J. Mycol. Pl. Pathol.* 34(2)654-655.

Integrated Disease Management of Tomato Late Blight[1]

Krishna K. Shrestha[1] and Richard Ashley[2]

[1] Plant Pathology Division, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal <krishnak85@hotmail.com>
[2] Department of Plant Science, University of Connecticut, USA

ABSTRACT

Tomato late blight caused by *Phytophthora infestans* (Mont.) de Bary is a serious disease of tomato and potato worldwide. The disease causes severe crop losses in the tomato growing regions of the world. Most of the cultivars grown in the world are reported to be susceptible to late blight. Systemic fungicides have been used widely in the past, but the disease has developed resistance over time. The present study was carried out to minimize fungicide use through integrated pest management. The bio-pesticides *Azadirachta indica* (Neem), *Artemisia vulgaris* (Mugwort) and *Trichoderma viride* were tested along with the fungicides Krilaxyl (metalaxyl 8% and mancozeb 64%) and Dithane M-45 (mancozeb 80%) in Nepal during 2000 and 2001. All three bio-pesticides were found more effective than the control in reducing development of the disease and they increased yield over the control by 17 to 41%. However, the fungicides, Krilaxyl and Dithane M-45 were observed even more effective. Two transplanting dates ie first and third week of July were tested against late blight in rainy season during 2000and 2001 and observed its effect on yields. Lower disease incidence and better yield were found in the second planting.

Key words: Dithane-M45, Krilaxyl, mugwort, Neem, *Phytophthora infestans*, *Trichoderma*

INTRODUCTION

Tomato late blight caused by *Phytophthora infestans* (Mont.) de Bary, is a destructive disease of tomato in many parts of the world. The disease also occurs commonly in potatoes, eggplant, nightshade and occasionally on peppers of the family, *Solanaceae* (Stevenson 1993). Wherever tomatoes are grown in tropics without excess use of fungicides, the disease is commonly present (Griffith et al 1995). The disease was reported having caused tomato and potato crops losses up to 100% (Fry 1999, Shrestha 1999, CIP 1999, Shrestha and Shrestha 1997, Pohronezny et al 1986, Sherf and Macnab 1986). In California, United States, late blight appears in all tomato growing areas particularly in sprinkler irrigated fields during prolonged periods of rain and /or fog with mild temperatures (Nunez and Voss 2001). An outbreak of tomato late blight occurred in Ontario, Canada in 1976 (Reyes et al 1977). Since 1990, severe outbreaks of late blight have been observed in commercial and home garden crops of potato and tomato in the United States and Canada (Rowe et al 2002).

At present, no tomato cultivars in the world are grown on a commercial scale without chemical protection from late blight disease. The interval between spraying and harvesting is usually 2 to 3 days in Nepal (SK Shrestha 2002, personal communication) and 0 to 5 days in the United States (R.

[1] The paper is based on PhD thesis of the first author submitted to University of Connecticut (UCONN), USA.

Ashley 2003, personal communication). The annual chemical application to tomato and potato crops is higher than any other food crops grown today (Niederhauser 1991). Such heavy use of pesticides could result in increased human health hazards and environmental pollution. Because of this, in recent years, cultural control and biological methods including botanical pesticides have been attracting considerable interest towards disease management (Upadhyay et al 1996).

The phenylamide systemic fungicide, metalaxyl, is highly effective in controlling late blight disease of potato and tomato. However, Goodwin et al (1996) reported that populations of *P. infestans* had developed resistance to this compound in different parts of the world. Neem (*Azadirachta indica* A. Juss) has shown insecticidal properties as well as antifungal activity (UCONN 2002). It is a perennial tree belonging to Meliaceae family and is grown in sub tropical and tropical regions. A botanical pesticide, azadirachtin, is derived in India from neem leaves and/or seeds and is now available in different formulations of commercial products. Several tests have indicated that it has considerable promise in controlling plant diseases (Ruskin 1992). Some reports have shown that it has reduced severity of diseases like late spot and rust of groundnut (Sachan 1990) and diseases of chilli (Maharishi 1993). Mugwort (*Artemisia vulgaris* L) an aromatic under shrub belongs to Compositae family. It grows wild in Nepal, United States and in many countries. The plant is reported having antiseptic, repellent and fumigant properties (Regmi and Karna 1988).

Trichoderma viride Pers. Ex Fr is a common saprophytic fungus, which can antagonize and also parasitize infections of plant pathogens like *Phytophthora, Pythium, Rhizoctonia* and *Sclerotium* (Wells 1988). The other species of the fungus, *T. harzianum* is also reported to have high potential as a bio-control agent of plant disease (Cook and Baker 1983).

Considering the growing importance of the late blight disease and the lack of information on integrated management, the present study was undertaken to develop integrated approaches to manage this disease. These approaches were cultural controls such as manipulation of date of sowing, choice of less susceptible cultivars, minimal use of fungicides and bio-control agents.

MATERIALS AND METHODS

Field Experiments

Field experiments were conducted during the tomato growing seasons of 2000 and 2001 at the research farm of the Plant Pathology Division, Khumaltar, Nepal. The experiments were laid out in a split- split plot design with three replications (Gomez and Gomez 1984). Main plots included 2 cultivars, Pusa Ruby (semi-determinate type) and CL 1131 (determinate type). Sub-plots had two dates of planting and sub-sub-plots had five treatments (Neem, Mugwort, *Trichoderma,* Krilaxyl and control) in 2000 and six treatments in 2001 in which Dithane M45 was included. Each sub-plot was 2- × 3-m with four rows. Each row was spaced 50 cm apart. The distance from plot to plot was 60 cm and from plant to plant 30 cm. The soil was sandy loam fertilized with a basal dose of NPK at the rate of 45-60-60 kg/ha during land preparation. A second dose of nitrogen, 45 kg/ha N, was applied as a top dressing 30 days after transplanting.

Two commercial susceptible cultivars of tomato, Pusa Ruby and CL 1131 as mentioned above were used in the experiments. Seeds of each cultivar were sown in the nursery beds on the first and third week of June and transplanted into the field on the first and the third week of July during both years. Ten plants per row and 40 plants per plot were maintained. Earthing up of the plants was done forty-five days after transplanting. Common cultural practices were followed during the cropping period. Besides late blight other diseases, insects and weeds were also monitored inspecting plants periodically in the fields.

Fresh leaves of neem and mugwort were collected and weighed into 100 g batches. Each batch was then macerated with 100 ml of water 1:1 (w/v) and an extract of aqueous stock solution was

obtained by straining through two layers of cheesecloth. The stock solution was diluted to 10% (10 ml stock solution + 90 ml water) before spraying in the field.

The spray schedule commenced soon after the appearance of disease in the plots. In the first crop season, the disease was observed on 12 July 2000 in the first planting and on 15 August 2000 in the second and the first spray was applied on 14 July and 17 August 2000, respectively. In the next season, the disease was observed on 7 July 2001 in the first planting and on 20 August in the second and the first spray was done on 9 July and 22 August 2001, respectively. Subsequent sprays were performed at the interval of 7 days. Altogether, five sprays were applied during each crop period.

In-vitro test

An in-vitro test was carried out in the laboratory of Department of Plant Science, University of Connecticut (UCONN) using neem (5% stock solution), mugwort (5% stock solution), Krilaxyl (0.75g/L) and Dithane M-45 (1.5g/L), to determine their effects on the development of mycelium of *P. infestans*. For this test, Jharrasi B isolate of *P. infestans* from Nepal was used. *Trichoderma* was test in dual culture with *P. infestans*.

Preparation of Rye A Agar (RAA) amended medium

Rye A Agar medium was prepared following protocol (Caten and Jinks 1968). The final volume of media was adjusted to 1 liter with de-ionized water and poured into 4 conical flasks each containing 250 ml. The flasks were autoclaved at 121^{o}C with 15psi for 20 minutes and allowed cool down to about 50-55^{o}C. Each botanical/ chemical pesticide, neem extract (20 ml), mugwort extract (20 ml), Krilaxyl (188 mg), Dithane M-45 (375 mg) was added to each flask and mixed thoroughly. The amended medium was then poured about 15 ml in each 90- × 15-mm plastic petri plates and the control set was also prepared.

Experiment setup

The experiment was set up with five plates for each of the five treatments and replicated three times. Uniform sized agar discs (5 mm) from actively growing *P. infestans* culture were cut from a 10-12 days old colony. One disc was put at the center of each plate. After inoculation, the plates were sealed with parafilm and incubated at 18-20^{o}C in darkness for two weeks. The colony diameter of each plate was measured on every 4th day from the date of inoculation for 4 times. The average diameter for each treatment was calculated.

Harvest

The first harvest was done in the first and third week of September in both years. Subsequently, matured fruits were harvested regularly and weighed. The total yield per plot was calculated at the end of the experiment.

DISEASE ASSESSMENT

The first disease scoring was done on 13 July and 16 August in 2000 and 8 July and 21 August in 2001 for the first and second dates of sowing, respectively. The disease was assessed visually on leaves, stems and fruits of all 40 plants of each plot following scale 0-6 (0 = no blight symptom and 6 = 90-100% plant damage/dead, AVRDC 1996). Subsequent assessments were done on every 7th day until total blight infestation had occurred in the control plot. In total, 6 observations were taken in each plot.

The percentage of disease for each plot was calculated using the formula of Townsend and Heuberger (1943):

$$P = \frac{\sum (n \times v)}{6N} \times 100$$

P = Percentage of disease
v = Numerical value of each index
6 = Total number of disease severity index
n = Number of plants in each index
N = Total number of plants

Meteorological data

Daily air temperature, precipitation and relative humidity were collected from the meteorological station located at the Khumaltar farm close to the experimental field.

Statistical analysis

Analysis of variance (ANOVA) was performed using the Statistical Analysis System (SAS 1985) and MSTATC statistical package. The least significant difference (LSD) range test ($P = 0.05$) was used for mean separation of late blight management treatments. Results from the field experiments are presented as highly significant ($P < 0.01$), significant ($P < 0.05$) or not significant (ns). None of the data was transformed.

RESULTS

Field experiments

At Khumaltar, in both years, the incidence of late blight disease caused by *P. infestans* in the experimental plots was high as expected for untreated tomatoes. The plants were naturally infected with late blight. The weather data showed that the monthly mean maximum temperature ranged from 26.3-28.3^{o}C, minimum temperature from 13-20.8^{o}C, relative humidity 78.4-87.4% and total rainfall 1.8-316 mm during June to October 2000. These observed weather conditions were found to be quite favorable for the development of late blight disease in both seasons.

During 2000, the symptoms of late blight on the plants from the first and second date of planting were first observed on 12 July and 15 August respectively. During 2001, the symptoms appeared on 7 July and 20 August for the first and second plantings, respectively. In the beginning, water soaked lesions appeared on the lowers leaves, which progressed further, showing brownish black lesions with fluffy, white, mycelial growth under the lesions of infected leaves. Symptoms were quite prominent on leaves, stems and green fruits in the control plots.

The disease spread rapidly in a spatial pattern and its intensity increased over time. A vertical gradient of disease infection developed in the plants. As the disease progressed, lesions appeared on the middle and upper leaves. There were distinctly visible effects of fungicides and bio-pesticides compared to control in both years. Disease progress was much higher in the control plots than in the treated ones. All plants in the control plots were blighted and defoliated within three-four weeks of the disease's appearance. In the treated plots, the disease progress in the plants was much slower and the severity of disease was much less than in control plots (Figures 1 and 2). The leaves remained green and attached to the plants for a longer period until senescence.

Statistical analysis indicated that the results were highly significant at $P < 0.001$. Table 1 and 2 showed the mean disease index, percent of mean disease severity, yield of tomato fruit and percent increase of tomato yield over controls for 2000 and 2001 respectively. It was noted that both fungicides and bio-pesticides provided better control of the disease in the treated plots than in the control plots. Krilaxyl was most effective in controlling late blight and was significantly different from other treatments at $P = 0.05$. In 2001, Dithane M-45 was included and it was also effective against the disease, and significantly different from other treatments except Krilaxyl. All three bio-pesticides (Neem, *Artemisia* and *T. viride*) were significantly better than controls as to the severity of disease in 2000 at $P = 0.05$. However, in 2001, *Artemisia* was at par with the control and also with other two bio-pesticides (Tables 1 and 2). There were no significant differences in effectiveness among the bio-pesticides. The effect of different treatments was supported by the results obtained from the in-vitro test (Table 3).

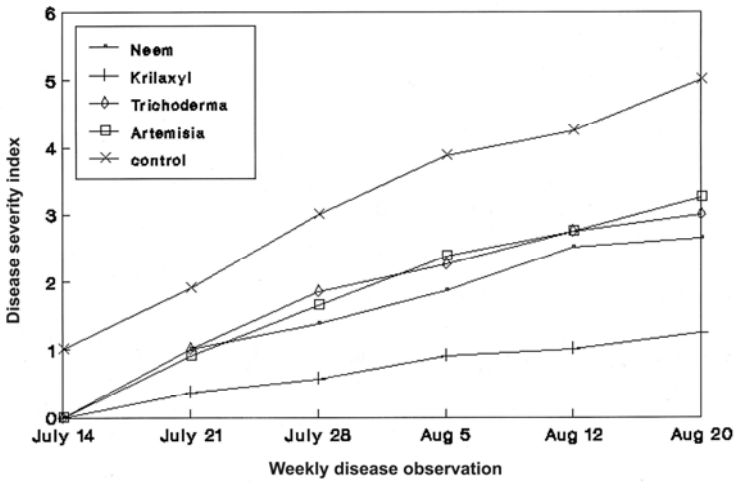


Figure 1. Effect of treatment on late blight disease progress in the first planting tomato cv. Pusa Ruby, 2000.

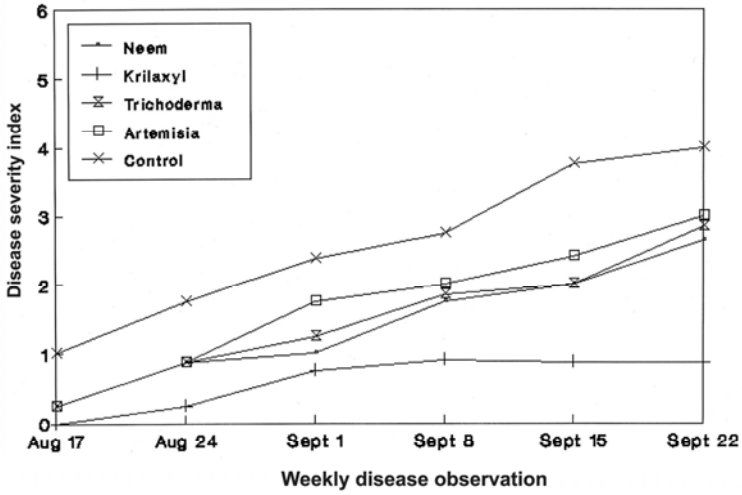


Figure 2. Effect of treatment on late blight disease progress in the second planting tomato cv. Pusa Ruby, 2000.

Table 1. Mean late blight disease index, mean disease severity, tomato fruit yield and yield increase over control at Khumaltar, Nepal, 2000

SN	Treatment	Mean disease severity index (DSI)	Mean disease severity, %	Tomato yield, t/ha	Yield increase over control, %
1	Neem	3.39b	56.53b	13.84b	17.49
2	Krilaxyl	1.29c	21.46c	26.61a	125.89
3	Trichoderma	3.49b	58.11b	14.40b	22.24
4	Artemisia	3.48b	58.00b	14.11b	19.78
5	Control	4.00a	66.61a	11.78c	-
	F-test	**	**	**	
	LSD at 0.05	0.47	7.90	1.86	
	CV, %	18.28	18.24	13.89	

Means in a column with different letters are significantly different at 0.05 level.

Table 2. Mean late blight disease index, mean disease severity, tomato fruit yield and yield increase over control at Khumaltar, Nepal, 2001

SN	Treatment	Mean disease severity index (DSI)	Mean disease severity, %	Tomato yield, t/ha	Yield increase over control, %
1	Neem	3.89b*	64.83b	12.12c	36.49
2	Krilaxyl	1.16d	19.38d	24.95a	180.97
3	Trichoderma	4.05b	67. 50b	12.57c	41.55
4	Artemisia	4.01b	66.83b	11.20cd	26.13
5	Dithane M-45	1.79c	29.81c	19.28b	117.12
6	Control	4.51a	75.17a	8.88d	-
	F-test	**	**	**	
	LSD at 0.05	0.318	7.15	2.77	
	CV, %	11.62	15. 90	22. 65	

Means in a column with different letters are significantly different at 0.05 level.

Table 3. Mean diameter of growth of *P. infestans* in amended rye A agar medium against pesticides and bio-pesticides in-vitro test, 2001

SN	Treatment	Jan 31 diameter, cm	Feb 4 diameter, cm	Feb 8 diameter, cm	Feb 12 diameter, cm	Average diameter, cm	T-test between treatment and control
1	Neem	0.50	2.17	4.17	4.87	2.93	3.826*
2	Krilaxyl	0.10	0.30	0.50	0.90	0.450	3.303*
3	Artemisia	0.50	2.40	4.48	5.42	3.20	4.256*
4	Dithane M-45	0.10	0.50	0.80	1.20	0.650	3.325*
5	Control	1.00	3.87	6.25	7.50	4.655	-

**, Significant at P 0.05.*

Tables 1 and 2 also show that the tomato yield was much higher (11-27 mt/ha) in the treated plots and significantly different from the yield in control plots (9-12 mt/ha) at P=0.05. The average increase in yield over controls was 46.4% in 2000 and 80.5% in 2001. Within the treatments, the total yield in Krilaxyl treated plots was much higher (25-27 mt/ha) and significantly different from other treatments at P = 0.05 in both years. This was followed by Dithane M-45 (19 t/ha) and bio-pesticides (11-14 t/ha). The yield among the bio-pesticides was not significantly different. The relationship between fruit yield and disease severity was calculated by using the Pearson Product Moment correlation efficient and a linear regression model. A highly significant negative correlation (r = -0.641) and (r = -0.820) between disease severity and fruit yield was observed in 2000 and 2001 respectively. Based on regression analysis, for an increase of one disease severity index of late blight, yield loss incurred was about 4.0 t/ha in both years (Figures 3 and 4).

With in-vitro test, considerable variation in the growth rate of the *P. infestans* was observed. No mycelial growth was observed on the medium containing Krilaxyl and Dithane M-45. The colony diameter on twelfth day of inoculation was 4.87 cm in Neem and 5.42 cm in *Artemisia,* which were less than 7.5 cm in the control (Table 3).

In the case of dual culture of *Trichoderma and P. infestans,* the colony diameter of *T. viride* was comparatively greater than *P. infestans.* After one week of inoculation, *T. viride* stopped and overlapped the colony of *P. infestans*. It indicates that *T. viride* inhibits the growth of *P. infestans* (not shown in the Table 3*)*.

In both seasons, measurements of disease severity in the cultivar, Pusa Ruby, appeared slightly higher than in CL1131, but statistically there were no significant differences in the disease severity and tomato yield between the cultivars at P = 0.05 (Table 4). The incidence of late blight disease and the tomato yield from the two dates of planting were analyzed. The mean disease severity index in the first date of planting was higher and significantly different from the second date in both years. The effect of disease was negative on the yield. Consequently, the mean yield in the second date of planting was higher and significantly different from the first date in the both years at P = 0.05 (Table 5).

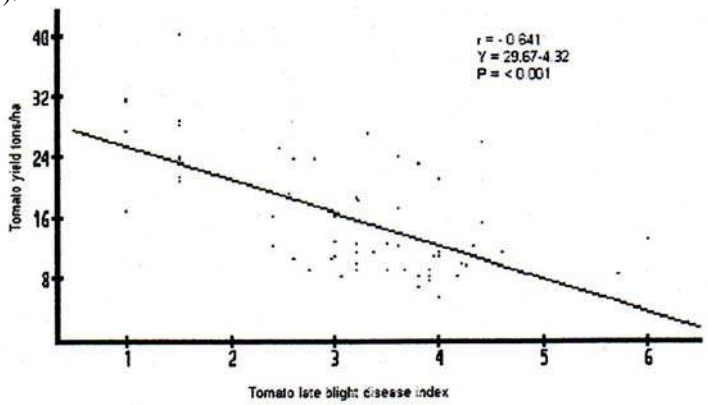


Fig.3. Relationship between tomato fruit yield and late blight disease severity, 2000.

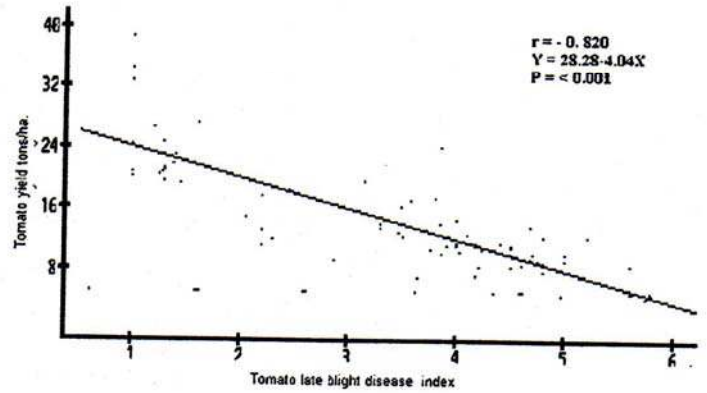


Fig.4. Relationship between tomato fruit yield and late blight disease severity, 2001

Table 4. Separation of means between tomato cultivars against ate blight severity and tomato yield in the experimental years, 2000 and 2001

SN	Cultivars	2000		2001	
		Mean disease severity index	Mean tomato yield, t/ha	Mean disease severity index	Mean tomato yield, t/ha
1	Pusa Ruby	3.22a*	17.91a	3.34a	15.80a
2	CL1131	3.03a	14.38a	3.31a	13.89a
	LSD at 0.05	0.8724	4.373	0.4636	2.54

**, Means in a column with same letters are not significantly different at 0.05 level.*

Table 5. Separation of means between the dates of tomato sowing against ate blight severity and tomato yield in the experimental years, 2000 and 2001

SN	Date of sowing	2000		2001	
		Mean disease severity index	Mean tomato yield, t/ha	Mean disease severity index	Mean tomato yield, t/ha
1	First	3.47a*	12.47b	3.68a	12.57b
2	Second	2.79b	19.82a	2.96b	17.12a
	LSD at 0.05	0.365	4.547	0.464	2.540

**, Means in a column with different letters are significantly different at 0.05 level.*

DISCUSSION

Tomato late blight disease (*P. infestans*) has become one of the most important constraints for successful cultivation of tomato in Nepal. In recent years, the frequency and severity of late blight epidemics in tomato crops has increased in tomato growing countries. Most commercial tomato cultivars are found to be susceptible to late blight. As a result, growers have been intensively using various chemical fungicides to control the disease. Today, it is environmentally, economically and scientifically unacceptable to rely primarily on fungicides for disease control. To minimize the use of fungicides against late blight disease, attempts are being made to develop integrated approaches to disease management. In the present study, different bio-pesticides extracted from Neem, Mugwort and *Trichoderma* were tested along with the chemical fungicides, Krilaxyl and Dithane M-45. Although these bio-pesticides were less effective than chemicals but they were found potential to provide some control of the pathogen. With applications of these bio-pesticides, yields were increased by 17 to 41% over controls. So far, there are apparently no other reports on these bio-pesticides being used in controlling late blight disease on tomato. Further testing of these bio-pesticides is needed to investigate the effect of concentration and ecological regions on their ability to control the pathogen. As these bio-pesticides become commercially available, their application in the field will be less expensive and more environmentally sound. Besides, these bio-pesticides may help to reduce application of some chemical pesticides.

Although metalaxyl is quite effective against late blight disease of tomato, the pathogen, *P. infestans* may soon develop resistance to this compound if it is solely used. There are some reports on using metalaxyl in combination with other protective fungicides to avoid the risk of developing resistance by pathogens. Gisi and Cohen (1996) reported that use of fungicide mixtures containing phenylamide compound and other protective fungicides are still an effective strategy for control of late blight of potato and tomato. Therefore in this experiment, Krilaxyl, a mixture of metalaxyl and

mancozeb was used as one of the treatments, and found quite effective. This result was also supported by the report of Ferro (1999) who found significant reduction of disease intensity with three or more applications of metalaxyl plus manzate (Ridomill Gold MZ) in the field.

In Nepal, Krilaxyl and other fungicides containing similar ingredients have been recently introduced. Although they are effective against late blight, they are quite expensive for the common farmers. The protective fungicide, Dithane M-45 was also found effective against tomato late blight. Therefore, if one of the tested bio-pesticides and a chemical fungicide are sprayed at least 2 times each during the cropping period, the disease may be reduced significantly. Doing so, it would help to minimize use of chemical fungicides in the field. Moreover, it would help reduce the production costs and environment pollution. However, more experiments are needed to establish the optimal number of bio-pesticides and fungicides applications with their dosage.

A second integrated approach is the manipulation of cropping patterns that has been developed to manage late blight disease. The results of this present study show that if the transplanting time is shifted 3 weeks latter than normal in rainy season, the severity of disease is significantly reduced. The reasons are probably due to less rainfall and more bright sunny days. The production and quality of tomato fruits from the latter plantings were also observed better than in the normal period. Statistically, susceptibility of Pusa Ruby and CL1131 were not different. However, the degree of susceptibility of Pusa Ruby was apparently slightly higher than in CL1131. Therefore, if the farmers like to use these cultivars for commercial cultivation, they should be advised to apply bio-pesticides to minimize the use of chemical pesticides.

In conclusion, late blight disease can be managed by rational using of bio-pesticides and fungicides. The bio-pesticides can help to reduce use of fungicides and the environmental pollution. In the rainy season, if the cropping period of tomato is shifted from early July to late July in the mid hills of Nepal, the disease intensity is reduced and also fruit yield is increased.

ACKNOWLEDGEMENTS

We would like to express our sincere appreciation to the members of Graduate Committee, Dr Sussane Von Bodman and Dr Ana Legrand, Plant Science Department, University of Connecticut for their encouragement, fruitful criticism, technical guidance and valuable suggestions. We are highly grateful to Mr Dhruva Joshi, former Executive Director, Nepal Agricultural Research Council (NARC) for his support and encouragement. We would also like to thank the faculty colleagues, staffs and field workers of the Department of Plant Science, University of Connecticut and Plant Pathology Division. NARC, Nepal for their sincere support and help. Our special thanks goes to Dr Sundar K. Shrestha, former Head of Plant Pathology Division, NARC for the permission to the first author to conduct field and laboratory research in Nepal. The first author is especially grateful to Dr Shrestha for his constant challenges and thoroughness while reviewing this paper.

REFERENCES

Caten CE and JL Jinks. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Canadian Journal of Botany* 46:329-348.

CIP. 1999. Integrated control of late blight. International Potato Center (CIP). http://cipotato.org/blight/lblight.htm.

Cook RJ and KF Baker. 1983. The nature and practice of biological control of plant pathogens. The American Phytopathological Society, Minnesota.

Ferro DN. 1999. Disease control. Tomato Outdoor. 1998-1999 New England Vegetable Management Guide.

Fry WE. 1999. Potatoes, once again under fungal Attack. http://ars.usda.gov/s/pr/potato0597.htm.

Gisi U and Y Cohen. 1996. Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. *Annu. Rev. of Phytopathol* 34:549-572.

Gomez KA and AA Gomez. 1984. *Statistical procedures for agricultural research*. Second Edition. Wiley Interscience Publication, John Wiley and Sons, IRRI, Philippines.

Goodwin SB, LS Sujkowski and WE Fry. 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and Western Canada. *Phytopathology* 86(7):793-800.

Griffith GW, R Snell and DS Shaw. 1995. Late blight (*Phytophthora infestans*) on tomato in the tropics. *Mycologist* 9:87-89.

Maharishi RP. 1993. Management of chilli (*C. annuum* L.) diseases by neem based preparation. **In:** *Proceedings of World Neem Conferences*, India. Pp. 809-811.

MSTATC. 1991. Microcomputer statistical program, experimental design, data management and data analysis. Michigan State University, U.S.A.

Niederhauser JS. 1991. *Phytophthora infestans*: The Mexican connection. **In:** *Phytophthora* (JA Lucas, RC Shattock, DS Shaw and LR Cooke, eds). Cambridge University Press, England.

Nunez J and R Voss. 2001. Late blight. Vegetable Information, University of California, Vegetable Research and Information Center. *http://vric.ucdavis.edu/veginfo/topics/disease/lateblight.html*.

Pohronezny K, VH Wadill, DJ Schuster and RM Sonoda. 1986. Integrated pest management for florida tomatoes. *Plant Dis.* 70:96-103.

Regmi PP and PL Karna. 1988. Weeds and other plants of pesticidal values in Nepal. National Conference on Science and Technology, Kathmandu, Nepal.

Reyes AA, JG Metcalf, JT Warner and LW Matheson. 1977. Outbreak of tomato late blight in Ontario. Canadian Plant Disease Survey. *CAB Abstracts* 57:13.

Rowe RC, SA Miller and RM Riedel. 2002. Late blight of potato and tomato. Ohio State University Extension Fact Sheet. *http://ohioline.osu.edu/hyg-fact / 3000 / 3102.html*.

Ruskin FR (ed). 1992. Neem: A tree for solving global problems. Report of an Ad Hoc Panel of the Board on Science and Technology for International Development. BOSTID. National Academy Press, Washington, D.C.

Sachan GC. 1990. Pest management through neem products. *Indian Farmer's Digest* 22:8.

SAS. 1985. SAS/STAT User's Guide vol. I, Fourth Edition, Cary, NC.

Sherf AF and AA Macnab. 1986. Vegetable diseases and their control. A Wiley Inter Science Publication, John Wiley and Sons, New York.

Shrestha SK and K Shrestha. 1997. An epidemics of late blight of tomato. *Nepalese J. of Agriculture* 18-29:141-144.

Shrestha SK. 1999. Status of ate blight of potato and distribution of mating types with genetic diversity in Nepal. **In:** *Proceedings of the Global Initiative on Late Blight (GILB) Conference*, 18-19 March, Ecuador.

Stevenson WR. 1993. Management of early blight and late blight. *Plant Health Management Series* (RC Rowe, ed). The American Phytopathological Society.

Townsend GR and JW Heuberger. 1943. Methods for estimating losses caused by diseases in fungicide experiments. *Plant Dis. Rep.* 27:340-343.

UCONN. 2002. Ignoring labels on organic products can create problems. IPM. University of Connecticut (UCONN). *http://www.hort.uconnedu/ipm/general/htms/orgprod.htm*.

Upadhyay RK, KG Mukerji, BP Chamola and OP Dubey. 1996. Integrated pest management: Concepts and prospects. **In:** *Integrated pest and disease management*. APH Publishing Corporation, New Delhi, India.

Wells HD. 1988. *Trichoderma* as biocontrol agent. **In:** *Bio-control of plant diseases* Vol I. (KG Mukerji and KL Garg, eds). CRC Press Inc.

Genetic and Non-Genetic Factors Affecting Reproductive Traits of Pakhribas Pig in Nepal

Shreeram P. Neopane

Animal Breeding Division, Khumaltar, PO Box 1950, Kathmandu <spneopane04@yahoo.com>

ABSTRACT

Pakhribas pig is a black colour pig and is highly preferred by the people in the Eastern hills of Nepal. This breed was developed in Nepal by three-way crossing of exotic breeds (Saddle back, Fayuen and Tamworth) at Agricultural Research Station (ARS), Pakhribas, the then Pakhribas Agricultural Centre (PAC). The data of 348 pigs, born over a period of fifteen years (1990 to 2004) were used to study the effect of non-genetic factors on reproductive traits and estimate their genetic parameters. The findings revealed that the overall gestation length and farrowing intervals were 113.7 ± 0.12 days and 182.5 ± 2.2 days, respectively. Season of birth and parity of dams were not important sources of variation for reproductive traits, where as year affected these traits significantly. Heritability estimates of gestation length was low (0.02 ± 0.094), but heritability for farrowing interval was moderate (0.14 ± 0.147) suggesting selection based on farrowing interval would bring the improvement in the trait.

Key words: Genetic and non-genetic parameters, hills of Nepal, Pakhribas pig, reproductive traits

INTRODUCTION

Pakhribas pig is a result of three-way crossing of exotic breeds (Saddle back, Fayuen and Tamworth) developed at Agricultural Research Station (ARS), Pakhribas, the then Pakhribas Agricultural Centre (PAC) (Oli 1986, Gatenby et al 1990, Aryal et al 1992). The breed is very popular in the eastern hills of Nepal because of its black colour and a good litter size at birth and weaning (Gatenby et al 1990). For religious purposes, black colour is preferred over other colours in the eastern hills. It is medium in size and can be maintained by the farmers in the hills.

The performance records indicate that they are good in productive characters including the reproductive parameters. A study carried out at On-farm situation aiming to compare the productivity of Pakhribas pigs with the local and its crossbred with Pakhribas pigs showed that the Pakhribas pigs produced 90% more meat than the local and 60% than its crossbreeds at the farmers' feeding and husbandry system (Oli 1986). This indicated that Pakhribas pigs are more productive and well suited to local environments.

Information on genetic and non-genetic parameters of litter traits for Pakhribas pigs is available (Neopane 2005). However, information on the non-genetic and genetic factors affecting reproductive traits is not available for Pakhribas pigs. The study was undertaken to estimate the non-genetic factors affecting reproductive traits and estimate their genetic parameters. In order to accomplish the study, a retrospective study was done and for this data obtained over fifteen years were analysed.

MATERIALS AND METHODS

The study was conducted at Pakhribas Agricultural Centre (PAC), Dhankuta district, in the Eastern mid hills of Nepal. The area was located at an altitude of 1740 m above sea level. The geographical location is 27^o 17' N and 87^o 17' E.

Records of pigs born from sixteen sows and four boars were used for the study. Pigs with piglets were kept at separate farrowing pen. Piglets were provided heat by electric bulbs. In farrowing pen rice straw was kept in order to make bed for the mother and also to maintain the temperature. Piglets were weaned at 6 weeks of age and at this age they were distributed to the farmers.

Data recorded over fifteen years (1990 to 2004) for reproductive traits of Pakhribas pigs at the centre were analysed to estimate genetic and non-genetic factors affecting the traits. For this least-squares analysis techniques based on unequal subclass (Harvey 1990) were performed using the following model;

$$Y_{ijk} = u + a_i + b_j + c_k + e_{ijkl}$$

Where , μ is overall mean
a_i is the effect of i_{th} parity of dam (i = 1, 2,…..14)
b_j is the effect of j_{th} season of birth (j = 1, 2, 3)
c_k is the effect of k_{th} year of birth (k = 1, 2,…..15)
e_{ijkl} is a random element assumed to be normally and independently distributed

An overall analysis, ignoring sires and dams, was used first to examine environmental effects. Then sires and dams were included in the model as random effects to estimate the genetic components of variance and to eliminate non-significant fixed effects (Harvey 1990).

RESULTS AND DISCUSSION

Non-genetic factors

Least-square means of reproductive traits are presented in Table 1. The least square mean of farrowing interval (FI) in the herd was 182.5 ± 2.2 days. This value corresponds to earlier reports for Pakhribas pigs (Aryal et al 1992, Shrestha 2000). The figure was slightly lower than the average records of 191 days by Dhaubdel and Pokharel (1997) using the three genotypes (Landrace, Yorkshire and their crosses) in Khumaltar, Kathmandu, Nepal. A higher value of farrowing interval was also reported for Landrace (196 days) and Yorkshire (206 days) in Kathmandu, Nepal (SARP 1992). These indicated that Pakhribas pigs are better than exotic breeds in terms of farrowing intervals.

The least square mean of gestation length (GL) in the herd was 113.7 ± 0.12 days. The value obtained was close to the records reported by Shrestha (2000) for Pakhribas pigs. This value also corresponds to the value reported by Fahmi and Bernard (1972) for European breeds of pigs. The value is well in range within values reported in the literature.

Parity of dam and season of birth were not important source of variation for reproductive traits (Farrowing interval and Gestation length). This suggested that the Pakhribas pigs can be reared up to 14th parity without any decline productivity level. Year was however, significant source of variation for farrowing interval ($P < 0.05$) and gestation length ($P < 0.001$). The probable reason for significant effect of year on farrowing interval was better environment (mild climate, less parasitic burden, less diseases, etc) in some years than others. Year itself is not a good environmental or non-genetic factors for any production traits (farrowing interval). However, its effect has to be corrected before heritability estimation of the trait is made.

Table 1. Least-squares means of reproductive traits (days) of Pakhribas pigs for different non-genetic factors

Factors	Farrowing interval			Gestation length		
	N	Mean	SE	N	Mean	SE
Overall	307	182.5	2.2	348	113.7	0.12
Parity of dams						
1	-	-	-	40	113.3	0.30
2	42	179.9	5.1	42	113.9	0.32
3	32	179.1	5.6	32	112.8	0.34
4	30	177.2	5.7	30	113.8	0.34
5	28	185.6	5.9	28	113.9	0.36
6	25	171.1	6.3	25	113.3	0.39
7	23	177.4	6.2	23	113.5	0.38
8	22	184.0	6.3	22	113.9	0.39
9	20	181.9	6.8	20	114.3	0.39
10	19	179.3	6.8	20	113.9	0.42
11	18	183.5	7.3	18	113.8	0.45
12	17	182.6	7.4	17	113.8	0.46
13	16	203.4	8.1	16	114.4	0.50
14	15	187.8	7.9	15	113.8	0.49
F-test		ns			ns	
Season of birth						
Early dry (Dec-Feb)	65	185.9	4.4	75	113.5	0.25
Late dry (Mar-May)	91	177.4	3.4	105	113.6	0.19
Wet (Jun-Nov)	151	184.2	2.6	168	114.1	0.15
F-test		ns			ns	
Year of birth						
1990	12	174.5	12.9	15	115.1	0.50
1991	13	182.9	8.1	14	113.6	0.49
1992	13	190.1	8.0	19	115.2	0.42
1993	15	198.5	7.6	15	113.9	0.47
1994	16	187.9	7.3	20	113.3	0.41
1995	22	186.3	6.4	25	113.1	0.38
1996	26	183.6	5.8	29	113.3	0.35
1997	28	193.5	5.6	30	113.9	0.34
1998	32	174.8	5.1	32	112.3	0.32
1999	30	166.9	5.4	30	113.1	0.34
2000	21	169.5	6.3	27	112.8	0.35
2001	22	186.6	6.2	24	113.0	0.37
2002	21	175.8	6.3	25	114.7	0.36
2003	19	184.6	8.2	23	114.8	0.46
2004	17	182.5	7.8	20	114.6	0.44
F-test		*			***	

N, Number of records. SE, Standard errors.

Genetic factors

Heritability estimates of reproductive traits are presented in table 2. Heritability estimates of gestation length was low (0.02 ± 0.094) but estimates of heritability for farrowing interval was moderate (0.14 ± 0.147) suggesting selection based on farrowing interval would bring the improvement. Crump et al (1997) reported moderate estimates of heritability for gestation length. Estimates of heritability for farrowing interval from the study are close to several research reports (Johansson 1981, Rydhmer et al 1995, Tholen et al 1996, Crump et al 1997). However, some other authors have reported lower estimates of heritability for farrowing interval ranging from 0.06 to 0.09 (Johansson and Kennedy 1985, Adamec and Johnson 1997, Oh et al 2005). Haley et al (1988) reviewed the estimates for farrowing intervals within a range of 0.04 to 0.22.

Table 2. Heritability estimates of reproductive traits of Pakhribas pigs

Trait	Heritability	Standard error
Farrowing interval	0.14	0.147
Gestation length	0.02	0.094

Low to moderate estimates of heritability indicated that some genetic variance is available for these traits and hence selection based on the trait (farrowing interval) may bring genetic improvement and consequently improve productivity by reducing farrowing interval.

CONCLUSION

Non-significant effect of parity on farrowing interval (1-14 parity) suggested that the pigs may be reared up to 14 parity without declined productivity level in terms of farrowing intervals. Moderate estimates of heritability for farrowing interval indicated that the dams if selected based on the shorter farrowing interval may improve the traits.

REFERENCES

Adamec V and RK Johnson. 1997. Genetic analysis of rebreeding intervals, litter traits and production traits in sows of the National Czech nucleus. *Livest. Pro. Sci.* 48:13-22.

Aryal IK, NP Shrestha, BB Kshatri, PP Rai and MP Shrestha. 1992. Some characteristics of Pakhribas pigs. *Veterinary Review* 7(10):21-23. Pakhribas Agricultural Centre, Dhankuta, Nepal.

Crump RE, CS Haley, R Thompson and J Mercer. 1997. Individual animal model estimates of genetic parameters for reproduction traits of landrace pig performance tested in a commercial nucleus herd. *Animal Sciences* 65:285-290.

Dhaubdel TS and PK Pokharel. 1997. Piglet mortality: A big economic penalty to the pig farmers. **In:** *Proceedings of the II nd National Workshop on Livestock and Fisheries Research*, 24-25 September 1997. National Animal Science Research Institute (NASRI), Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal. Pp. 175-178.

Fahmy MH and CS Bernard. 1972. Reproductive performance of gilts from lives selected from feed utilisation and carcass score. *Canadian Journal of Animal Sciences* 52:267-271.

Gatenby RM, PB Chemjong and SK Chimariya. 1990. Reproduction of pigs in the Koshi Hills. *PAC Technical Paper* No 125. Pakhribas Agricultural Centre, Dhankuta, Nepal.

Haley CS, E Avalos and C Smith. 1988. Selection for litter size in the pigs. *Anim. Breed. Abst.* 56:317-332.

Harvey WR. 1990. *User guide for LSMLMW PC-2 version*. Ohio State University Press Columbus, USA.

Johansson K and BW Kennedy. 1985. Estimation of genetic parameter for reproductive traits in pigs. *Acta Agric Scand*. 34:421-431.

Johansson K. 1981. Some notes concerning the genetic possibilities of improving sow fertility. *Livest. Prod. Sci.* 8:431-447.

Neopane S P. 2005. Genetic and non-genetic factors affecting litter traits in Pakhribas black pigs. *Nepalese Veterinary Journal* 28:51-57. Nepal Veterinary Association, Tripureshwor, Kathmandu, Nepal.

Oh SH, DH Lee and MT See. 2005. Estimates of genetic parameters for reproductive traits between first and later parities. *Animal Science Departmental Report 2004-2005*. North Caroline State University.

Oli KP. 1986. Comparative study of carcass yield of different breeds of pigs in the eastern hills of Nepal. *PAC Technical paper No 99*. Pakhribas Agricultural Centre, Dhankuta, Nepal.

Rydhmer L, N Lundeheim and K Johansson. 1995. Genetic parameters for reproduction traits in sows and relation to performance-test measurements. *J. Anim. Breed. Genet.* 112:33-42.

SARP. 1992. *Annual report*. Swine and Avian Research Programme, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Kathmandu, PO Box 1950, Nepal.

Shrestha NP. 2000. Characterization of a pig developed at Pakhribas. **In:** *Proceedings of the Fourth Global Conference on Conservation of Domestic Animal Genetic Resources.* Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal. Pp. 194-196.

Tholen E, KL Bunter, S Hermesch and HU Graser. 1996. The genetic foundation of fitness and reproduction traits in Australian pig populations. 2. Relationships between weaning to conception interval, farrowing interval, stayability, and other common reproduction and production traits. *Australian Journal of Agricultural Research* 47(8):1275-1290.

Use of Rice Straw and Black Gram Straw in Fodder Based Goat's Diets in the Hills of Nepal

Chet R. Upreti[*1], Bahadur S. Kuwar[2] and Shambhu. B. Panday[3]

[1] Agricultural Research Station (Goat), Bandipur, Tanahun
[2] Agricultural Research Station (Goat), NARC, Bandipur, Tanahun (formerly)
[3] Nepal Agricultural Research Council, Singhadurbar Plaza, Kathmandu

* Current address: Animal Nutrition Division, Khumaltar, Lalitpur, PO Box 1950, <crupreti@yahoo.com>

ABSTRACT

The aim of this study was to investigate the possible use of crop residue such as rice straw and black gram straw (Kushauro) in hill goats' diet. Twenty male and female goats of age 6-9 months were divided into 4 dietary groups. Animal of first diet groups (D_1) were fed with fodder leaves (Khanyu) only, second diet group (D_2) with 20% rice straw + fodder tree foliage, third diet group (D_3) with 20% rice straw + fodder tree foliage + concentrate @ 1% of body weight, and fourth diet group (D_4) fodder tree foliage + 15% rice straw + 5% black gram straw + concentrate @ 1% of body weight meet to the daily requirement. These rations were fed to animals of individual diet group for 150 days. Daily feed intake, daily weight change was recorded. Body weight was taken at the beginning of the experiment, at the end of experiment and 15 days interval. The result showed that there was significant difference ($P < 0.05$) between different dietary groups for average daily gain (ADG). The highest ADG was observed in D_3 (19.6±4.33), followed by D_2 (12.67±1.38 g/d) and D_4 (11.73±1.78 g/d). The lowest ADG was found in D_1 (10.8±1.87 g/d). The result indicated that 20% rice straw can be used in goat diet with supplementary diet of concentrate @ 1% of body weight, and foliage of Khanyu as a basal diet. Feeding only fodder tree foliage is not suitable to get higher ADG in stall-feeding management.

Key words: **A**verage daily weight gain, black gram straw**, f**odder tree foliage, rice straw, stall-feeding

INTRODUCTION

Rice straw is a crop residue derived from rice production. It serves as a major feed for ruminants. (Dixon 1985). Annually 10.7 million metric ton rice straw is produced in the country, out of which the large ruminants such as cattle and buffalo use 82.4%. The remaining balance 17.6% rice straw is available for other uses. If this surplus straw could be used for goat feeding, it will substantially reduce the feed scarcity problem in the country (Pande 1997), particularly during the winter. But the available straw is low in nutrients content and is highly lignified (Sharma et al 1995, Chenost and Kayouli 1997). Therefore, there is a need to improve in quality in terms of its use and make use of straw in animal diet. Therefore it is important to supplement tree foliage and concentrate feed (compounded cereal grain, legume and their by-products) to make the best utilization of the crop residue. Work carried out in other countries have shown that feeding 30 percent rice straw with *leucaena* leaves (50%) and rice bran (20%) as supplements can to maintain 68.6 g/d daily weight gain (Rasjit and Perez 1980). Upreti 2004 recorded that if straw is treated with urea and fed *ad lib* with 20% Ipil-Ipil green foliage supplementation the ADG of 41.11 can be obtained in stall feed management system. Further, goats has higher rate of dry matter digestibility (DMD) with rice straw (Devendra 1988) that indicate possibility of incorporating rice straw in goat diet in certain proportion. Therefore, a study was conducted at ARS Bandipur using goats to investigate on the use of crop residues particularly rice straw (*Oryza sativa*) and black gram straw (*Vigna mungo*) with khanyu (*Ficus semicordata*) as basal diet.

MATERIALS AND METHODS

Feeding experiment was conducted to evaluate the possible use of crop residue particularly the rice straw and black gram straw on the goats for stall-feeding system at ARS Bandipur during winter 2000. Tree foliage, Khanyu (*Ficus semicordata*) was fed as basal diet. Concentrate feed was offered @ 1%of body weight of experimental goats.

Experimental animals

Sixteen female and four castrated male goats of 6 to 8 months of age were used in the experiment. The initial body weight (IBW) of animals ranged from 11 to 12 kg. The breeds of goats were Khari, Jamunapari crosses (50% Jamunapari × Khari), and Barbari crosses (50% Barbari × Khari). They were drenched seven days before the experiment against internal parasite using Albendazole at recommended dose. Animals were also dipped against external parasites seven days before the drenching. Faecal sample were analyzed after seven days of drenching to assess the parasitic burden. These animals were then grouped into four groups on the basis of body weight in such a way that each breed and sex represents in each treatment group to reduce their possible effect of the sex and breed in the treatment. Animals were individually housed in metabolic crates affixed inside the experimental shed. The crates were measured 1.0 meter length, 0.45 m width and 1.0 meter height.

Experimental diet

Following diets were fed to the experimental animals to know the level of rice straw (*Oryza sativa*) and Black gram Kunauro (*Phaseolus mungo*) intake and body weight change.

D_1 = Conventional feeding with fodder leaves only
D_2 = 20% Rice straw + fodder leaves
D_3 = 20% Rice straw + fodder leaves + concentrate 1% of body weight
D_4 = 15% Rice straw + 5% black bean straw + concentrate 1% of body weight

Locally available rice straw (87.5% DM) was chaffed (6 cm long) and incorporated in the diets at the percentage described in individual diet based on the daily DM requirement of animal. Feed formulation was done according to NRC recommendation to meet the daily nutrients requirement of DM = 3% of body weight and CP = 3 g/kg body weight per day. Khanyou (*Ficus semicardata*) twigs with fresh green foliage were offered as main diet. Nutrient content of different feedstuffs is given in Table 1. Chaffed straw, fodder twigs and concentrate were offered in certain hourly interval in wooden box fixed at brisket height of metabolic pens. Fresh water was offered tree times a day. Daily intake of rice straw, fodder, black gram straw and concentrate was recorded for an experimental period of 150 days. A fixed level of concentrate was offered in D_3 and D_4 @ 1% body weight daily.

Body weight measurement

Measurements taken

The trial period was of 150 days after an adaptation period of 15 days. At the end of trial a digestibility trial was conducted for 7 days. Total feed intake was recorded daily in the morning at 09 hrs. Faeces and urine excreted was collected individually and recorded for the last 7 days of experimental period. The body weight of individual animal was taken at 15 days interval in the morning before feeding.

Chemical analysis

Dry matter (DM) content of offered feed and faeces voided were determined by drying at 100^{o}C for 24 h and organic matter (OM) content was determined by ignition in the electronic furnace at 500^{o}C for 6 hours. Representative samples of the feeds, faeces and urine were analyzed for proximate composition by AOAC 1990 and Goering and Van Soest (1970). The chemical composition of different feed ingredients used in the experiment is given in Table 1.

Data were analyzed using analysis of variance as suggested by Snedecor and Cochran (1968) using Statistix version 1.0 Statistical package.

RESULTS AND DISCUSSION

Feedstuffs were analyzed for nutritive value using facilities available at Animal Nutrition Division, Khumaltar. The CP of rice straw was similar to the average figure as reported by Upreti and Shrestha 2006 and the CP content of black gram straw was (*Vigna mungo*) recorded 1% higher as reported by same authors.

Table 1. Chemical composition of different feedstuffs (%DM basis)

Ingredients	DM	CP	OM	EE	NDF	ADF	ADL	H.CL.	CL
Khanyou	38.8	14.1	88.84	0.82	53.24	47.53	28.3	5.71	19.23
Concentrate mixture	87.53	15.3	93.53	4.84	-	-	-	-	-
Rice straw (RS)	87.5	4.5	81.31	0.36	74.91	50.54	21.12	24.37	29.42
Black gram straw	85.0	12.5	87	1.0	-	-	-	-	-

DM, Dry matter. CP, Crude protein. OM, Organic matter. EE, Ether extract. NDF, Neutral detergent fiber. ADF, Acid detergent fiber. ADL, Acid detergent lignin. H.Cl, Hemi cellulose. Cl, Cellulose.

Feed intake

The nutrient intake of different diet groups is shown in Table 2. The DM intake was significantly different ($P > 0.05$) between treatment groups. The highest DM intake (503.85 g/d) was found in D_3 followed by D_4 (491.2 g/d), and D_2 (484.1 g/d). Significantly lower DM intake was found in D_1 (440.31 g/d). These DM intakes were 3.63, 4.0, 3.85 and 3.95 percent of body weight of Diet 1, 2, 3 and 4 respectively. It indicated that incorporation of rice straw at the level of 20% of DM requirement did not affect significantly ($P > 0.05$) on DM intake in goats similar as reported by Rasjit and Perez (1980).

The DM intake per kg live weight gain was significantly ($P < 0.05$) lower in D_3 (25.71 kg), which was followed by D_2 (38.22 kg) and D_1 (40.77 kg). The highest DM intake per kg live weight gain was found in D_4 (41.86 kg). The lower DM required per kg live weight gain in D_3 would be due to inclusion of concentrate mixture in the diet that has improved the digestibility of nutrients (Agrawal et al 1989).

Table 2. Nutrient intake of animals (g) of different treatment groups on DM basis

Nutrients	Diets			
	Fodder only D_1	RS 20% + Fodder 80% D_2	RS 20% + Fodder (55%) + Concentrate D_3	RS 15% + Fodder (80%) + BGS (5%) D_4
DM	440.31^a	484.12^b	503.85^b	491.18^b
CP	57.24^a	49.54^b	34.04^b	50.75^b
Ca	9.64	7.88	5.91	7.74
P	1.01	1.11	1.75	1.08
% DM I	3.63	4.0	3.85	3.95
DMI (g)/W $^{0.75}$	67.74	74.36	73.23	74.31
Nutrient intake (kg) per kg LW gain				
DM	40.77^a	38.22^a	25.71^b	41.86^a
CP	5.3^a	3.91^a	1.74^b	4.32^a

RS, Rice straw. BGS, Black gram straw. Value in the rows having different subscript were significantly different ($P < 0.05$) and without superscript were not significant ($P > 0.05$).

The crude protein (CP) requirement per kg live weight was significantly lower ($P < 0.05$) in D_3 (1.74 kg), followed by D_2 (3.91) and D_1 (5.3 kg). These requirements were substantially higher than the requirement reported by ARC (ARC 1980) for a growing goat (0.388 g CP/gram gain).

Growth rate

Body weight of different treatment groups at different interval, average daily weight gain (ADG) and change in metabolic body size is presented in Table 3. There was significant difference ($P < 0.05$) between diets groups for total weight gain and ADG. The highest total weight gain for an experimental period of 150 days was obtained in $Diet_3$ (2.94 ± 0.65 kg), followed by D_2 (1.9 ± 0.21 kg), D_4 (1.76 ± 0.27 kg) and D_1 (1.62 ± 0.28 kg). Average daily weight gain was found significantly higher ($P < 0.05$) in D_3 (19.6 g/d), followed by D_2 (12.67 g/d), D_4 (11.73 g/d) and D_1 (10.8 g/d). These growth rates of a growing goats of 6-9 month age were substantially lower than the growth recorded at Goat Research Station, Bandipur over past five years (ARS 2000) that was 39.33 g/d, and Rasjit and Perez (1980) when animal were fed with 20 percent rice straw with *Leucaena* and rice bran.

Table 3. Weight gain of goats in different dietary treatments

Nutrients	Diet groups			
	Fodder only D_1	RS 20% + Fodder 80% D_2	RS 20% + Fodder (55%) + Concentrate D_3	RS 15% + Fodder (80%) + BGS (5%) D_4
Initial body weight (kg)	11.5 ± 0.97a	11.425 ± 0.95a	12.46 ± 1.24a	11.8 ± 0.97a
Final body weight (kg)	13.12 ± 1.0a	13.321 ± 0.11a	15.4 ± 1.7	13.56 ± 1.17
Total weight gain (kg)	1.62 ± 0.28a	1.9 ± 0.21ab	2.94 ± 0.65	1.76 ± 0.27
Average weight gain (g)	10.8 ± 1.87	12.67 ± 1.38ab	19.6 ± 4.33b	11.73 ± 1.78ab
Initial metabolic body size ($W^{0.75}$)	6.24 ± 0.59	6.21 ± 0.87	6.63 ± 1.10	6.37 ± 0.87
Final metabolic body size ($W^{0.75}$)	6.89 ± 0.89	6.97 ± 0.98	7.78 ± 1.43	7.07 ± 1.02
Change in metabolic body size ($W^{0.75}$)	0.65 ± 0.26	0.76 ± 0.16	1.15 ± 0.51	0.7 ± 0.20

Value in the rows having different superscript were significantly different ($P < 0.05$).

The average daily weight gain of goats fed with different rice straw based diets is shown in Figure 1.

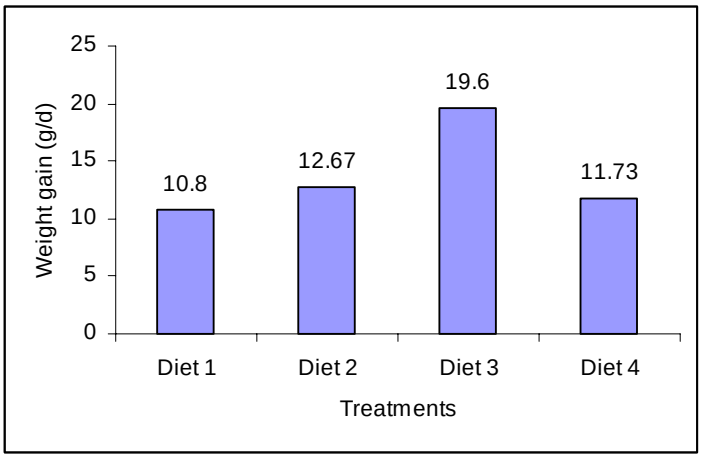


Figure 1. Average daily weight gain of goats fed with different rice straw based diets.

The growth pattern of experimental animals was similar to the expected growth curve of animals (Figure 1). The growth rate of animals of D_3 was higher than other groups, while that of Diet 1, 2 and D_4 were similar. This indicated the importance of incorporating concentrate in the diet that improves the digestibility of nutrients. The decreased growth rate of animals of Diet between 145 to 150 days of trials was due to diarrhea in one animal. From this experiment it could be advised that rice straw can be incorporated in the diet up to 20 percent of dry matter requirement of animals without effect in growth. Though lower growth rate, D_3 could be utilized as a maintenance ration in the feed scarcity period.

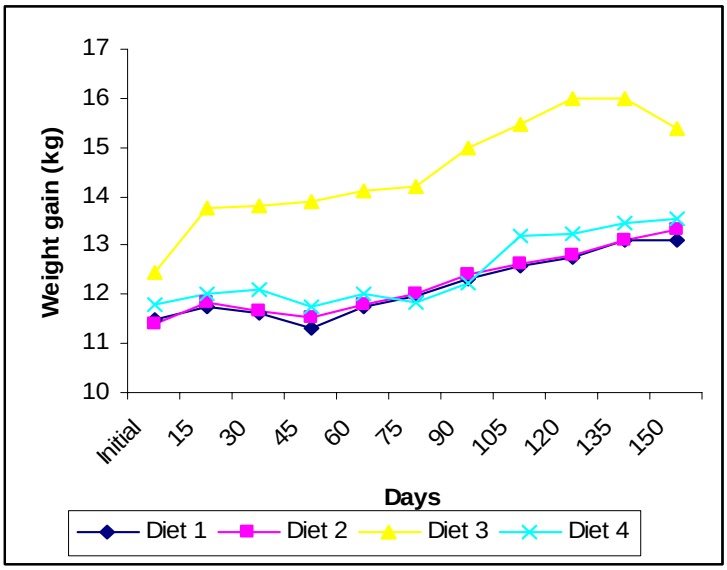


Figure 2. Average body weight gain patterns of goats fed with different rice straw based diets at 15 days interval.

Meat type goat could be raised in stall feeding system by feeding straw up to 20 percent of dry matter requirement in combination of fodder and concentrate at the rate of 1% body weight. Feeding only with tree foliage is not enough to get higher growth performance in stall feeding management in the Hills of Nepal. Therefore, during the winter, goat can be fed with certain level of rice straw in stall-fed management system.

ACKNOWLEDGEMENTS

The authors would like to thank to HARP for funding the experiment. The authors are grateful to all the staff of ARS Bandipur who helped to conduct the experiment and to technical staff of Animal Nutrition Division Khumaltar for timely analyzing of feed samples. Special thanks goes to Dr. W. Stewart-Jones, HARP Research Advisor ARS Lumle for his regular visits, supports, comments and moral support, Dr NP Shrestha, Director Livestock and Fish Research, NARC for his encouragement, Professor Dr M Sapkota for helpful comments and regular technical assistance and Dr SM Pradhan for critical comments.

REFERENCES

Agrawal IS, ML Verma, AK Singh and YC Pandey. 1989. Feeding value of ammonia treated straw in terms of milk production. *Indian J. Anim. Nutr.* 6:89.

AOAC. 1990. *Official methods of analysis*. Fourteenth Edition. Association of Official Analytical Chemist, Washington. DC.

ARC. 1980. *The nutrient requirement of ruminant livestock*. Commonwealth Agriculture Bureoux, Slough, Berk, UK. Pp. 349.

ARS. 2000. *Annual report 1999/2000*. Agriculture Research Station (Goat), Bandipur, Tanahun. Pp. 4-17.

Chenost M and C Kayouli. 1997. Roughage utilization in warm climates. FAO 135:1-225.

Devendra C. 1988. Nutrition and meat production, Goat meat production in Asia. **In:** *Proceedings of a Workshop* (C Devendra, ed), 13-18 March 1988, Tando Jam, Pakistan. Pp. 30-34.

Dixon RM. 1985. Maximizing the rate of fiber digestion in the rumen. **In:** *Proc. of the 6th Annual Workshop IPD*. Pp. 49-67.

Goering HK and BS Van Soest. 1970. Forage fiber analysis. *Agricultural Hand Book No. 379*. USDA, Washington, DC.

Pandey RS. 1997. *Fodder and pasture development in Nepal*. Udaya Research and Development Service (Pvt) Ltd., Kathmandu. Pp. 4-5.

Rasjit S, CB Jr. Perez. 1980. Fattening goat with ipil-ipil (*Leucaena leucocephala*) and rice straw plus rice bran or molasses. *Philippine Journal of Veterinary and Animal Science* 6:500-507.

Sharma DD, DV Rangnekar and M Singh. 1995. *Physical of chemical treatment of fibrous crop residue to improve nutritive value:* A review. ICAR Publication. NDRI, India. Pp. 263-276.

Snedecor GW and WG Cochran. 1968. *Statistical methods*. Sixth Edition. Oxford and IBH Publishing Co., Calcutta, India.

Upreti CR and BK Shrestha. 2006. Nutrient contents of feed and fodder in Nepal. Nepal Agricultural Research Council, Kathmandu, Nepal.

Upreti CR. 2004. Rice bran and *leucaena* supplementation on the growth and digestibility by goats fed urea treated rice straw. *PhD Thesis*. CLSU, The Philippines. Pp. 43-44.

Evaluation of Selected Species of Fodder Trees Cultivated for Feeding Ruminant Animals in the Hills of Nepal

Ramesh C. Khanal[1] and Chet R. Upreti[2]

[1] Animal Nutrition Division, Nepal Agriculture Research Council, Khumaltar
[2] Agricultural Research Station (Goat), Bandipur, Tanahun

ABSTRACT

Leaves and twigs from four species of tree fodders, *Aetocarpus lakoocha* (AL), *bauhinia purpurea* (BP), *Garuga pinnata* (GP) and *Ficus sroxburghii* (FR), cultivated widely for feeding ruminants in the hills of Nepal and maintained at the Agriculture Research Station (Goat), Bandipur were investigated for there chemical composition, and nutrient intake, digestibility, and growth rate in growing female goats. Differences occurred between species of tree fodders on nutrient composition, which contained 286, 342, 312, and 263 g/kg DM; 174, 98, 116 and 178 g/kg total ash; 137, 163, 140, and 119 g/kg CP; 440, 458, 437, and 451 g/kg NDF; 383, 407, 382, and 406 g/kg ADF; 177, 135, 181, and 143 g/kg ADL; 19.5, 21.6, 20.9, and 29.3 g/kg Ca; and 2.2, 2.8, 2.4, and 2.5 g/kg P on DM basis for AL, BP, GP, and FR, respectively. The DM intake was higher for goats fed AL (432 g/d) and GP (428 g/d) than BP (342 g/d) or FR (306 g/d). Nutrient digestibility was higher for goats fed AL and GP, except Ca, which was higher for goats fed FR. Similarly, highest daily weight gain was observed for goats fed AL (71 g/d) and GP (64 g/d) than either of BP (54 g/d) or FR (30 g/d). Overall, leaves and twigs from these tree fodders supported moderate growth, but may not be advisable for maximum production.

Key words: Digestibility, goat, nutritive value, performance, tree fodders

INTRODUCTION

Fodder trees are grown in the hills of Nepal from the immemorial. Their importance in supplying fodder, fuel wood, protection of soil erosion and environment, and some other household needs has been well documented (Pandey 1982). Inadequate feed supply and poor nutrition during the dry winder and early summer season (mid-January through mid-May) are the major constraints to increase ruminant production in the hill of Nepal (Kiff et al 1999). Of the 75 administrative districts in the country, 50 were found to be less than 80% sufficient in supplies of livestock feed (Schreier et al 1991). Leaves and twigs collected from several species of fodder trees cultivated in a great variety of soil and climatic condition are a main source of green forage for ruminants during dry winter and early month and help maintain the body condition and production of ruminants.

Khanal and Subba (2001) described the nutritional characteristics of leaves and twigs from 31species of fodders trees cultivated in the hill of Nepal. Khanal et al (1999) have determine the proximate composition, detergent fibers, and Ca and P contents of both cultivated and uncultivated tree fodders and browse plants available mostly in the western hill of Nepal. Similarly, Subba (1998) has determined the detail chemical composition of leaves from those available in eastern hills of Nepal. Subba (1999) also ranked them according to various nutritional characteristics. On the other hand farmers have their own preference based on there age-old practices in the cultivation and feeding of leaves and twigs from various species of tree fodders. Realizing this, Thorne et al (1999) and Walker et al (1999) attempted to derive a biological interpretation of indigenous knowledge system with regard to the quality of tree fodders and consistency and discriminatory power of indigenous and laboratory assessment of the nutritive values of tree fodders available in Nepal. Thorne et al (1999) also attempted to find the potential complementarities between indigenous and laboratory-based indicators of tree fodder quality. However, part of there interpretation was based not on actual animal performance, but on in vitro studies. As a result, several of these authors pointed out the need to investigate on the quality of various fodder trees and shrubs based on the

actual performance of ruminants. This would ultimately help reduce the severity of scarcity of feed and fodder and maintain ruminant production. Therefore, objective of this study was to investigate the performance of goats upon feeding leaves and twigs from four species of selected fodder trees commonly cultivated in the hills of Nepal.

MATERIALS AND METHODS

Sixteen female growing Khari goats with an average initial weight of 8.89±0.6 kg and age of 140 ± 20 days were selected for the study. Animals were blocked according to there initial body weight. Within block animals were allotted at random to one of four treatments, ie species of tree fodders selected for the study. Tree fodder species were Badahar (*Artocarpus lakoocha;* AL*),* Tanki (*Bauhinia purpurea;* BP), Dabdabe (*Garuga pinnata*; GP) and Nemaro (*Ficus roxburghii*; FR), selection of which was based on their widespread availability across the low and mid hills of Nepal. While AL is regarded as the one of the most nutritious fodder tree species by the farmers, BP is a leguminous fodder and expected to have higher protein contents. The GP is a relatively large with small thin leaves, whereas FR has big, rounded, and thick leaves with a relatively large mid-rib. Leaves and twigs from the fodder trees were collected from the same north facing fodder block maintained in Agriculture Research Station (ARS, Goat), Bandipur at an altitude of 875 m from the sea level. Soil had a pH of 5.64 with 3.2% organic carbon, 174.3 ppm of available phosphorus, and 0.27% total nitrogen. The station received an annual rainfall of 2000 mm with an average of 85% relative humidity, and minimum and maximum temperature of 8 and 32^{o}C, respectively.

Experimental goats were housed in individual cages in the same shade with facilities for collection of feed, orts and feces, but not urine. Goats were allowed to have an *ad Libitum* access to leaves and twigs from one of the four fodder trees mentioned above. Water was offered twice daily at 1000 h and 1700 h while tree fodder leaves together with twigs and petioles were offered twice daily at 0900 h and 1600 h. Orts were collected 2 h after feeding. All animal were de-wormed before allotting them to the experiment and further medication, if any, was administered as per the suggestion of a veterinarian.

Total experimental period was 74 days including a 14-day adaptation period, which was conducted during Feb-May 2000. Growth was monitored every fortnight. Feed offered and orts were collected daily for determining the daily feed and nutrient intakes. Total fecal output was measured during the final 8 days of the experiment and their samples collected during the total collection period. Dry matter (DM) content of the feeds offered and orts collected as well as fecal samples was determined daily during the last 8 days of digestibility study.

Laboratory analyses of the tree fodder leaves and twigs offered and fecal samples collected was done for DM, crude protein (CP) and total ash (TA), contents as per AOAC (1980). Neutral detergent and acid detergent fiber (NDF and ADF) as well as acid detergent lignin (ADL) were determined as per (Van Soest and Robertson 1985). Samples of leaves and twigs were analyzed for their chemical composition every fortnight during the experiment. These samples were air dried at ARS (Goat), brought to Animal Nutrition Division Laboratory, and ground to pass through a 1-mm sieve for further laboratory analyses. Wherever appropriate, all values are expressed on DM basis.

Data were statistically analyzed in SAS using PROC GLM (SAS 1999). Model included treatment and block as the independent variables. In case of final body weight, initial body weight was used as the covariate. Means were separated using REGWQ.

RESULTS AND DISCUSSION

Nutrient composition of the leaves and twigs from selected species of fodders trees is given in Table 1. The DM content was higher for BP ($P < 0.05$) than AL and FR, but there was no difference ($P > 0.05$) between the other species of tree fodders. The values were comparable to the earlier reports for the same species of tree fodder harvested at similar times of the year (Khanal and Subba 2001,

Throne et al 1999, Wood et al 1994). The DM content would increase with the increasing maturity, which was probably another reason why it is higher for BP that was approaching fruit bearing stage. Crude protein content varied significantly ($P < 0.05$) among species, highest being for BP, which is a leguminous tree fodder. Crude protein content in all of them was slightly lower than reported previously (Khanal and Subba 2001, Khanal et al 1999, Subba 1998). It was probably because of the difference in sampling procedures employed between the studies. While leaves and twigs were included in the current study to represent the actual feeding practices of the farmers, it was only the leaves that were used to determine CP content previously (Khanal and Subba 2001). Time of harvest also affect the CP content with the same species of tree fodder varying in CP content by about 30 to 40% when harvested at different times of the year (Topps 1992, Wood et al 1994). Nonetheless, all of them could be regarded to have medium CP content, which could make a valuable source of protein for ruminants. Moreover, Subba (1999) has shown that a higher proportion of CP present in this tree fodders is actually present in the form available to ruminants. The ash content was also different ($P < 0.05$) among the four species of tree fodders with smaller values for BP and GP than either of the AL or FR. While Wood et al (1994) reported higher ash contents for AL and FR, Khanal and Subba (2001) reported slightly lower ash content for BP and GP. Such variations may exist owing to the difference in harvesting season, fodder maturity, leaf : twig ratio and even soil condition and topography.

Table 1. Nutrient composition (g/kg) of leaves and twigs from selected species of fodder trees[1] used for feeding ruminant in the hills of Nepal

Nutrient	Fodder tree species				SEM[2]
	AL	BP	GP	FR	
DM	286[b]	342[a]	312[ab]	263[b]	17.4
Total ash	174[a]	98.2[b]	116[b]	178[a]	13.5
CP	137[bc]	163[a]	140[ab]	119c	9.3
NDF	440	458	437	451	27.1
ADF	383	407	382	406	35.0
ADL	177	135	181	143	32.1
Ca	19.5	21.6	20.9	28.3	3.2
P	2.2	2.8	2.4	2.5	0.5

Figure in the same row with different superscripts differs ($P < 0.05$). [1] Fodder tree species used were AL, Artocarpus lakoocha. BP, Bauhinia purpurea. GP, Garuga pinnata, FR, Ficus roxburgii. [2] Standard error of mean.

No significant difference ($P > 0.05$) in detergent fibers or Ca and P content was observed among species. The NDF content was lower than previous reports for AL and BP, but it was similar in case of GP and FR (Khanal and Subba 2001). Thorne et al (1999) reported higher NDF and ADF values for FR than was observed in the current study. AS expected, ADL content was high for all species. Relatively higher ADL and lower hemicellulose content (NDF minus ADF) is probably one of the characteristics of tree fodder and shrubs fed to ruminants. Similar results of high ADL and LOW hemicellulose content have been reported previously for these tree fodders cultivated under similar condition (Subba 1998). Calcium content was close to or more than 2% on DM basis. High Ca and medium to high CP content is worth considering, because hill farmers of Nepal give a priority to feed tree fodder leaves and twigs to lactating ruminants, primarily buffaloes that have greater demand for CP and Ca contents during early lactation. This may also be useful while making feeding packing and supplementation strategies for ruminant production in the hills of Nepal, particularly during drier months. The P content was within the normal range of requirement in the forages (McDowell 1997).

There was a significant difference ($P < 0.05$) in the nutrient intake of animals (Table 2), lowest nutrient intakes being for FR group. The DMI, DMI/100 kg bwt and DMI/kg $w^{0.75}$ was lower for BP and FR group than AL and GP groups. Large thick leaves and twig like mid rib in case of FR and thinly distributed leaves that would increase the overall proportion of twigs in case of BP might have contributed to the lower DM intakes. It was observed during the experiment that goats did not relish FR and had palatability problems. Aryal et al (1994) have observed higher intakes of DM with 620 g/d when male goats weighing 7.6 kg initially were given various tree fodders (depending on the

availability round the year) percent supplemented with concentrate (16% CP) at 1% of their body weight. They have observed an intake of 3.8 kg DM/100 kg BW and 76.2 g/kg $w^{0.75}$, which was similar to that of AL and GP groups but higher than BP and FR groups. However, higher CP content (Table 1) led the similar CP intake in BP group compared with AL and GP groups. Other studies related to nutrient intakes of female goats fed such tree fodder leaves and twigs exclusively are limited. A dramatic increase in feed intake (and feed conversion ratio) in both sheep and goats have been observed when the basal diet was supplemented with rumen undegradable protein and starch (Throckmorton and Leng 1984). Since rumen undegradable protein and starch was not supplemented in the current study, their positive effects on intake and digestibility of nutrients could not be ascertained.

Table 2. Nutrient intake (g/kg) by female goats fed leaves and twigs from selected species of fodder trees[1] used for feeding ruminant in the hills of Nepal

Nutrient	Fodder tree species				SEM[2]
	AL	BP	GP	FR	
DM	431.8^{a}	341.7^{b}	427.9^{a}	305.8^{c}	9.98
DM, % BW	3.95^{a}	3.27^{b}	3.98^{b}	3.14^{a}	0.09
DM, /kg $w^{0.75}$	72.0^{a}	59.0^{b}	72.2^{a}	55.5^{b}	1.69
OM	356.8^{b}	306.3^{c}	378.2^{a}	251.3^{d}	7.38
Total ash	75.0^{a}	35.3^{d}	48.7^{c}	54.5^{b}	1.71
CP	50.5^{b}	52.4^{ab}	55.8^{a}	30.3^{c}	1.54
Ca	8.42^{b}	5.82^{c}	8.94^{a}	8.96^{a}	0.17
P	0.95^{a}	0.96^{a}	1.03^{a}	0.76^{b}	0.03

Figure in the same row with different superscripts differs ($P < 0.05$). [1] Fodder tree species used were AL, Artocarpus lakoocha. BP, Bauhinia purpurea. GP, Garuga pinnata, FR, Ficus roxburgii. [2] Standard error of mean.

Digestibility of nutrients was affected by the species of tree fodder and could be considered relatively low (Table 3). Inclusion of twigs with leaves may have contributed to the lower digestibility of nutrients. Higher ADL contents (Table 1) may also have rendered relatively poor digestibility of nutrients from these tree fodders since it forms complexes with hemicellulose, which would otherwise be digestible. Previous studies on tree fodder leaves have shown higher neutral cellulose, DM, and OM digestibility (Khanal and Subba 2001). Presence of antinutrient factors such as tannins may also have contributed to poor digestibility, because all but BP are shown to contain >2% tannin in there leaves (Khanal and Subba 2001, Subba 1998, Wood et al 1994). Though not measured, tannin content in the current study might have been higher because of the inclusion of twigs. Digestibility of nutrients for AL and GP was similar and significantly higher ($P < 0.05$) than that of BP and FR, which were not different ($P > 0.05$) from each other in most of the cases, except digestibility of total ash and Ca. However, Ca digestibility was highest ($P < 0.01$) for FR than for the rest, reasons for which were not clear.

Daily weight gain, feed: gain ratio, and final body weight of female goats are given in Table 4 while the growth pattern every fortnight is presented in Figure 1 and 2. Higher nutrient intake accompanied by better digestibility of various nutrients might have had positive effect on the significantly ($P < 0.05$) better growth rates of female goats fed AL and GP than BP or FR. Goats in AL group tended to grow faster ($P = 0.08$) than goats in GP group even though the digestibility and intakes of nutrients in these two groups were similar. Farmers, however, consider AL as the most *Posilo* (nutritious) tree fodder, regard it as the king of tree fodders, and often feed it to lactating buffaloes only. Two of the four animals fed FR lost their body weight during the 2nd and 3rd fortnights of the experiment. This must be due to poor intake and digestibility of the fodder species concerned, which was probably not sufficient to meet the maintenance requirement of the animals. Growth rate of other groups of animal was also relatively poor, because high growth rates cannot be supported to the products of fermentative digestion and that rumen undegradable protein supplements are essential to take advantage of the energy absorbed from volatile fatty acids (Preston 1998). Another reason may be the sex of the animals since females grow at slower rates than males at similar age (Anous and Mourad 1993).

Table 3. Nutrient digestability (%) by female goats fed leaves and twigs from selected species of fodder trees[1] used for feeding ruminant in the hills of Nepal

Nutrient	Fodder tree species				SEM[2]
	AL	BP	GP	FR	
DM	53.4[a]	46.5[ab]	58.8[a]	44.8[b]	3.02
OM	58.9[a]	46.1[b]	65.5[a]	46.0[b]	3.73
Total ash	58.4[a]	49.3[b]	47.0[b]	34.5[c]	3.89
CP	53.2[a]	45.0[b]	57.0[a]	44.1[b]	2.26
NDF	50.3[b]	40.8[a]	50.3[a]	42.5[b]	2.32
ADF	45.2[a]	40.2[ab]	49.4[a]	38.7[b]	3.50
Hemicellulose	49.3[b]	38.2[c]	60.4[a]	41.0[c]	2.06
Ca	60.7[ab]	47.7[c]	55.5[b]	65.7[a]	1.88
P	47.9[ab]	38.1[c]	50.7[a]	41.6[bc]	2.62

Figure in the same row with different superscripts differs (P < 0.05). [1] Fodder tree species used were AL, Artocarpus lakoocha. BP, Bauhinia purpurea. GP, Garuga pinnata. FR, Ficus roxburgii. [2] Standard error of mean.

Table 4. Final body weight, average daily gain, and feed gain ratio of female goats fed leaves and twigs from selected species of fodder trees[1] cultivated in the hills of Nepal

Nutrient	Fodder tree species				SEM[2]
	AL	BP	GP	FR	
Final BW, kg	13.0a	12.1b	12.7ab	10.6c	0.31
Daily gain, g/d	71.1a	53.9b	64.4a	29.7c	3.24
Feed:gain ratio	6.07a	6.34a	6.64a	10.3b	0.87

Figure in the same row with different superscripts differs (P < 0.05). [1] Fodder tree species used were AL, *Artocarpus lakoocha*. BP, *Bauhinia purpurea*. GP, *Garuga pinnata*. FR, *Ficus roxburgii*. [2] Standard error of mean.

An overall weight gain of 81 g/d was obtained under Indian conditions when Sirohi, Marwari and Kutchi goats were raised between 3 to 6 months of age (Nagpal et al 1995), all of which are heavier breeds than Khari used in the current study. A maximum growth of 66 g/d was observed between 6 to 12 months of age in Nepali hill bucks (Khari) in eastern Nepal when goats were allowed to have *ad libitum* access to tree fodders and supplemented with corn at 25 g/d per bwt (Shrestha et al 1990). However, the growth declined to 48 g/d when supplemental corn was reduce to 10 g/d per kg body weight. Growth rate was even less at 41.3 g/d per kg bw for male kids that weighed 7.6 kg initially and reared for one year on *ad libitum* access to tree fodders supplemented with concentrate at 1% of there body weight (Aryal et al 1994). Although daily weight gain obtained in the current study was better than or comparable tot hat observed previously in the same breed (Upreti et al 1999, Upreti and Khanal 1998) Aryal et al 1994, Shrestha et al 1990), it was poorer than many other international findings (Penning et al 1996, Mahgoub and Lodge 1996, Nagpal et al 1995). Supplementation of rumen undegradable protein would probably have increased their growth rate.

Weight gain was highest during the first fortnight of the experiment and significant difference ($P < 0.01$) existed in the growth rates during all stages of growth, from the beginning to the end of the experiment. Consistent with the daily gain, final weight was also higher for goat fed AL and GP than BP or FR. Final weight at 6 month of age in the current study was similar to that observed previously at the same age of the same breed maintained at Lumle Farm in the western hills of Nepal (Khanal et al 2005). Lower final weight and poor growth rate led to poorer feed: gain ratio for goat fed FR than goats fed the rest of fodders. Overall the growth rate was poor for goats irrespective of the species of tree fodder and raising goats solely on such fodder and raising goats slowly on such fodder tree leaves and twigs may not advisable for optimum production.

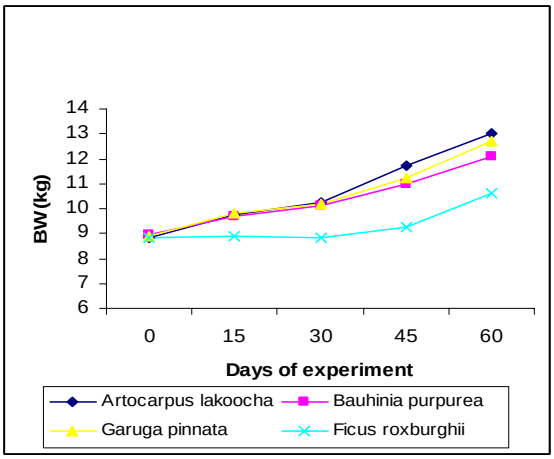


Figure 1. Growth pattern of female goats fed with different tree foliage.

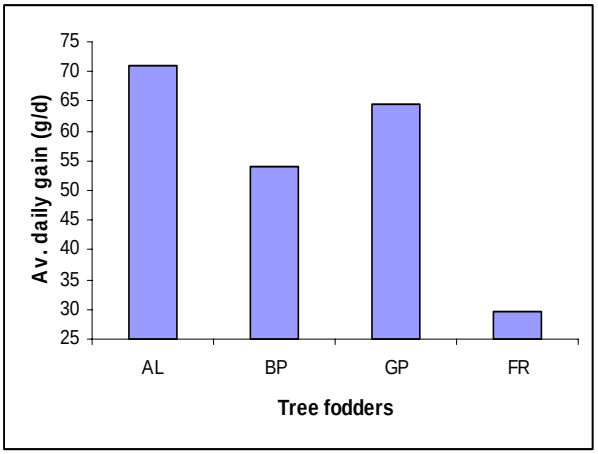


Figure 2. Average daily weight gain of female goats fed with different tree foliage.

CONCLUSIONS

Feeding of leaves and twigs from different species of fodder tree to growing female goats showed the difference existed not only in the nutrient composition among individual fodder trees species, but also in the intake, digestibility and performance of the animals. *Artocarpus lakoocha and Garuga pinnata* were better in terms of overall response by the animals. *Bauhinia purpurea,* though highest in crude protein content, was not as good as *Artocarpus lakoocha and Garuga pinnata* in terms of animal performance. *Ficus ruxburghii* was merely able to maintain the animals. Based on the performances of female goats in the current study, feeding of these species of tree fodder is probably not sufficient to sustain higher production from ruminants. Further studies on the effects of supplementing this vital source of fodder with rumen undegradable protein, starch or both on nutrient intake, digestibility, and performance of the animal is important.

ACKNOWLEDGMENTS

We acknowledge Nepal Agriculture Research Council for making research fund available, DK Dewan and KB Neupane for lab works and MP Saha for care and management of experimental animals. A critical comment made by Dr BR Joshi is highly appreciated.

REFERENCES

Anous MR and MM Mourad. 1993. Cross breeding effect on reproductive traits of kids. *Small Rumin. Res.* 12:141-149.

AOAC. 1980. *Official methods of analysis*. 13th Edition. Association of Official Analytical Chemists, Washing DC.

Aryal RC, NP Shrestha, SP Neopane, HB Gurung and AR Gurung. 1994. Effect of feeding Banmara *(Eupatarium adenophorum)* on fattening goats. *PAC Technical Paper No. 158*. Pakhribas Agricultural Center, Dhankuta, Nepal.

Khanal RC and DB Subba. 2001. Nutritional evaluation of leaves from some major fodder trees cultivated in the hills of Nepal. *Animal Feed Science Technology* 92:17-32.

Khanal RC, DP Rasali, TS Dhaubhadel, BR Joshi and NPS Karki. 2005. Comparative performance of indigenous Khanri and Kahri * Sinhal goats at Lumle farm. *Nepal. J. Biol. Sci.* 5:124-128.

Khanal RC, SB Panday and SK Khanal. 1999. Nutrient content in the fodder trees and shrubs found in various parts of Nepal. *Paper presented in the 1st Convention of the Society of Agriculture Scientists/Nepal*, 9-11 June, Kathmandu.

Kiff E, P Thorne, BH Pandit, D Thomas and SM Amatya. 1999. *Livestock production systems and the development of fodder resources for the mid hills of Nepal*. Department of Forestry Research and Survey, Natural Resources Institute and Nepal Agroforestry Foundation. 78 p.

Mahgoub O and GA Lodge. 1996. Growth and body composition in meat production of Omani Batina goast. *Small Ruminant Res.* 19:233-236.

McDowell LR. 1997. *Minerals for grazing ruminants in tropical regions*. University of Florida, Institute for food and Agriculture Science in USA.

Nagpal AK, VSP Singh and PC Jain. 1995. Effect of weaning age and feeding system on growth performance and carcass traits of male kide in three breeds in India. *Small Rumin. Res.* 17:45-50.

Pandey KK. 1982. *Fodder trees and tree fodder in Nepal*. Swiss Development Cooperation. Berne, Switzerland.

Penning PO, RH Johnson and RJ Orr. 1996. Effect of continuous stocking with ship or goats on sward composition and animal production from a grass and white clover pasture. *Small Rumin. Res.* 21:19-29.

Preston TR. 1998. Tropical animal feeding: A manual for research workers. *FAO Animal Production and Health paper No. 126*. Food and Agriculture Organization of the United Nations, Rome.

SAS. 1999. SAS user's guide. Release 8.2. SAS Inst., Cary, NC.

Schreier H, S Brown, G Kennedy and PB Shah. 1991. Food, feed and fuel wood resources of Nepal: A GIS evaluation. *Environment Management* 15:815-822.

Shrestha NP, SP Neopane and HB Gurung. 1990. Effect of athelminthic treatment and feeding regimes on growth rate of local goats. *PAC Technical Paper No. 127*. Pakhribas Agricultural Center, Dhankuta, Nepal.

Subba DB, 1999. Tree fodder and browse plant as potential nutrition suppliers for ruminants. **In:** *Proc. 3rd Natl. Workshop Lives. Fisheries Res. Nepal* (RC Khanal and SP Neopane, eds). National Animal Science Research Institute, Nepal Agricultural Research Council (NARC), Khumalter, Lalitpur, Nepal. Pp. 1-14.

Subba DB. 1998. *Chemical composition and nutritive value of feed of East Nepal*. Pakhribas Agricultural Center, Dhankuta, Nepal.

Thorne P, DB Subba, DH Walker, B Thapa, CD Wood, FL Sinclair. 1999. The basis of indigenous knowledge of tree fodder quality and its implications for improving the use of tree fodder in developing countries. *Animal Feed Science Technology* 81:119-131.

Throckmorton JC and RA Leng. 1984. Effect of bypass protein and bypass starch on milk yield and bodyweight gain in dairy cows. *Animal Production Aust.* 15:628-630.

Topps JH. 1992. Potential, composition and use of legume shrubs and trees as fodders for livestock in the tropics: Review. *J. Agriculture Science* 118:1-8.

Upreti CR and RR Khanal. 1998. Study on kids rearing – Effect of weaning of day on the growth performance of kids. *Annual report - 1997/98*. Agriculture Research Station (Goat) Bandipur, Tanahun, Nepal.

Upreti CR, SB Panday and MS Sapkota. 1999. Study on the effect of rice straw, tree leaves and concentrate feeding on goats. *Annual report - 1998/99*. Agriculture Research Station (Goat), Bandipur, Tanahun, Nepal.

Van Soest PJ and JB Robertson. 1985. *Analysis of forages and fibrous foods: A laboratory manual for animal science 613*. Cornell University, Ithaca, USA.

Walker DH, PJ Thorne, FL Sinclair, B Thapa, CD Wood and DB Subba. 1999. A systems approach to comparing indigenous and scientific knowledge: Consistency and discriminatory power of indigenous and laboratory assessment of the nutritive value of tree fodder. *Agriculture System* 62:87-103.

Wood CD, BN Tiwari, VE Plumb, CJ Powell, BR Roberts, VDP Sirimane, JT Rossiter and M Gill. 1994. Interspecies differences and variability with time of protein precipitation activity of extractable tannins, crude protein, ash, and dry matter content of leaves from 13 species of Nepalese fodder trees. *J. Chem. Ecol.* 20:3149-3162.

Marketing Constraints to Goats in the Western Hill of Nepal

Megh B. Nepali*[1], Megh R. Tiwari[3], Sudha Sapkota[2], Hari P. Poudel[3], Budhi R. Acharya[3] and Sudeep Gautam[1]

[1] Socio Economics and Agricultural Research Policy, Division, Khumaltar
[2] Nepal Agricultural Research Council, Singha Durbar
[3] Regional Agricultural Research Station, Lumle

ABSTRACT

Goat marketing study was conducted in eleven districts with the objectives of exploration the existing goat marketing system in western hills of Nepal. The methodology of the study was households survey, Rapid Marketing Appraisal and use of secondary data. Some of the marketing centers are at the village level, catchments, terminal level markets and district headquarters. There are some marketing agents dealing with the goat marketing from village to the municipalities. Goat marketing agents mainly found farmers and middlemen at the village level and catchments markets. In terminal markets mainly wholesalers and middlemen were involved where as in district headquarters and municipalities middlemen, wholesalers and butchers were dealing the goat business. 59.3% respondents believed that middlemen visit in the village for goat marketing. Almost all farmers sell their goats on the basis of estimated price. Major constraints on goat marketing were lack of public goat marketing centres, lack of communications about goat marketing and lack of infrastructure etc in all the domestic markets. In the western hills of Nepal main goat marketing centre was found in Pokhara sub municipality where traders/whole sellers bring their goats to sale from different catchments. Government has the provision of establishing new goat marketing centers in each VDCs and district headquarters.

Key words: Agents, collection, demand, price, transportation

INTRODUCTION

Goat farming is an important component of subsistence farming system in the western hills of Nepal for cash generation of resource poor farmers who are unable to invest in large ruminants. Most farmers raise goats because the demand of goat is high throughout the year. Currently goat marketing management in Nepal is poor, which is creating the problem of over and under supply in different times and places. Raising different breeds of goats is commonly practiced under the traditional management either in sedentary or migratory system in the rural areas of Nepal. While looking at market situation of goat in the remote rural area, the price hiking is chaotic. Goat market price is not uniform. Demand of goat is high not only in the festival time but also throughout the year. When there is high demand, question arises why it is not possible to fulfill the demand domestically. Every year Nepal imports about 266 thousand goats either from India or Tibet to fulfill the demand of goat meat (Ministry of Finance 2006).

For the improvement of living standard of hill and mountain people Agricultural Prospective Plan (1995) has given considerable emphasis on improvement in livestock production especially milk and meat which might improve the cash generating opportunity of the farming families. APP (1995) has estimated the contribution of livestock GDP (Gross Domestic Production) to increase from 31% of before APP level to 45% in the last period of APP in which the highest growth is estimated in the mountains and hills regions of the country. As envisaged in APP, NARC has given priority for Goat Research Program. However, despite of 7.4 million goats in the country, the urban need is generally met with import from the neighboring countries.

Most of the farmers in the western hills raise either local Khari or improved goat, in a flock or a few in their home yard or in stall feeding system. The total number of goats in Nepal is 7,421624,

however in western hills of Nepal the goats population is 995,144 (MoAC 2002). Goat meat production in Nepal is 42,820 mt in 2006. During FY2002/003, meat production was increased by 2.5 percent and reach to the level of 204000 mt. Goat contributed about 12% to livestock GDP. Economic return from goat farming is comparatively higher than other sector of livestock (Pandey 1998). The average income from goat sale was estimated to be Rs 11,299 per household (Thakur et al 2003). If proper production and marketing supports are provided, there is vast potential for growth of goat enterprises in Nepal. After buffalo, goat is a second biggest source of meat in Nepal. In such situation the organized marketing network is virtually lacking in the country. Total amount of Nepalese currency that goes India from Nepal appears to 50 crores each year (Kharel 2000). The western zone does not have large, organised goat markets of the number and size as in the eastern and central zone. Animal protein is supplied by the goat to human being, without competing with humans for cereal grains. The goat is a prolific animal and can be raised cheaply by the farming households. Under sedentary management system 65% goats were raised, Livestock management Plan (1993) of which more than 50% are reared in the hills. Farmers were getting the price of their goat even the less than the cost of production though consumers are paying high price for the goat meat. In order to understand the marketing problem, this research was conducted in western hill districts to collect information from rural farmers, middlemen, wholesaler, butchers and consumer.

METHODOLOGY

Data were collected from 11 districts (Gorkha, Lamjung, Tanahaun, Kaski, Syanga, Palpa, Arghakhanchi, Gulmi, Baglung, Parbat and Myagdi) using the Participatory Rural Appraisal (PRA) and Rapid Marketing Appraisal (RMA) checklist as well as household questionnaires for the study in 2003. Four goat raising VDC's from each district were purposively selected with the consultation of District Livestock Offices from each district. In each VDC one goat-raising ward was selected on the basis of commercial goat raising. From the each ward about 5% commercial goat raisers were randomly selected for the interview. In the case of consumers in urban areas information was collected from different institutions specially police camp, army camp, boarding school, hospitals, hotels and restaurants and 20 respondents from other consumers randomly selected from each district. Similarly, the information was collected from middlemen, wholesalers, retailers, traders, market centre and catchments in each district in clusters. The data was tabulated, cross tabulated and analyzed in excel program.

FINDINGS

General socio-economic information

In the study area, family size ranged from 6 to 8, with a mode household size 6. Male and female ratio is about equal. In the surveyed area average land holding in *khet* land is 4.68 *Ropani, Bari* land 5.36 *Ropani, Bhir* land 3.32 *Ropani* and pasture land 3.51 *Ropani.* Farmers were generating household income per annum from different sources. Figure 3 gives the percentage of different sources of income. Income derived from goat is 11%, fruit 4%, vegetables 3%, cereal 18%, remittance 14%, labor 5%, pension 10%, service 27% and from other animals 8% (Figure 1).

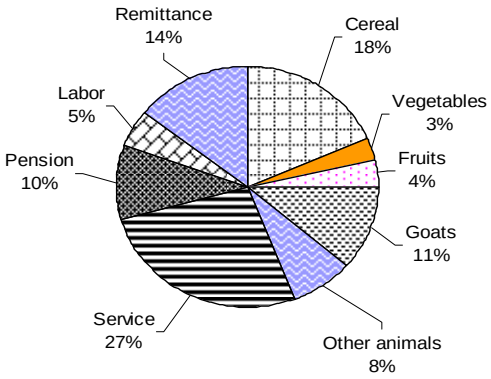


Figure 1. Average income generation per year.

Table 1. General socio-economic information in the study area

Average	Arghakhachi	Palpa	Gulmi	Syangja	Parbat	Kaski	Baglung	Myagdi	Lamjung	Gorkha	Tanahun
Family size	7	8	7	7	6	6	7	6	6	6	6
Landholding (Ropani)											
Khet land	6	2	4	4.5	8	4	3	5	6	6	3
Bari land	7	5	14	5	3	2	3	5	2	7	6
Bhir land	9	4	4	1	2	-	0,1	13.5	1	1	1
Pasture land	20	4	1	2,2	1	0.5	0.6	1	1	0.3	7
Goat population per households	9	7	8	6	3	7	3	6	11	8	7
Sales of goats per households per year	3	3	2	2	2	4	2	2	2	1	2
Market agents											
Wholesalers	2	2	1	4	1	14	2	1	2	2	2
Middleman	4	6	4	4	5	6	4	4	4	4	4
Butchers	6	8	11	6	9	36	9	6	4	5	6
Number of goats collected											
Villages	986	1440	2160	96	0	3240	720	900	1080	216	334
Catchments	310	365	0	120	0	3240	360	360	360	4320	1440
Others	1406	2322	1242	1542	2337	18522	2323	1550	1602	2321	1576

Khet land, Wetland paddy area. *Bari* land, Rainfed upland. *Bhir* land, Sloppy land. 1 hector, 20 *Ropani*

Goat marketing system

Goat marketing survey was conducted in 11 western hill districts of Nepal in the farming system of agriculture based livestock as well as agro-forestry in the different agro ecological zones ranging from 250-2300 meters above the sea level which covers river basin, low hills, mid hills and high hills (Nepali et al 1998). Most of the farmers are raising their goats either in grazing or stall feeding in the western hills of Nepal. The supply of goat is not meeting the demand of goat meat. In the study area goats were marketed through different marketing channels. When we discuss the marketing channels these consist of local markets, catchments/ terminal markets, district market and municipality market. A local level village market is the market where goat producer sale their goats with in the village for consumption or breeding purpose as well as to the middleman. This study resulted that about 61% goats were sold with in the village markets. Middlemen and farmers were the other marketing agents to collect goats from different villages and made sales them to the catchments markets. Wholesalers purchase goats from terminal markets to supply goats. Chanauta, Butawal, Mugling and Lother from where wholesalers load the goats on bus roof or use trucks to transport goats to Pokhara. District headquarters markets and municipalities were the main marketing centres where wholesaler and middlemen supply the goats for marketing. Pokhara sub-municipality market is the main goat marketing centre in Western Nepal. The study revealed that there is lack of public goat marketing centres either at the village level or in the district headquarters and municipality.

EXISTING GOAT MARKET CHANNELS

In the existing goat marketing system in western hills it consists of farmers, wholesaler, middlemen, retailers and consumers. A wholesaler deals with the business of more than 40 goats collecting from the catchments market and terminal markets and transport them to the district head quarters. The wholesalers made sale to the retailers and sometimes middleman at a time where as middlemen deal with small numbers (10 to 15) of goats collecting from house to house. The retailers deal with the business of few goats collecting from the neighboring villages or purchase a few goats from the wholesaler/middleman then slaughter the goats and sale meat to the consumers. In the existing goat marketing system in many cases farmers themselves were involved for the sales of goats within the village. Wholesalers and middlemen were purchasing 46.96% of goats in the villages and middlemen were very near to them for collecting from the villages and selling them in headquarters areas (Table 1). Besides middlemen, retailers too were involved in goat marketing because they were the one who collect local goats and bring them in district headquarters and sale meat in share price not in weight because consumers preferred such type of meat even though the price is high. In the surveyed area wholesaler, middlemen, butchers and farmers were involved for the goat marketing. Figure 1 illustrates the major goat marketing channels. Goats were marketed by the wholesalers 46.96%, middlemen 45.34%, butchers 4% and others 3.7% respectively.

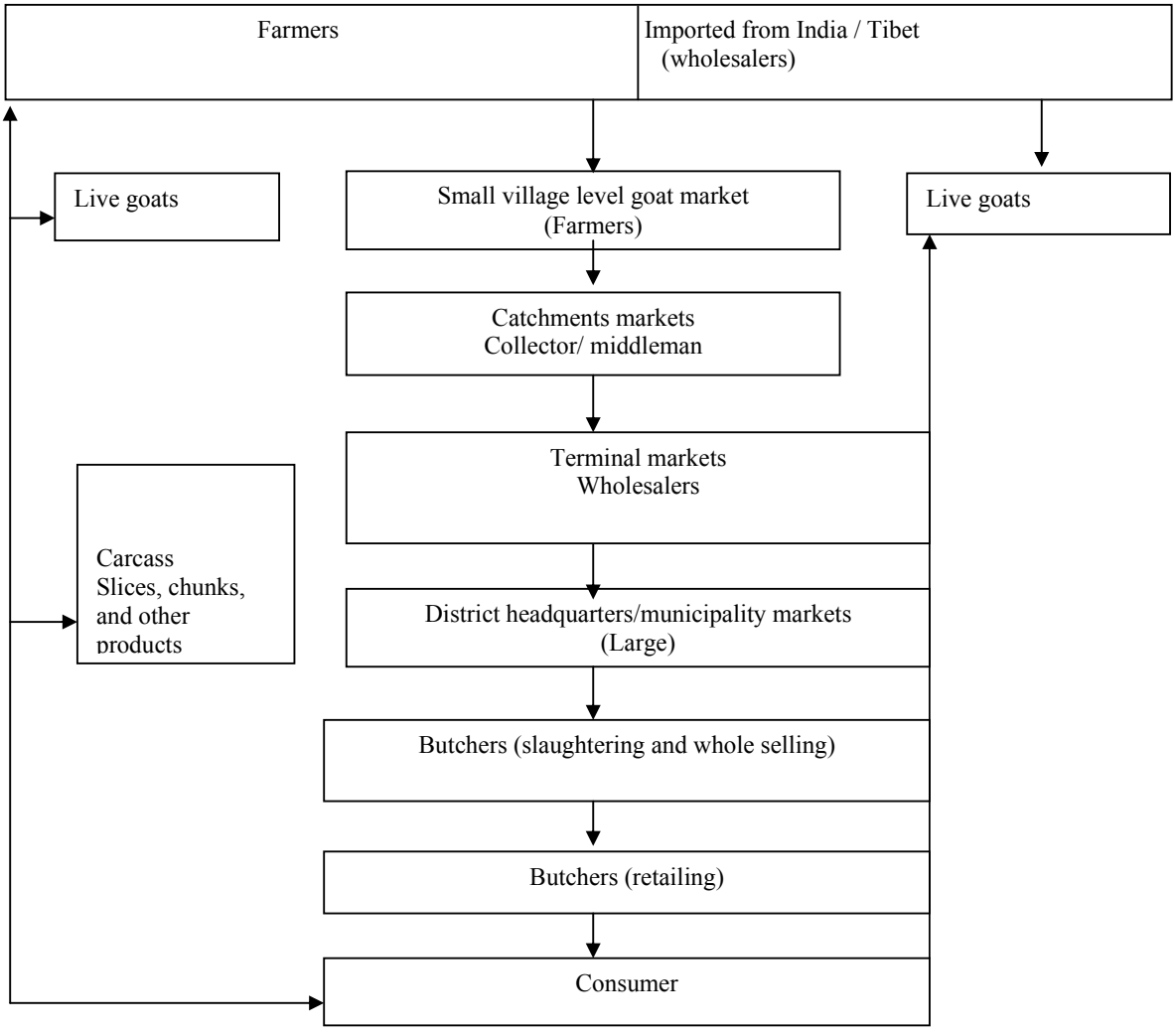


Figure 2. Major goat marketing channels in the western hills of Nepal.

Goat marketing centres and catchments

In the study area, although there is no marketing infrastructure existed in public sector either in the VDCs level or in district headquarters and municipalities, the goats produced were marketed through unorganized marketing centers. There are some marketing agents in the goat production area such as local markets, catchments (where farmers bring their goats on the way to market)/terminal markets (goats are collected to transport to the large markets), district market and municipality market. This study resulted that about 61.7% goats were sold with in the village markets. Middlemen and farmers collect goats from different villages and made sales them to the catchments markets.

There are some terminal markets from where the wholesalers purchase goats from the middleman and supply to the district headquarters and municipalities. From the terminal markets wholesalers load the goats on bus roof or use trucks to transport goats to Pokhara as well as other district headquarters. District headquarters markets and municipalities are the main marketing centers. According to the wholesalers they were bringing the goats from Lahan, Lother, Arghakhachi, Dang,

Surkhet, Jagarkot, Sallyan, Myagdi, Butawal and supply to the district headquarters and municipality markets. Traditional traders especially Muslims bring the goats from Indian boarder side such as Kanpur, Lakhnow, Hariana and Punjab. Those traditional traders supply goats to Sandhikharka, Tansen, Putalibazar, Walling, Damauli, Pokhara, Kushma, Baglung and Beni. Besides the Muslims, middlemen, farmers and local butchers also supply goats in the district headquarters. Local butchers purchase goats either from catchments or from rural area. Traders from Mustang bring mountain goats in thousand numbers around October to December in Pokhara valley. The mountain sheep and goats cover the local market of Beni, Baglungbazar, Kushmabazer, and Pokhara municipality. Study found that 60% goats were supplied from India to Nepal specially from Kanpur, Bareli, Lakhnow, Kalpe, Barabanki,Punjab and Hariyana. About 23% from village and 17% from catchments goats were supplied to the market area of Western Nepal. Middlemen, farmers and butchers were the main agents to supply goats from catchments and rural areas of Nepal.

Availability of goats in the existing market

In the study area, especially in district headquarters, the responses of butchers regarding the availability of goats as required were found only 52.82%. This is because of irregular supply of goats either importing from neighboring country or irregular market mechanism in the study area. Goats were available in Kaski district highest 100% followed by 75% each in Arghakhachi and Palpa districts. In Kaski district, highest availability was found due to good goat market mechanism. Wholesaler, middlemen and retailers were equally active for the goat marketing in this district.

Goat marketing at farm level

The analysis of data showed that 64% farmers marketed their goats at the local level, 3% marketed their goat in district headquarter, 3% sold to the whole seller, 29% to the middleman, 1% at Municipality. Less than 1% was sold for breeding purpose and for sacrificing the goat to the god and goddesses (Figure 3).

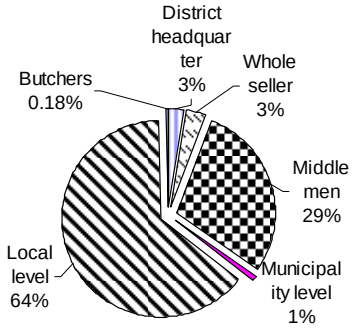


Figure 3. Goat marketing at farm level.

Visit of the middlemen in the village for goat marketing

Visit of the middlemen in the village for goat marketing was one of the important parts of marketing. In Western hills, 59.3% respondents reported that middlemen visited in the village for goat marketing. In Lamjung district, highest respondents (77%) reported that middlemen visited frequently in the villages to purchase goats and in Kaski district lowest (28%) followed by (42%) respondents in Parbat district (Table 1).

Average sales of goats per households per year

Goat keepers were selling their goats in average 2.3 per annum. Responding farmers reported that the selling of goats ranged from 1 to 4 goats per annum per households. Similarly, responding

farmers reported that they were getting average Rs 3802.6 per annum per household by selling their goats. Annual selling was ranged from Rs 375 to Rs 6023 per households.

Price determination of live goat

In the study area the price of live goat was determined through estimate (looking goat's physical appearance) system. In most cases, goat raising farmers were cheated because they need money immediately to support their family and were bound to sold their goats when the customers visit around their farm yard otherwise they do not have any option to sold their goats because of there is no public goat marketing centres. There was no any mechanism to minimum support price for goat to the goat raising farmers. Due to the lack of marketing centres there was no communication between the farmers and marketing agents related with the goat availability and the price. For price determination, there is bargaining between buyers and sellers but when buyers do cash down then unconsciously farmers accept the given price. In the study area almost farmers sell their goats on the basis of approximation price.

Physical market infrastructure

In the study area there is lack of physical market infrastructure and weak marketing channels. Specially, the village markets were very weak and hardly functional. When we say village market that is a place having tea shops. Local people get together, where farmers bring their goats around the village tea stalls and grocery shops where they may meet to the middleman or there may be in need of attention program to slaughter the goat and share the meat around the participating villagers. Catchments markets are identified places where the buying and selling of goat takes place in between farmers, middlemen and wholesalers but the date and time was not fixed. Government intervention is needed to occupy the public land and regulate for the marketing centers and strengthen such type of catchments by supporting the fenced. Farmers bring their goats and keep them in it. Physical market infrastructure might be the shelter for marketing agents. Marketing agents may have the facility of balance equipments and other necessary information for goat marketing. In terminal market individual farmers and middleman bring their goats for marketing. It is the identified place but there is lack of marketing physical infrastructure and not regular schedule of date and time of gathering of buyers and sellers may occur. In such markets mainly wholesalers' purchase goats from the middlemen and transport to the municipality and district headquarter markets. Market channels are not regulated and sustainable due to the lack of set of laws and regulations and other government support system.

Transportation of goats from collection to the market centres

Every year at least 42916 goats were sold in districts headquarters in the study area. About 20158 goats (47%) were brought from India and 22758 (53%) goats were collected from different villages. From the different districts the collection of goats around the road access area by the middleman, farmers and butchers transported to district headquarters through either trucks or sometime through the roof of bus. On an average about 200 goats were brought in Pokhara by the wholesaler at a time. The wholesalers/middleman reported that goats were collected from different study area districts. Traditional traders (Muslim) brought goats from India especially from Bareily, Etawah, Kalpi, Lukhnow, Barabanki, Kanpur and Punjab. These goats were supplied in headquarters of western hill districts of Nepal. Traders transported their goats from long distance by using the means of transportation. Mainly middleman and butchers drive their flocks from near villages of the district head quarter where farmers transported their goats to the market centres on foot. Marketing agents were using the vehicles for transportation for collecting goats. In the study area 49% marketing agents use the means of transportation, 41% drive the flocks and 10% agents draw goats by tying rope on neck. The use of means of transportation was high 80% in Lamjung district followed by 66% in Myagdi, Gorkha, Parbat and Palpa. Most of wholesalers transport goats from Indian boarder site to the market centres. About 39.64% butchers and middlemen mainly drive their flocks to the market centres. Driving of flocks was found high in Syangja district (100%) followed by in Tanahun district (56%).

Sanitation of meat stalls (shops)

In general most of the Nepalese people preferred to consume goat meat even it is more expensive as compared to buffalo meat, pork and chicken. The demand of goat meat during festivals like Dashain, Tihar and ceremony was found higher than in other times.

Especially in districts headquarters the meat stalls were observed regarding the sanitation of the stalls and environments of the surroundings. Better sanitation of the stalls, open and flies around the stalls, and polluted environment around the stalls were taken in to observation. About 55.54% meat stalls were found in better sanitation, 25.81% dirty and flies around and 16.90% were of dirty environment.

Goat marketing constraints

The marketing constraints existing in Nepal were lack of basic rules like minimum support price to the goat raising farmers, certification for the slaughter, infrastructure of market centres and lack of public awareness about the price of live weight of the goat such as kg. There was not enough well developed infrastructure for animal marketing even at metropolitan cities, sub metropolitan cities, municipalities, district market, and village level markets. Some times the animal may get sick from Pneumonia or lack of shade at night or change in climate/place. In such situation, farmers are bound to sell their goat for whatever price they get. Most of the farmers in rural area are less educated. Therefore, farmers have to loose their expected price. Underdeveloped marketing system was the major marketing constraints besides that production and management constraints were other types. In goat production and management predators, infertility, disease, parasitic, grazing, grass and feed were the other constraints that lead under supply of goats in the markets.

Table 2. Number of respondents involved in different surveyed districts

Particular	Arghakhachi	Palpa	Gulmi	Syangja	Parbat	Kaski	Baglung	Myagdi	Lamjung	Gorkha	Tanahun
Availability of goats	6(75)	2(50)	6(75)	4(50)	3(66)	36(100)	1(17)	3(43)	2(50)	2(33)	2(22)
Farmers knowledge on live weight	74(100)	77(100)	78(90)	83(100)	46(96)	77(100)	74(100)	76(100)	79(100)	79(99)	81(100)
Visit of the middle in the village	51(69)	59(77)	45(56)	44(53)	19(42)	22(28)	48(65)	29(37)	61(77)	59(74)	60(74)
Price determination of live goat n	74(100)	77(100)	81(100)	83(100)	46(100)	80(100)	74(100)	76(100)	79(100)	80(100)	81(100)
Transportation of goats	2(25)	4(50)	0	4(44)	2(50)	24(30)	1(14)	2(66)	4(80)	2(50)	1(11)
Vehicle Driving flocks	2(25)	3(38)	7(100)	3(33)	2(50)	11(14)	1(17)	1(33)	1(20)	2(50)	5(56)
Others	4(50)	1(13)	0	2(22)	0	2(3)	0	0	0	0	0
Sanitation of meat	2(33)	8(100)	8(73)	2(33)	3(33)	30(83)	3(33)	2(33)	2(50)	2(40)	6(10)
Open and flies around	2(33)	0	2(18)	2(33)	3(33)	1(3)	6(66)	2(33)	1(25)	2(40)	0
Dirty environment	2(33)	0	1(9)	2(25)	3(33)	0	0	2(33)	1(33)	1(20)	0

The number in parenthesis indicates percentage.

CONCLUSION

Most of the goat raising farmers, marketing agent like middlemen and wholesalers realized that there is lack of proper goat marketing centers in the VDCs level or in district headquarters/ municipalities of western hills of Nepal. There are some weak goat marketing centers and channels functioning from village to the district headquarters. Middlemen, wholesalers and butchers were

dealing the goat business in district headquarters and municipalities. Regarding the meat stalls, more than fifty percent of the respondents confessed there is lack of proper sanitation. Mainly, goat marketing constraints in western hills are goat production, marketing, appropriate marketing channels, improved breed, predators, infertility, diseases, parasites, grazing land, labors, fodder and forages, concentrate feed and appropriate shed. All the stakeholders including goat raising farmers have to overcome from these constraints through the package of goat production and marketing program which leads to improve the goat marketing condition in Nepal. This study strongly recommended that government should have make policy for the provision of establishing new goat marketing centers in each VDCs and district headquarters. Marketing channels agents' likes wholesaler, middleman and retailers must hold the license to deal business with goat.

ACKNOWLEDGEMENT

The support of Regional Agricultural Research Station, Lumle for providing an opportunity to carry out this study is gratefully acknowledged. The support of National Agriculture Research and Development Fund (NARDF) for funding this research as a part of project is gratefully acknowledged. We would like to extend our special thanks to Mr IP Gautam, for encouraging proposal writing, carryout the survey and editing this part. Thanks are due to Mr BS Shrestha. Thanks are also due to farmers for their valuable attention and pertinent information during field survey.

REFERENCES

Kharel M. 2000. Glimpse of goat marketing situation in Nepal. **In:** *Proceedings of the Third Environmentally Sustainable Livestock Production for Poverty Alleviation Animal Science Convention*. Nepal Animal Science Association (NASA). Lalitpur. Nepal.

MoAC. 2002.*Statistical information on Nepalese agriculture 2001/2002*. Agri-Business Promotion and Statistics Division, Ministry of Agriculture and Cooperatives, Singha Durbar, Kathmandu, Nepal.

MoF. 2006. *Annual commodity description*. Department of Custom, Ministry of Finance, Government of Nepal.

Nepali MB, B Shrestha, RP Bari and KN Dhakal. 1998. *Baseline and need identification survey of agro-ecological research site at Maduwa of Syangja district representing low hills in Western Nepal*. Agriculture Research Station, Lumle, NARC, Nepal.

Panday N. 1998. *Technical report on gender dynamic in goat production*. Outreach Research Division (ORD), Khumaltar, Lalitpur, NARC, Nepal.

Thakur NS, M Joshi, MR Tawari, B Shrestha and L Pandey. 2003. *Socio-economic study on goat farming: A case study of command area under RARS (Goat), Bandipur*. Outreach Research Division (ORD), Khumaltar, Lalitpur, NARC, Nepal.

Measuring Returns from Improved Rice, Maize and Wheat Research in Nepal

Naresh S. Thakur*[1], Min N. Paudel[2], Devendra Gauchan[1] and Bhawana Shrestha[3]

[1] Socio-economics and Agricultural Research Policy Division, NARC, Nepal
[2] Outreach Research Division, NARC, Nepal
[3] Monitoring and Evaluation Division, NARC, Nepal

ABSTRACT

Studies on returns to research have been established in different countries to justify research funding and allocation of research priorities. However in Nepal, there are no scientific studies carried out recently on return to research investment in major crops and commodities, despite priority given in agricultural sector. This paper has summarized the research investment with respect to major cereals viz; rice, maize, and wheat and income generated by the impact of improved varieties of these crops in Nepal. Internal rate of return for major cereal crops (rice, maize, and wheat) was estimated using time series data of 1995 to 2005. The findings revealed that there is a negative annual growth rate (-0.59%) of NARC budget during 1998-2004. There was a food deficit during mid nineties, however, in the late nineties while despite tremendous curtailing of budget for research, a surplus of food grain was observed. Although the situation of positive food grain balance is not the result of current year's efforts on research it could be the impacts of years of efforts that were in a continuous pace in the past for agriculture R&D coupled with the impacts of extension related activities in the country. Food surplus is mainly contributed by the efforts of massive release of improved varieties of crops by NARC during late nineties. The internal rate of return (IRR) of these cereals for a decade (1995-2004) was in the range of 84-105%, which is around Rs 235673 millions of income generation while investment for their research was Rs 559 millions (0.24% of their revenue). This indicates that there is gross under funding on research for major crops, despite their significant contribution in national economy, food security, and livelihood improvement in Nepal. Finally paper suggests for increased investment in agricultural research for meeting increasing needs of food, income, and employment of growing population as well as enhancing and sustaining future agricultural R&D in the country.

Key words: Agriculture, food crops, impact, internal rate of return, livelihood, research investment

INTRODUCTION

Agriculture is the largest sector of Nepalese economy, which contributes 39% to the total gross domestic product (GDP) and 65% to the employment of the economically active population (MoAC 2005). Crop coverage is dominated by rice (35%), followed by maize (20%), wheat (16%), cash crops (10%), legumes (7%), minor crops (7%), and horticultural crops (5%). Area coverage by improved seeds of rice, maize and wheat are 74, 69 and 91%, respectively (CBS 2004, MoAC 2005). Likewise, these crops are cultivated in

15,41,729 ha, 8,49,892 ha and 6,75,807 ha with respective productivity of 2.8 t/ha, 2.0 t/ha, and 2.1 t/ha, (MoAC 2005).

Agricultural research potentially helps the poor by augmenting producer's income, reducing food prices to consumers, enhancing the demand for labor, and stimulating growth linkages in the economy (Walker 2000). Agricultural research is an investment for future food production, productivity, and food security. New technologies in agriculture are developed by research while extension takes them to the end users. There have been serious efforts to introduce latest technologies in agriculture research. Technologies are introduced in Nepal by international, regional, and local networks of research activities. In a country like Nepal it is very difficult to conduct basic research and most of technologies are adaptive type and only a few of them are basic ones. In comparison to the countries of South and Southeast Asia the productivity of agriculture in Nepal is very low for cereals (less than 2.8 t/ha).

Agricultural research in Nepal dates back to early 1960s with the establishment of agricultural research stations and commodity research programs in various agroecological zones of the country. These research stations and commodity programs were under same umbrella with agricultural extension despite there were several reorganization of the agricultural departments within the Ministry of Agriculture. Until 1990 the Department of Agriculture (DoA) and the Department of Livestock Services (DoLS) mainly carried out agricultural research under the ministry of Agriculture. In order to focus research on major commodities and enhance the national capability in agricultural research, the Government of Nepal under an Act in 1991 created the Nepal Agricultural Research Council (NARC) an autonomous institution with a national mandate for agriculture research in Nepal.

> NARC's attention is focused on improving access and uptake of complete package of improved technologies in farmers' level that is directed by Agriculture Perspective Plan (1995), National Periodic Plans and NARC Vision (2002). These have laid emphasis to poverty reduction by increasing agricultural productivity and farm income through adoption of improved technologies. To address APP priorities, Government of Nepal has allocated a sizable amount of resources to improve food security as well as the living standards of the rural masses. Major source of resources for agricultural gross domestic product (AGDP) in Nepal comes from APP priority commodities especially cereals such as rice, maize and, wheat, which are the three prime staples in the country. NARC has also given a high priority for research in these crops. However, investment in research in Nepal is very low as compared to its contribution in national economy. Available data show that investment in agriculture research in many part of the world is around from 0.5 to 2% of AGDP while in Nepal it is less than 0.2%.

Studies on returns to crops have been established in different countries. Echevrria (1990) has done work on return to investment on wheat in different countries and documented the internal rate of returns as 90% (Mexico), 30-35% (Bangladesh), 97% (USA), 30-39% (Canada), 58% (Pakistan), 18-36% (Peru), and 110% (Brazil). Similarly Mruthyunjaya et al (2004) estimated IRR of 24-67% for cultivation practices of rice with other crops on rainfed technology in India. In Nepal, return to investment was estimated with IRR of 74% for wheat research and about 84% for wheat breeding during seventies to nineties (Morris et al 1994). They also projected IRR of 49% for future wheat breeding in Nepal. A few studies conducted in Nepal have indicated that the investment on the crops research is worth paying because of a very positive internal rate of return as considerable farm families have been benefited from these crop technologies (Hocking et al 1995). However, recently (after 1990s) there are no scientific studies dealing with impact of research investment in major crops such as rice, wheat and maize in aggregate to justify future research resource allocation and funding priorities in Nepal. Considering the importance of research investment and justification for future allocation of research resources in major crops, it has therefore become imperative to know how much budget is allocated for agriculture research and what the internal rate of return is for the investment as a whole for these major as well.

The prime objective of the study is to provide information for major food crops (rice, maize, and wheat) with respect to economic parameters, which could provide justification of expenditure incurred on these crops in Nepal. However, the specific objectives are to provide scientific information about economic tools that could help provide planners and decision makers to allocate appropriate funds for attaining fruitful return from research and development (R&D) of these crops in days to come.

MATERIALS AND METHODS

This paper tries to gather information for major food crops with respect to actual expenditure on research that could explain what percentage of internal rate of return (IRR) and growth rate (g) are achieved by the expenditure incurred on them. Ultimately findings could be used as tools for convincing policy makers, donors, research managers, and related authorities in order to decide an appropriate amount of funds allocated for NARC to conduct meaningful research for these crops as well as setting future priorities in agriculture R&D. This will also help justify research work of NARC effectively in future because there is a dearth of resources in NARC as compared to its contributions for the country as a whole. Since NARC technologies are being disseminated and their adoption have been picking up in the farmer's field, the economic impacts are visible from various sources. In today's world of scarce public funding and greater accountability, governments, donors and research managers are increasingly demanding assessment of the economic returns to their investments in research (Maredia et al 2000).

Rice, maize, and wheat crops are selected purposively to calculate economics parameters of IRR, from time series data of 1995 - 2005. Actual expenditure incurred for these crops was taken from the expenditure of respective national research programs, percentage of actual expenditure in Agronomy (rice 70%, wheat 20%) and Botany (rice 40%, wheat 30%, and maize 20%) Divisions, Khumaltar, and cost of cultivation based on the average cost of cultivations reported by the Economic Analysis Division of Department of Agriculture for the districts situated for commodity programs of these crops. Percentage of area covered by improved varieties of these crops was recorded from the time series data of statistical survey of Agriculture of the Ministry of Agriculture and Cooperative (MoAC 2005). Production of improved varieties for these crops was estimated with respect to their respective percentage of areas covered by improved varieties times productivity of improved variety. In the areas grown for improved varieties, a projection of production for local variety was estimated by multiplying productivity of local variety. The actual production for improved varieties was the difference between the productions of improved varieties less projected productions of local varieties in the areas grown for improved varieties. The analytical tools for financial analysis for IRR and annual growth rate (Gittinger 1972) were estimated from the difference of production between improved varieties and projected production of local varieties obtained from the areas grown for improved varieties. This production difference was again estimated based on the current market price of the respective crops that gave total production in prevailing market price. A period of ten years (1995-2005) is considered for economic analysis. Market prices are used for computing the value of production because price and transportation subsidies on inputs have been removed by the government of Nepal. A discounting factor is used for estimating the present value of costs and returns to be used for calculating NPV.

There are several options for evaluating the stream IRR and annual growth rate (G). The best approach is to use a combination of IRR and G calculations to summarize the relevant information on the total returns to research. These economic parameters are and the standard procedures are outlined below:

Where,

a) The IRR is defined as the rate of an investment which we equate the present value of benefits and costs. It is found by an iterative process and is equivalent to the discount rate (r) that satisfies the following relationship:

$$\sum\left[(Bt - Ct)/(1 + IRR)t\right] = 0$$

Where, IRR represents the internal rate of return, B benefits from the crop commodity programs, and C actual expenditure of the crop commodity programs plus cost of cultivation of crop in question.

Bt = Benefit in year t

Ct = Research costs in year t
t = year goes from 0 to n< ∞
b) The annual growth rate;

$$G = \left[(vt/v0)^{1/t} - 1 \right] \times 100$$

Where; G be the growth rate; vt = Value in time t, and v0 = Starting value in (time = 0),
This ration compares the discounted benefits to discounted costs.

RESULTS AND DISCUSSION

Area and production

Analysis of the data revealed that the total area of rice, maize, and wheat has increased by 12.7%, 10.2%, and 8.1%, respectively in 2005 as compared to 1995. It could be due to expansion of net areas, which brought new land into cultivation and consequently increased in cropping intensity. The most significant change has been observed in 2004 (15,59,436 ha) in areas planted to rice. The area increases to maize and wheat were not substantial like that of rice in the same period. Similarly, increased productivity of rice, maize, and wheat is estimated by 30.9%, 19.6%, and 41.1%, respectively in the 2005 as compared to 1995 (Table 1). The rate of change in wheat productivity is highly significant over the period of 10 years because of many improved technologies adopted by farmers. The increase in cereal crop productivity is mainly due to improved quality seeds and complete package of practices of technologies generated and developed by respective commodity research programs. However, the component of increase into area and yield indicates that the area expansion play a significant role to enhance the productivity in cereals because of widespread prevalence of local production practices. Significance increases in agricultural production, however, are possible with the development, production and distribution of new variety seeds and improved technologies.

Table 1. Trend in cereal grains production for 1995 and 2005

Crop	Total cultivated area, ha			Productivity of improved variety, t/ha		
	1995	2005	% Change	1995	2005	% Change
Rice	1368423	1541729	12.7	2.623	3.037	15.8
Maize	771410	849892	10.2	1.881	2.009	6.8
Wheat	624329	675807	6.4	1.598	2.134	33.5

Source: MoAC 2005.

There is a high potential for spreading improved varieties of major cereals because of R&D activities, which has been developing full package of technology through NARC, extension and I/NGOs in farmer's level. Total released varieties of rice, maize and wheat are 49, 17 and 28 respectively during the study period 1995-2004. Farmers had increased options for choosing and selecting seeds of improved varieties after the inception of NARC as per their local socioeconomic needs and to fit in their cropping systems in a given recommended domain. Productivity change brought about by improved varieties ranged from 15-33%, which is very encouraging as compared to other countries in Asia. Pandey and Rajatatsereekul (1999) reported that in Thailand increase in rice yield from improved varieties over currently grown varieties was 10%. This could be because of wide spread cultivation of improved varieties in Thailand compared to few improved varieties grown in Nepal. The use of improved seeds in Nepal is gradually increasing for wheat, rice and maize (Table 2). Wheat has maximum coverage of improved varieties followed by maize and rice indicating the respective comparative advantage of these crops with respect to generation and adoption of these varieties in farmers' fields. Data indicate that only limited percent of farmers still continue to grow indigenous varieties from their own sources in these crops.

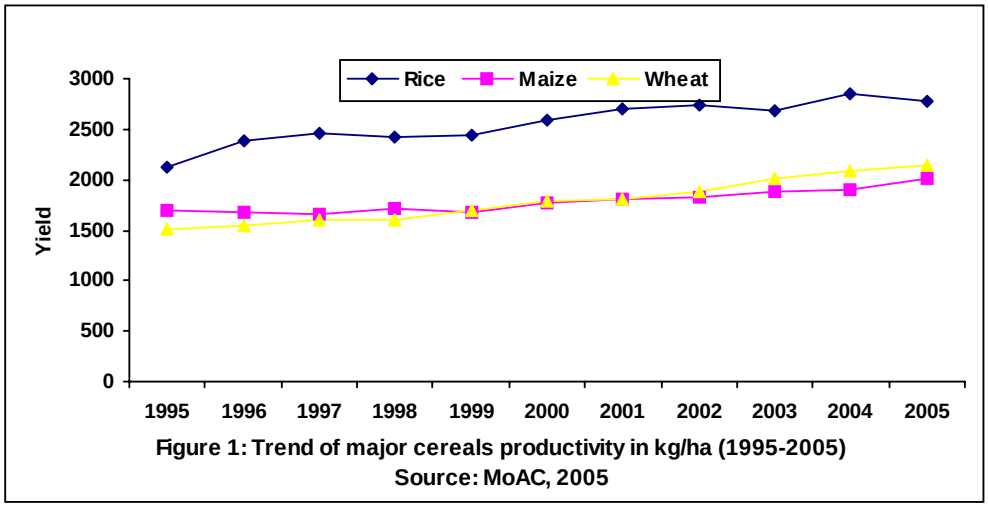


Figure 1. Trend of rice, maize and wheat productivity from 1995 to 2005.

TABLE 2. AREA COVERED BY HYVS OF MAJOR STAPLES IN 1995 AND 2004

Crop/year	Total area coverage, ha		Area under HYVs, ha		% Area under HYVs	
	1995	2004	1995	2004	1995	2004
Rice	1369423	1559436	767014	1291837	56.01	82.84
Maize	771410	834285	448035	709309	58.08	85.02
Wheat	624329	664589	527808	633951	84.51	95.39

Source: MoAC 2005.

For these crops the income generation from improved varieties is extensively higher than that of local. However, there is a remarkable contribution of local varieties to generate income as well. The maximum contribution of improved varieties coverage for these crops is about 80-95% (Table 2) and there is a corresponding contribution of income generation by them. Income generated by the impact of improved varieties of these crops from 1997/98 to 2004/05 was estimated Rs 235673.54 millions while investment for their research was only of 0.0025% of their revenue (Paudel et al 2006). At present, budget allocation for full phase research in NARC is not up to the extent what it should be. However, the impact of research in cereals has been clearly visualized by positive change in income in current year as compared to 1995, which was before inception of NARC. For a meaningful contribution of these crops to the national AGDP, the budget allocation for research should be allocated at par with developing countries where 0.56 percentage of AGDP was put for research in 51 developing countries around 20 years ago (Thapa 1996). In present context, budget allocation to research in neighboring countries including India has increased more than 0.5 percent of AGDP while in Nepal this is hovering around 0.016 percent of AGDP (MoAC 2005, ITAD/New Era, 2005). This clearly showed that agriculture research in Nepal is grossly under funded.

To cite an analogy of expenditure on research, income obtained from improved varieties, proportion of research expenditure to income obtained, and IRR for major cereal crops has been illustrated (Table 3). Research cost for rice, maize, and wheat plus cost of cultivation for districts where these crops commodity programs are situated is considered as the actual cost of research spent by national crop commodity programs of these crops plus actual expenditure incurred for Agronomy and Botany Division, Khumaltar during the period. So far as research investment with respect to income obtained from improved varieties is concerned it is the difference between the productions obtained from the areas grown for improved varieties less projected production of local varieties from the areas grown for improved varieties. Therefore, the percentage of proportion with respect to research investment is the income solely obtained from the income of improved varieties of cereals that have covered certain percentage of total areas.

A comparison of trend in major cereals production with respect to area cultivated and productivity of improved variety in 1995 and 2005 has shown that both the area and productivity of major cereals have been increased (Table 1). The increase in productivity for maize is found less than increase in cultivated areas during ten years while increase in productivity for rice and wheat is significantly more than that of areas for the same period. This suggests that there has been remarkable impact of technologies to yield a

positive effect on productivity for rice and wheat compared to maize in which increase in production was associated with increase in cultivated area. This may be because of the condition that maize is comparatively grown in less fertile land than that of rice and wheat, which are grown in more fertile land. At the same time maize is a crop of poor man who lives in hills where the impact of new technologies are less effective compared to rice and wheat.

Table 3. A comparison of expenditure, return, and internal rate of return from 1995 to 2004

Crop	Research expenditure (million Rs)	Income obtained from improved variety (million Rs)	Proportion of research investment with respect to income obtained from improved variety, %	Internal rate of return (IRR), %
Rice	167.12	151036.64	0.1106	105.00
Maize	182.89	38299.17	0.4775	84.04
Wheat	209.54	46337.73	0.4522	103.02
Total	559.55	235673.54	-	-

Source: ITD/New Era 2005.

Our concern is that out of millions of income generated by the impact of new technologies at least 1%, which is equivalent to Rs 2356.7 million at present value, should be invested for research (Table 3). But research investment as of now for these crops is 0.24% of the income obtained from improved variety. Although there are numerous commodities for which new technologies have been generated by agriculture research only the most important cereals is shown for an example. Because these commodities cover around 99% of the total cultivated areas in Nepal. The IRR of cereals for a decade is in the range of 84-105%, which is a very encouraging for investment, where agriculture is the predominant sector for employment generation and GDP contribution.

New technologies primarily come through NARC however, some of the farmers bordering to neighboring countries get improved technologies. The number of released varieties of rice, maize, and wheat are 8, 5 and 6, respectively during 1995-2004 (Table 4). Farmers groups in different locations also produce HYVs in participatory approach in their fields for seed multiplication. Productivity of improved varieties is far more than the national productivity of these crops. This potentiality of productivity could be enhanced by effective dissemination of such technologies in farmer's field in wider scale.

Table 4. Improved varieties of rice, maize and wheat released during 1995-2004

Variety	Released year	Productivity, t/ha	Recommendation domain
I. Rice			
a. Early			
1. Hardinath-1	2004	5.0	Tarai, inner Tarai, valley and river basin areas
b. Main season			
1. Chandannath-1	2002	6.0	Jumla valley and similar high hills (2300 m)
2. Chandannath-3	2002	6.0	Jumla valley and similar high hills (2300 m)
3. Khumal-11	2002	10.0	Kathmandu valley and similar areas
4. Manjushree-2	2002	8.3	Kathmandu valley and similar areas
5. Rampur Masuli	1999	5.7	Tarai, inner Tarai, foothills in Central and Western development regions (> 900 m)
6. Khumal-6	1999	7.8	Kathmandu valley and similar areas
7. Machhapuchhre-3	1996	5.0	Mid to high hills for cold climates
II. Maize			
1. Gaurav Hybrid	2003	9.0	Tarai, inner Tarai and foothills (summer and winter)
2. Manakamana-3	2002	10.6	Mid-hills of Eastern, Central and Western regions
3. Ganesh-1	1997	5.0	High hills
4. Rampur-1	1995	3.8	Tarai, inner Tarai
5. Arun-1	1995	4.0	Tarai, inner Tarai
III. Wheat			
1. Gautam	2004	5.0	Timely and late sown irrigated condition of Tarai, Taars and lower valleys

Variety	Released year	Productivity, t/ha	Recommendation domain
2. BL-1473	1999	4.0	Irrigated medium to high fertility condition of whole Tarai, Taar and low altitude
3. Kanti	1997	5.5	All hill areas
4. Pasanglhamu	1997	6.7	Mid-hills (Kathmandu, Jumla)
5. Rohini	1997	4.1	Tarai, Taar and < 1000 m
6. Achyut	1997	4.5	Tarai, Taar and < 1000 m

Research expenditure

The study revealed that the proportion of research investment for NARC in comparison with national and MoAC budget has declined and reached climax in 2002. It is important to note that the share of NARC to the national and MoAC budget has increased after 2002 and reached up to the level of 0.58% and 14.71%, respectively. National and MoAC budget have increased in a small pace for seven years (1998-2005) but there is a negative allocation of budget for NARC during the same period. The annual growth rate of budget for NARC is estimated to be -0.59% whereas national and MoAC budget is 7.63% and 2.17%, respectively from 1998 to 2005 (Tables 5). However, APP has set a target of doubling agricultural research budget from the current year level of Rs 180 million to Rs 360 million per year for the next 20 years (APP 1995). Low budget allocation for NARC indicates less priority for research in the country. Such a negative trend of budget for research can result into low productivity in the long run, which should not be the case of agro-based country like Nepal. There has been significantly decrease of donor support for NARC due to political instability, resource crunch and insurgency in the recent years in the country.

Table 5. Comparison of NARC budget with national and MoAC budget ('000 NRs)

Year	National budget	MoAC budget	NARC budget	Proportion of NARC budget to national budget, %	Proportion of NARC budget to MoAC budget, %
1995	-	-	205382	-	-
1996	21904189	2388779	195412	0.89	8.18
1997	-	-	207393	-	-
1998*	62022294	2267193	326422	0.53	14.40
1999*	69693337	2350968	315706	0.45	13.43
2000*	77238226	2857489	322597	0.48	13.04
2001*	91621335	3360573	486586	0.53	14.48
2002*	99792219	3927556	577760	0.58	14.71
2003*	96124796	2423526	297780	0.31	12.29
2004*	102400000	2472945	300575	0.29	12.15
2005*	111689900	2692284	311249	0.28	11.56
Growth rate %	7.63	2.17	(-) 0.59		

*Source: * ITAD/New Era 2005.*

A ten year's edible grain production and requirement balance sheet of Nepal has indicated that from the year 1994/95 to 1998/99, there was a food deficit while from the year 1999/00 to 2003/04 there is a food surplus (Table 6). This shows that the impact of research in Nepal has shown its effect on food grain production with a positive food balance in the recent years. Despite a tremendous curtailing of budget for research, the growth rate of a budget for NARC being negative (-.059%) while it is positive for national budget (7.63%) and budget of MoAC (2.17%) for the same period, NARC is still giving vital technologies to agriculture in Nepal. The positive impact of research can not be visualized in few years' efforts rather it is the impact of years' that were in a continuous pace in the past. At the same time agriculture in Nepal is dependent on monsoon because of massive rainfed nature farming in the country. Because the final outcome of research are only visible during a long period of time, may be tens of years of investment on research could only be visible. Therefore, here

comes the importance of continuous efforts on agriculture R&D, which has a pivotal role for maintaining food sustainability in a country whose economy is dependent on agriculture.

Table 6. Edible cereal grain production and requirement of Nepal (1995-2004)

Fiscal year	Total production, ton	Total requirement, ton	Total balance, ton
1994/95	3397760	3882915	(-) 485155
1995/96	3913878	3948229	(-) 34351
1996/97	3972587	4079135	(-) 106548
1997/98	4027349	4178077	(-) 150728
1998/99	4097612	4279491	(-) 181879
1999/00	4451939	4383443	68496
2000/01	4513179	4430128	83051
2001/02	4543049	4463027	80022
2002/03	4641466	4565820	75646
2003/04	4835973	4683272	152701

[a] Source: Ministry of Agriculture and Cooperatives 2005.

The data presented in this study show that agricultural research that will benefit small farmers through the availability of daily staples, nutrition, low food prices and income in a poor developing country like Nepal is severely under funded. Social rates of return to most past investments in agricultural research have exceeded 20 percent a year. For developing-country governments this is a most worthwhile investment. Yet low-income developing countries invest less than 0.5 percent of the value of farm production in agricultural research, compared with 2–5% in higher-income countries. Since research is typically a public good, it needs to be financed to a large extent by the government. In view of declining donor support for agriculture research, NARC needs to be proactive for looking funds through various sources including private sectors, I/NGOS, bilateral donors, international organizations, etc. The changing socio-economic and institutional context of R&D, the changing nature of clients, partners and funding sources are to be understood by the NARC to make its research system effective, responsive and dynamic in partnerships with multiple stakeholders (Gauchan et al 2003). There is an urgent need to improve efficiency, management, and the incentive in research in order to maximize benefits from limited government budget provision to research. This is essential not only for improving the performance of agricultural sector but also to have a sustainable funding source.

Estimating the actual return to investment in agricultural research is complicated by the dispersed area over which research impacts may occur and the large number of external factors in addition to research induced technical changes affecting production, productivity, income and employment etc. Estimated rates of return to research are distorted by problems of attributing the credit for particular research-induced productivity increases, among research expenditures undertaken at different times, in different places, and by different agencies (Alston et al 1998).

In addition, since separate aggregated data on improved varieties were not available, the data used for estimating returns here are mainly of general time series data on production and productivity where improved variety data were imputed from area on overall crop varieties. Similarly the increased production and productivity of crops are combined effect of research and extension interventions, where here only research costs particularly of major commodity programs and two of the disciplinary divisions are included. Moreover, the study is primarily based on secondary information without using primary field survey data on adoption of improved technologies, costs, market price, yield, elasticity and other data from experimental trials. This study also does not take into account of effects of other policy and programs for which dynamic analytical methods such as economic surplus measures and econometrics technique could be used.

Agricultural research generates several indirect economic effects, including labor-market effects and growth linkages of technical change, that go beyond the cost-benefit analysis using IRR, NPV, BCR and use of standard measures of consumer and producer surpluses. The effect of technology adoption on demand for labor has potentially major indirect benefits. Overall effects of technical change as a result of research ultimately depend on general growth and income effects induced throughout the economy by consumption, input and output linkages. A full accounting of these effects requires a general equilibrium (GE) model (Maredia et al 2000). In the future rigorous analytical methods combining technical, scientific, and economic information from a number of sources could be taken into consideration while estimating rate of return on agricultural research.

CONCLUSION

The area and productivity have been positively increasing during 1995-2005 due to improved quality seeds and complete package of practices generated and developed by NARC research programs. Productivity of cereals has brought to change by improved varieties from 15 to 22%, which is very encouraging as compared to other countries in Asia. Income generated by the impact of improved varieties of major cereals during 1998-2005 was estimated Rs 235673.54 million but investment for the research was only 0.0025% of their income. The total budget allocation for NARC is hovering around 0.016% of AGDP. This clearly indicates that agricultural research in Nepal is grossly under funded. For agriculture research there should be at least 1% of the total income generated by the impact of new technologies at present value. But now, research investment for these crops is 0.24% of the income obtained form improved varieties during a decade. The estimate of IRR of major cereals is in the range of 84-105%, which is a very encoring for investment where agriculture is the main livelihood sector of masses especially the poor. During a decade (1995-2004) the budget growth rate for Government of Nepal (7.62%), MoAC (2.17%) is positive while for NARC it is negative (-.0.59%). The low budget allocation for NARC indicates less priority for research investment in the country. Such a negative growth rate of budget allocation for research can result into low productivity in the long run, which should not be the case of agro-based country like Nepal.

There is an urgent need to significantly increase NARC budget allocation for research in order to generate new technologies with active participation of farmers aimed at increasing agricultural production and productivity. Government of Nepal should provide budget allocation aimed at achieving the minimum target of investing at least 1% of the AGDP in agricultural research in periodic five-year plans from now onwards. In current situation, government of Nepal should also declare strong commitment to agricultural research by substantially increasing budget allocation in order to attract increasing donor assistance. Price and transportation subsidies on inputs were removed in Nepal as part of the fiscal burden by the government. Fund saved through this measure can be used for NARC research through farmers' participation in research. In view of declining donor support for agriculture research, there should be functional linkage among public private and partnership principles of concerned stakeholders.

ACKNOWLEDGEMENT

We are thankful to Agriculture Botany and Agronomy Divisions for providing annual budget incurred for cereal crop research activities at Khumaltar. We appreciate the help and co-operation provided by Senior Account Officers, NARC, Mr Manas Kumar Kandel and Mr Dev Raj Dhakal who were instrumental for giving annual time series budget expenditure on cereals.

REFERENCES

Alston JM, GW Norton and PG Pardey. 1998. *Science under scarcity: Principles and practice for agricultural research evaluation and priority setting*. Ithaca, NY: Cornell University Press.

APP. 1995. *Agriculture prospective plan*. John Miller Inc., National Planning Commission and Agricultural Projects Service Center (APROSC), Nepal.

CBS. 2004. Central Bureau of Statistics, National Planning Commission, Nepal.

Echevrria RG. 1990. Assessing the impacts of agriculture research. **In:** *Methods for diagnosing research systems constraints and assessing the impact of agriculture research II. ISNAR* (RG Echevrria, ed). The Hague, The Netherlands.

Gauchan D, M Joshi and S Biggs. 2003. A strategy for strengthening participatory technology development in agricultural and natural resources innovations systems: The case of Nepal. *International Journal of Technology Management and Sustainable Development* 2(1):39-52.

Gittinger JP. 1972. Economic analysis of agricultural projects. The Economic Development Institute, International Bank for Reconstruction and Development. The John Hopkin University Press, Baltimore and London. Pp. 221.

Hocking A, Hocking D and SB Mathema. 1995. An analysis of research management at Lumle and Pakhribas Agricultural Centers in Nepal. *A consultant report to the Overseas Development Adminstration (ODA)*, UK.

ITAD/New Era. 2005. A review of research impact, responsiveness and future priorities in the agriculture sector in Nepal. *Final Report*. ITAD, New Era, Nepal.

Maredia M, D Byerlee and J Anderson. 2000. Ex post evaluation of economic impacts of agricultural research programs: A tour of good practice. Paper presented to the *Workshop on The Future of Impact Assessment in CGIAR: Needs, constraints and options*, 3-5 May, Rome. Standing Panel on Impact Assessment (SPIA) of the Technical, Advisory Committee, Rome.

MoAC. 2005. *Statistical information on Nepalese agriculture: Time series information (1991-2004)*. Ministry of Agriculture and Cooperative, Kathmandu, Nepal.

Morris ML, HJ Dubin and T Pokhrel. 1994. Returns to wheat breeding research in Nepal. *Agricultural Economics* 10:269-282.

Mruthyunjaya SP, LM Pandey and AK Jha. 2004. Impacts of selected technologies refined under NATP. *Agricultural Economics Research Review* 17:1-16. National Centre for Agricultural Economics and Policy Research (NCAP), New Delhi.

NARC Vision. 2002. Nepal Agricultural Research Council (NARC), Kathmandu, Nepal.

Pandey S and S Rajatasereekul. 1999. Economics of plant breeding: The value of shorter breeding cycles for rice in Northeast Thailand. *Field Crops Res.* 64:182-197.

Paudel MN, NS Thakur, D Gauchan and RP Upreti. 2006. A retrospective and perspective of outreach research in Nepal. *Agricultural Development Journal* 3:1-19.

Thapa GB. 1996. *Resource allocation to agriculture research in Nepal*. Agro-enterprise and Technology System Project (ATSP), NARC, Khumaltar.

Walker T. 2000. Reasonable expectations on the prospects for documenting the impact of agricultural research on poverty in ex-post case studies. *Food Policy* 25:515–530.

RESEARCH NOTE

Identification of Suitable Planting Method for Potato + Maize Intercropping System in the Hill of Eastern Nepal

Tanka P. Barakoti

Agricultural Research Station, Pakhribas, Dhankuta, Nepal Agricultural Research Council
<drtp.barakoti@gmail.com>

ABSTRACT

Field experiments to identify suitable planting method under potato + maize system in the high hill (2200 m) of eastern Nepal Sindhuwa, Dhankuta, was conducted during 2000 and 2001 seasons for the potato variety Hale and maize variety Ganesh 1. Of the different planting methods tested, significantly the highest yield of potato (15.5 t/ha) was recorded from the alternate row planting followed by recommended practice and flat row planting. Early emergence (by 7-12 days) was in farmers' practice, whereas uniform plants (1-5 scale) observed in flat row and double row planting. Maize did not follow this trend. Double row and farmer's practice favored good ground coverage (80-95%) by potato plants, which attained height from 49.8cm (farmer's practice) to 56.8 cm (flat row). Number of main stems/plant was higher in all treatments, except farmer's practice. Similar trend was followed in tuber numbers. Maturity of crops did not depend on planting methods. Final stand of potato ranged between 46.9 (alternate row) and 68.6 thousand/ha (farmer's practice). Late blight and bacterial wilt infection was higher in the farmers' practice. The height of maize plants was significantly differed between sole cropping (220 cm), which yielded higher followed by alternate row (183 cm) and flat row plantings. Gross income from maize and potato was higher in flat row planting. The data revealed that alternate row and flat row planting methods were superior over the common farmers' practice and the recommended practice, so the identified planting methods were recommended.

Key words: **Effect, intercropping, maize, planting method, potato**

INTRODUCTION

Potato (*Solanum tuberosum* L.) and maize (*Zea mays* L.) are the main staple food crops in the hills of Nepal. In the mid hills, maize ranks first in terms of area and production, similarly potato is the dominating crop in the high hills (above 2000 m). Inter-cropping of these two crops is common in the potato-based cropping system adopted by the farmers (above 1800 masl) as potato + maize system. The average yields of potatoes (9.50 t/ha) and maize (1.60 t/ha) in the eastern hills are lower than the national average (10.5 and 1.77 t/ha respectively). One of its reasons identified was lack of suitable planting techniques and spacing (Barakoti 2001a). One of the research areas identified by Pakhribas Agriculture Centre was to identify suitable husbandry practices for potato + maize system (Chand et al 1993). In general, potato and maize are compatible, depending upon the varieties, as the farmers are aware of the possible competition (Chand et al 1993), as they are traditionally grown in the eastern and central regions since decades. Luxuriantly growing potato varieties suppress maize during initial growth period. Shade of maize plants adversely affect yield of potato. However, their yields might depend on planting methods, and varieties that compete for nutrient elements, space, water, light etc. Therefore, inclusion of some legumes such as peas and beans were started and found profitable (Poudel 2001) under potato + maize system. Result of participatory rural appraisal in the eastern hills identified more severe incidence of diseases and pests in this system, whereas plant protection measure applied is rare. Lack of technical know-how and technology is one of the limiting factors (Barakoti 2001b). Haphazard planting without following recommended row and plant spacing without ridging is common for both maize and potato in the hills. This may create

unfavorable environment for growth and development to either crop. Earthing-up is not practiced for potato planted in flat. Peak growing period of both these crops is summer to rainy season, when there is development and attack of diseases and insect pests in the agro-crops. It is one of the reasons that the yields of maize and potato are lower in the high hills compared to Tarai. In the plain, potato is planted in narrow spacing (60- × 25-cm). Kushwah (2001) reported that planting distance of 50- × 15-cm and 50- × 20-cm was economically suitable in Gujarat condition, India.

MATERIALS AND METHODS

The experiments were conducted in Sindhuwa, Sub-station of the Agricultural Research Station (ARS) Pakhribas, Dhankuta district, in the high hill condition 2200 masl, East Nepal, during 2000 and 2001 cropping seasons. Recommended variety of maize- Ganesh 1 (white grain) and potato- Hale local (red skinned, well stable and popular in the area) were included in the study. It was assumed that this variety might have brought from Holland and so named as Hale. Recommended rates of chemical fertilizers, i.e. half dose of Nitrogen and full dose of Phosphorus and Potash @ 40:60:60 $N:P_2O_5:K_2O$ kg/ha and farmyard manure (FYM) @ 20 t/ha were applied as basal at the time of planting. Recommended seed rate of potato was medium sized tubers @ 1.2 to 1.5 t/ha depending on row spacing. Maize was sown @ 18-20 kg/ha as per number of rows and spacing. Plot size was 3- x 3.5 m. Trials were laid out in RCB design in 4 replicates. Details of different planting methods tested were as following:

1. **Alternate row planting (ARP):** Potato and maize planted alternately at 60 cm row distance.
2. **Double row planting (DRP):** Two rows of potato planted at 30 cm in between maize rows of 90 cm.
3. **Flat row planting (FRP):** Potato planted at 60 cm without ridging at planting and tuberization.
4. **Farmer's practice (FP):** Haphazard planting of cut pieces and small tubers, making pits with spade, applying double handful of FYM and a teaspoonful of urea per pit.
5. **Recommended practice (RP):** Planting potato and maize in rows of 60 cm and 75 cm respectively as intercropping alternately and slight ridging.
6. **Sole potato:** Planting potato only in its recommended spacing of 60- × 25-cm.
7. **Sole maize:** planting maize only in its recommended spacing of 75- × 25-cm.

Row spacing of potato and maize and ridging for potato differed between the planting practices. The intra row for all treatments, except FP was 20 cm. Potato was planted during first half of February, common time for the variety and maize planted/dibbled during fourth week of March each year, after emerging out of the seed tubers. Harvesting of potato was done in third week of July and maize during second to third week of October both years, three months later than potato. Intercultural operations: two weeding, thinning (for maize) and earthing-up were done as recommended and normally practiced by the farmers. Top-dressing with Nitrogen @ 40 kg/ha was done during the tuberization. No pesticide was applied against diseases and insect pests to observe the effect of treatments under varied spacing and ridged conditions. Late blight was scored in 1-9 rating according to CIP scale.

Parameters recorded in the experiments were: emergence, plant height, uniformity, ground cover, plant stand, maturity, yields, cost of cultivation, and severity of major diseases and insect pest. Tubers were analyzed based on size class to identify the ratio of seed size tubers. Analysis of variance was performed through Genstat for F-test, SED and CV.

Potato and maize planted and harvested situation in the experimental plots (3- × 3-m)

Treatment	Crops	Planted	Harvested
Farmers' practice	Potato	Without row	Whole plot
	Maize	Without row	Whole plot
Recommended practice	Potato	5 rows	3 rows
	Maize	4 rows	4 rows
Alternate row planting	Potato	3 rows	3 rows
	Maize	4 rows	4 rows
Double row planting	Potato	6 rows	4 rows
	Maize	3 rows	3 rows
Flat row planting	Potato	5 rows	3 rows
	Maize	5 rows	5 rows
Potato sole	Potato	5 rows	3 rows
Maize sole	Maize	4 rows	4 rows

RESULTS AND DISCUSSION

The mean data on major agronomic traits recorded in potato are presented in Table 1a and 1b. Length of tuber sprouts at planting was normal (2.4-3.0 cm) differing slightly. The tubers took long time (above 70 days) for attaining more than 90% emergence due to cool winter period. Earlier emergence was observed in farmer's practice (FP) due to cut tubers. Relatively higher emergence of tubers in FP followed by recommended practice (RP) was due to higher number of hills, which was due to close (15-25 cm) and uneven planting without rows, and the least number in ARP was due to less number of rows (4) compared to other planting practices. The ground coverage by the plants as an important agronomic parameter was visually estimated at 18th weeks after planting. The data were highly significant ($P < 0.01$) attaining 80% (ARP) to 95% (sole cropping). The ground cover in ARP was lowest because potato and maize were planted widely at 60 cm rows alternately. Uniformity of plants visually scored at initial tuberization, showed superior in flat and double row plantings. Uniformity was homogenous in all treatment plots both years. The uniformity was good (4 scale) according to the standard ranking. The data did not give significant result. The plant height of potato measured at harvesting time did not vary markedly. The results were not significant. Similar trend was found in the maturity days and main stem number, which were too non-significant. The No of main stem was highest in RP (4.3), whereas it was least (2.3) in the FP, which might be due to close or dense plants. Planting method did not show significant variation on plant height, number of stems and maturity period, which might depend genetically as compared to environmental factors. Number of harvested plants varied significantly ($P < 0.01$), where FP and DRP were at par and significantly differed with the remaining planting practices, which were also at par (Table 1b). Total number of tubers/plot was also found non-significant.

Table 1a. Agronomic traits of potato under potato + maize cropping system in the eastern Nepal's hill (2200 masl), Sindhuwa, Dhankuta district, 2000 and 2001

Planting methods	50% emergence DAP	Emergence at 80 DAP, %	Ground cover at 125 DAP, % (2nd wk, May)	Uniformity (1-5 scale)* at 110 DAP (3rd wk, April)	Plant height at harvesting, cm	Maturity days from planting
Farmers' practice (FP)	66.8	99.0	87.5	3.8	49.8	155
Recommended practice (RP)	74.0	97.5	92.5	3.8	55.0	155
Alternate row planting (ARP)	76.5	93.0	80.0	3.8	52.8	156
Double row planting (DRP)	75.5	95.6	90.0	4.3	53.0	155
Flat row planting (FRP)	74.3	95.5	92.5	4.5	56.8	157
Potato sole	74.8	96.3	95.0	4.5	49.8	156
F-test	ns	ns	***	ns	ns	ns
SED	4.15	3.30	2.71	0.40	3.11	0.61
CV, %	4.30	7.52	1.90	13.9	8.30	3.10

*DAP, Days after planting. ns, Non significant. * 1, Very poor. 2, Poor. 3, Fair. 4, Good. 5, Excellent.*

The number of plants in FP was highest but the number of tubers was the lowest. The plant numbers of FP and DRP varied highly significantly between the other treatments. But the numbers of main stems were not significant. Similarly, the number and weight of tubers showed non-significant

results. Slightly lower weight was in ARP due to less number of rows and plants and slightly higher was in the FRP (Table 1b).

Table 1b. Agronomic traits of potato under potato + maize cropping system in the eastern Nepal's hill (2200 m), Sindhuwa, Dhankuta district, 2000 and 2001

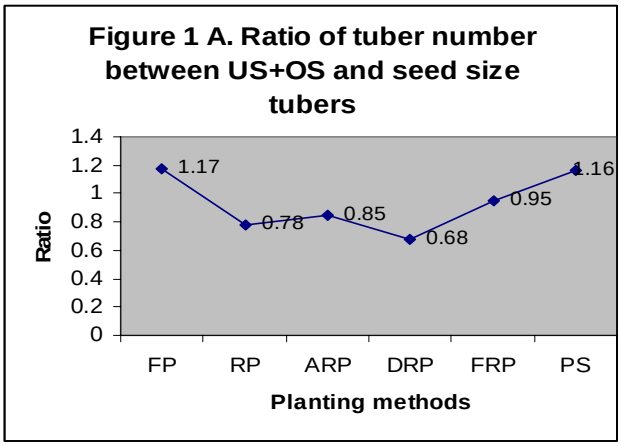
Planting methods	Main stem, no/plant	Number of harvested plants/plot	Total tubers, no/plot	Total weight of tubers, kg/plot	Plant stand, no/ha
Farmers' practice	2.3	44.0	201	7505	68604
Recommended practice	4.3	33.5	258	7655	61383
Alternate row planting	3.8	31.2	243	6866	36941
Double row planting	3.5	44.2	290	7656	49162
Flat row planting	3.8	33.7	253	7820	61660
Potato Sole	3.5	33.2	249	7328	60272
F test	ns	***	ns	ns	
SED	0.70	1.14	36.3	1137	
CV, %	28.3	4.40	20.6	21.5	

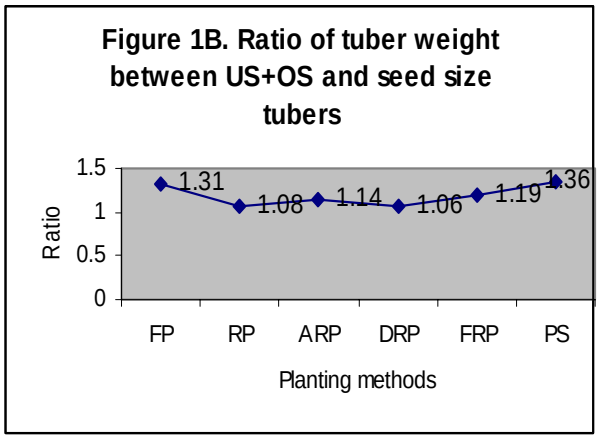
Potato tubers in Nepal are graded into three sizes since the then National Potato Research and Development Program (NPDP) as shown in Table 2. Normally, the consumers prefer medium size tubers, which are regarded as seed size and recommended for seed purpose. The number and weight of each size were recorded for each treatment. The data revealed that seed size tubers of 25-50 g had the highest No (86-141) and weight in all treatments followed by under size (< 25 g). But the result was non-significant. The weight of oversize tubers was higher in all treatments, except alternate row planting. Tuber number and size varied depending upon planting practices. The highest number of seed size tubers and their weight was found in double row planting followed by recommended practice. However, Sood and Singh (2003) did not find any variation in plant growth and tuber yield in the raised bed double row system in comparison to single row.

The proportion and weight of the tubers in the treatments is presented in Figure 1. The plant stands calculated according to the area planted/covered varied (36941-ARP, 68604-FRP) per hectare. The stands were also less in the DRP plots. The highest No in the FP was due to close planting without maintaining spacing. The lower stands were due to low No of rows/ plot. The yield of potato tubers ranged from 12.95 t ha^{-1} (FP) to 15.54 t ha^{-1} (ARP) based on planting practices. However, the data gave non-significant results. The arithmetic means were higher for the recommended and flat row planting also, than the sole planting. Therefore it was obvious that planting potato in the high hill condition was beneficial with maize rather than sole crop. Both the crop showed synergistic properties by each other. Therefore, the practice has been common in the eastern and central region hills of Nepal above 1800 m.

Table 2. Number and weight of tubers, and yield under potato + maize cropping system in the eastern Nepal's hills (2200m), Sindhuwa, Dhankuta district, 2000 and 2001

Planting Methods	Under size (< 25 g)		Seed size (25-50 g)		Over size (> 50 g)		Tuber yield, t/ha
	Number/ plot	Weight, kg/plot	Number/ plot	Weight, kg/plot	Number/plot	Weight, kg/plot	
Farmers' practice	84	1.50	86	3.58	29	2.68	12.95
Recommended practice	106	1.53	124	4.29	28	1.82	14.20
Alternate row planting	109	1.96	111	3.66	18	1.17	15.54
Double row planting	131	1.84	141	4.56	19	1.26	13.10
Flat row planting	102	1.50	114	3.98	34	2.30	14.67
Potato Sole	106	1.44	109	3.64	42	2.77	13.32
F-test							ns
SED							1.95
CV, %							19.7





There are some economic diseases and insect pests of potato in the hill conditions. Late blight caused by *Phytophthora infestans* is the number one disease commonly occurring every year when the weather favors its development (Poudel 2001). In the potato + maize cropping system, microclimate differs due to maize plants, which not only competes with potato, but also provides environment for the development of diseases and pests during the summer-rainy season. Late blight (LB), bacterial wilt, potato virus (PV) and aphid infestation were observed in the experiment. Very few symptoms of LB as 2 scales were observed in the plants on third week of May, whereas its incidence rose to 10-25% after one month. Then it spread rapidly and within 10 days attained 6-7.2 score scale equivalent to 50-95% at maturity. The severe infection was observed in the farmers' practice, which might be due to contamination from cut tubers as well as close plants. Due to early variety, tubers were already matured at that time and so there was no harm to the yield of potato. Similarly bacterial wilt (BW) was found highest in the FP (6 n/plot). Potato virus-like symptoms were observed in small scale, and early blight (EB) was negligible. Aphid observation taken on two dates did not exceed 5% (FP) infestation. It was the least (2.7%) in the sole cropped plots. Higher population of potato and maize enhanced aphid population.

Table 3. Aphid infestation and late blight incidence in potato under potato + maize system in the Eastern region high hills (2200 m), Sindhuwa, Dhankuta district, 2000 and 2001

Planting methods	Aphid (Aphis sp.) damage, %		Late blight (1-9 scale)* (*Phytophthora infestans*)			Bacterial wilt incidence, %
	May, 100 DAP	June, 130 DAP	3 wk May, 100 DAP	2 wk June, 125 DAP	4 wk June, 140 DAP	2 wk June, 125 DAP
Farmers' practice	5.0	3.0	2	4.0	7.2	6
Recommended practice	4.5	2.7	2	3.5	6.2	0.5
Alternate row planting	3.7	3.0	2	4.0	6.2	0.8
Double row planting	4.0	3.0	2	3.5	6.0	0.0
Flat row planting	4.2	3.2	2	3.5	6.0	0.3
Potato Sole	2.7	2.7	2	3.7	6.0	1.2
Maize Sole	-	-	-	-	-	-

** 1, no symptoms. 9, all leaves and stems dead.*

Maize parameters in the potato+maize husbandry trial are presented in Table 4. The maize variety Ganesh 1 emerged out about 3-4 times latter than Tarai due to low temperature in the high hill. Plant heights of maize were measured thrice during the vegetative period (at peak tuberization, silking and harvesting). The tallest plants were formed in sole cropping. Analysis of variance of the treatment means showed highly significant ($P < 0.01$) differences at silking stage, when potato tubers were harvested. It followed the previous trend. The second highest was in alternate row planting. The height recorded finally at harvesting varied 187-232 cm, the second highest was in double row planting.

Final plant population of maize varied significantly between the treatments. The stands in all treatments were maintained close to the recommended number (53333). Means of grain and stover yields were highly significant, where the highest yield was recorded in sole cropping. In potato + maize planting practice, significantly higher yield of maize grain (7083 kg/ha) was obtained from flat row planting followed by farmers practice. The highest yield of stover was found in alternate row planting.

Table 4. Maize parameters in the husbandry trial under potato + maize system in the Eeastern region high hills, Sindhuwa Sub-station, Dhankuta, 2000-2001

Treatment	50% emergence, days	Plant height of maize		Plant population/ha	Stover yield, t/ha	Grain yield, kg/ha
		At silking, cm	At harvesting, cm			
Farmers' practice	28.0	144	193	51944	9.444	6072
Recommended practice	27.5	138	193	51666	8.639	5494
Alternate row planting	26.5	182	196	48889	9.666	4880
Double row planting	28.0	138	201	40555	6.361	4277
Flat row planting	28.5	132	187	49166	8.944	7083
Maize Sole	28.0	220	232	49722	12.416	8016
F-test	ns	***	**	*	**	***
SED	0.61	12.6	10.3	3130	1.37	516
CV, %	3.10	11.2	12.5	9.10	20.9	11.9

ESTIMATED AVERAGE INCOME FROM POTATO AND MAIZE

The income from potato and maize intercropping was calculated based on the then mean prices of the commodities (Table 5). The total gross income value showed that flat row planting (FRP) appeared superior over other treatments. It might be due to the better environment for longer period drought during tuberization as the stress was less in the non-ridged plots. Similar income from the farmers' practice compared to alternate row and recommended practices seems at par however while judging from the disease and quality points of views, farmers' practice seems inferior and other practice/ methods need to select. Flat row planting may not be appropriate for early rainfall year because of excess moisture in the soil.

Table 5. Income from potato and maize estimated based on yield produced and market price

Planting methods	Income from		Gross income, Rs
	Potato, Rs	Maize, Rs	
Farmers' practice	77,700	50,700	128,400
Recommended practice	84,200	44,900	129,100
Alternate row planting	93,240	38,800	132,040
Double row planting	78,600	32,800	121,400
Flat row planting	88,020	56,800	144,820
Maize sole planting	-	76,200	76,200
Potato sole planting	79,920	-	79,920

Traditional planting method of potato and maize is inferior to tested planting methods. Row planting in appropriate spacing is beneficial where most agronomic parameters are superior, and diseases and pests severity is lowered due to better environment. Yield and biomass are increased. Intercropping is more beneficial over sole cropping due to combined yields of two crops. Alternate row planting (ARP) of potato and maize at 60 cm row spacing can be recommended to the growers. Recommended and flat row planting are equally suitable after ARP.

ACKNOWLEDGEMENTS

The author would like to thank Drs JB Abington and SB Mathema, HARP Director and Manager respectively and thankful to the then Chiefs Dr KR Regmi and Mr PL Karna of Agricultural Research Station Pakhribas for their managerial support. Sincere thanks are due to all support staff mainly sub-station based Mr JB Basnet, who directly helped in conducting the research trials.

REFERENCES

Barakoti TP. 2001a. Development of improved package of practices for increased potato production in the Eastern hills of Nepal. *Project Completion Report*, Hill Agriculture Research Project (HARP) ARS, Pakkhrbas, Dhankuta, Nepal.

Barakoti TP. 2001b. Factors affecting maize production technology adoption by the farmers of Eastern Nepal. **In:** *Sustainable maize production systems for Nepal* (NP Rajbhandari, JK Ransom, K Adhikari and AFE Palmer, eds). Proceedings of a maize symposium, NARC and CIMMYT, Nepal. Pp. 268-273.

Chand SP, DP Sherchan, RK Shrestha and P Paudyal. 1993. Traditional potato + maize mixed cropping systems in the Koshi hills. *PAC Working Paper No. 86*. Pakhribas Agriculture Centre, Dhankuta, Nepal.

Kushwah VS. 2001. Optimization of inter and intra row spacings for potato cultivar Kufri Jyoti. *J. Indian Potato* 28:58-59.

Poudel MN. 2001. Inclusion of legumes in potato + maize system in the hills. **In:** *Annual report, 2057-058*. Agricultural Research Station Pakhribas, NARC, Pakhribas, Dhankuta, Nepal. Pp. 13-14.

Sood MC and N Singh. 2003. Effect of N fertigation and planting methods on potato growth and yield in Shimla hills. *J. Indian Potato* 30:65-66.

RESEARCH NOTE

Pollen and Spikelet Analysis in F_1 Rice Hybrids and their Parents

Bal K. Joshi*, Laxmi P. Subedi, Santa B. Gurung and Ram C. Sharma

Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal

* Current address: Biotechnology Unit-NARC, Khumaltar, PO Box 1135, Katmandu-Nepal

<joshibalak@yahoo.com>

ABSTRACT

Pollen analysis can be used to discriminate between different species, identify possible interspecies hybrids, identify restorer and maintainer lines, useful to study genetics of restorer gene, interaction between chromosome and cytoplasm and relationship between parents. Pollen abortion system of male sterility is an important tool in hybrid rice production and spikelet is the major yield components. Nine improved cultivars, six landraces and three wild aborted cytoplasmic-genetic male sterile (CMS) lines were used to analyze pollen and spikelet in F_1 rice hybrids and their parents. The frequency of pollen categories and its relationship to spikelet fertility were investigated. Pollen sterility of the F_1s was determined by staining pollen grains in 1% potassium iodide-iodine (I-KI) solution. Spikelet fertility was determined by counting the total number of seed set in proportion to the total number of spikelets. Correlation and regression coefficients for some traits were computed. In hybrids, pollen fertility ranged from 0.5 to 82% and spikelet fertility from 0 to 87%. Pollen fertility varied from 28 to 97%, while spikelet fertility from 73 to 91% in pollen parents. The highest and the lowest percentages of pollen fertility were found in Chaite-6 and Chiunde cultivars respectively. Spikelet fertility percentage varied widely among hybrids and many hybrids had lower spikelet fertility percentage than their parents. Therefore, it is of practical importance to understand the causes of high spikelet sterility in hybrids for possible increase in spikelet fertility. Highly significant positive correlation was found between stained round fertile (SRF) pollen and spikelet fertility. The positive value of correlation and regression coefficient on SRF and spikelet fertility were found between F_1 and mid parent, and F_1 and male parent. High fertility of cross may be due to the presence of a wide compatibility gene or restorer genes in the cultivar. Lower pollen and spikelet fertility of the crosses was attributed to effect of the genetic background of the tester parent.

Key words: Correlation, F_1 rice, pollen category, regression coefficient

INTRODUCTION

Many scientists had studied the sterility in the inter-varietal hybrids of cultivated rice (2n = 2x = 24). Oka (1974) proposed a duplicate gametophytic lethal model and assumed that the sterility in the inter-varietal hybrids is controlled by multiple sets of duplicate genetic lethal genes. Ikehashi and Araki (1986) adopted the one locus sporo-gametophytic interaction model and postulated that the spikelet sterility in Hsien Keng hybrids is caused by the allelic interaction at the S-5 locus (Ikehashi 1991). The degrees of pollen sterility in the F_1 hybrids depend both on the number of heterozygous locus for F_1 pollen sterility and on the heterozygous degree between alleles at the locus. The sterility genes determined by allelic interaction seem to be of wide occurrence (Sano 1990) between distantly related taxa and serve as one of the genetic mechanism for hybrid sterility (Sano 1983, Scoles and Kibirige-Sebunya 1983). Two out of three different genes causing F_1 hybrids sterility between *O. sativa* and *O. glaberrima* (Sano 1983) acted as gamete eliminator and the other acted as pollen killer, which suggests that gametic abortion due to allelic interaction frequently occurs between them.

Pollen analysis can be used to discriminate between different species, identify possible interspecies hybrids (Mariani and Tavoletti 1993), identify restorer and maintainer lines (Ikehashi and Araki 1984), useful to study genetics of restorer gene, interaction between chromosome and cytoplasm, and relationship between parents. Cultivated rices are grouped into two types Indica and Japonica based on character associations and hybrid sterility (Kato 1930). Ikehashi and Araki (1984) subdivided the Indonesian varieties into four types based on spikelet fertility in F_1s. The characters of pollen grains had been used as taxonomic traits (Small et al 1981). Due to the haploidy nature of pollen, it has many advantages in genetic and breeding studies. Male sterility in rice results due to abnormality at any stage from microsporogenesis to pollen maturation. Pollen abortion system of male sterility is an important tool in hybrid rice production. Many factors affect the pollen abortion system. Pollen can be categorized either on the basis of number of nuclei or on the basis of shape and staining behavior (Chaudhary et al 1981). Shape and staining behavior system of classifying pollen is simple, which have been used widely for identifying restorers and maintainers and to evaluate CMS lines (Joshi 2001). Here, pollen and spikelet were analysed in F_1 rice hybrids and their parents. The frequency of pollen categories and its relationship to spikelet fertility were investigated.

MATERIALS AND METHODS

Nine improved cultivars, six landraces and three wild aborted cytoplasmic-genetic male sterile (CMS) lines (Table 1) were used in this experiment, which was conducted at the Institute of Agriculture and Animal Sciences (IAAS), Rampur, Nepal during the dry and wet seasons of 1999. The improved cultivars and landraces were obtained from National Rice Research Program, Hardinath, and IAAS, Rampur, respectively. The CMS lines were obtained from International Rice Research Institute (IRRI), the Philippines.

Approach method (Erickson 1970) was used for pollinating the seed parents. F_1 seeds were produced in greenhouse. Field, consisting of 14 F_1s, 14 pollen parents, three CMS lines and one check cultivar, Masuli was divided into 32 plots of 0.8 m^2 size each. The pollen parent was planted beside their F_1 and CMS planted after the pollen parent. Masuli was planted in the middle plot. Field was fertilized as recommended. Twenty-one-day old seedlings were transplanted in the field in two rows with 10 hills per row at spacing of 20- × 20-cm. Single seedling was planted to each hill. Each plot had 20 plants.

Pollen sterility

Pollen sterility of the F_1s was determined by staining pollen grains in 1% potassium iodide-iodine (I-KI) solution (Dalmacio et al 1995, Virmani et al 1997, Chaudhary et al 1981, Sohu and Phul 1995). Pollen sterility was determined in F_1s and their parents. At heading about 15 spikelets from each experimental unit were collected in the morning just before their blooming and fixed in 70% alcohol. All the anthers from six spikelets were taken out and placed in the stain. Pollen grains were released using a needle. After removing the debris these were observed in microscope (10x). The pollen grains were counted in 3 random microscopic fields and classified based on their shape, size and extent of staining (Virmani et al 1997, Chaudhary et al 1981) as follows:

CATEGORIES OF RICE POLLEN AND THEIR FEATURES

Categories of pollen	Shape and staining behavior	Classification
Unstained withered sterile (UWS)	Withered and undeveloped, unstained	Sterile
Unstained spherical sterile (USS)	Spherical and smaller, unstained	Sterile
Stained round sterile (SRS)	Round and small, lightly or incompletely stained, rough surface	Sterile
Stained round fertile (SRF)	Round and large, darkly stained, smooth surface	Fertile

In case of CMS lines and some hybrids, pattern of pollen abortion was classified as follows (Chaudhary et al 1981):

Type 1: Almost all pollen grains appear as UWS and USS

Type 2: Majority of the pollen grains appears as USS (51%) followed by SRS (36%) and UWS (14%).

Type 3: Majority of the pollen grains are SRS (52%) however UWS and USS are 20-25%.

Spikelets fertility

Five panicles from each experimental unit were bagged before flowering for spikelet fertility analysis. At maturity, the bagged panicles were examined for seed set. Spikelet fertility was determined by counting the total number of seed set in proportion to the total number of spikelets.

F_1s were also classified on the visual basis as seed set as male parent or weaker than male parent and anthers whether plumpy yellow or white shriveled. Data from the field were analyzed and correlation and regression coefficients for some traits were computed using MS Excel (2000) and MINITAB (12.32) software.

RESULTS AND DISCUSSION

In hybrids, pollen fertility ranged from 0.5 to 82% and spikelet fertility from 0 to 87% (Table 1a and b). Pollen fertility varied from 28 to 97%, while spikelet fertility from 73 to 91% in pollen parents. The highest and the lowest percentages of pollen fertility were found in Chaite-6 and Chiunde cultivars (Table 1a) respectively. Chaite-6 had the lowest spikelet per panicle and IAR-97-34 had the highest number of spikelet and percentage of filled grain. IR58025A/Sabitri had the highest percentage of pollen and filled grains. Most of the hybrids had higher spikelet number than their parents. The highest spikelet number was found in R58025A/IAR-97-34 (Table 1b). Half of the hybrids had set the seed as their pollen parents. Only a hybrid, IR68888A/Khumal-7 had white shriveled anthers on visual basis. Bindesowari had the highest frequency of SRF and Masuli had lowest among the pollen parents (Table 2). The highest and the lowest frequency of SRF was found in IR58025A/Sabitri and IR68888A/Chiunde among hybrids respectively.

Table 1a. Pollen and spikelet fertility of the pollen parents and CMS lines, Rampur, Nepal 1999

		Pollen		Spikelet		
SN	***Line/cultivar***	Counted, n	Fertility, %	No. panicle^{-1}	Fertility, %	r†
	CMS line					
1	IR58025A	385	1	178	0	-0.49**
2	IR62829A	268	12	150	0	-0.71**
3	IR68888A	238	0	134	0	0
	Cultivar					
1	Bindesowari	468	97	162	83	-0.26*
2	Chiunde	403	56	211	73	-0.31*
3	Chaite-6	283	95	141	86	0.98**
4	Deharadune	338	70	181	82	-0.79**
5	Gogi	440	81	194	67	-0.42**
6	IAR-97-34	496	67	234	91	-0.06
7	Janaki	521	80	145	75	0.65**
8	Kanchan	427	84	176	73	0.77**
9	Khumal-4	553	79	179	89	0.92**
10	Khumal-7	457	77	146	79	0.97**
11	Kature	547	63	154	87	-0.3
12	Masuli	266	28	172	84	1
13	Ratodhan	455	83	156	78	-0.78**
14	Radha-11	493	74	188	84	-0.73**
15	Sabitri	587	67	148	86	-0.61**
	Range	266-587	28-97	141-234	67-91	-0.79-1
	Mean	423.61	61.89	169.39	67.61	-0.01
	SE	107.12	30.63	26.25	31.72	0.69

**, **, Significant at 5% and 1% level respectively. † Simple correlation coefficient between fertile pollens and fertile spikelets.*

Table 1b. Pollen and spikelet fertility of hybrids, Rampur, Nepal, 1999

SN	Hybrid	Pollen		Spikelet		r†	Seed set as‡	F/S§
		Counted, n	Fertility, %	No panicle^{-1}	Fertility, %			
1	IR58025A/Janaki	374	49	165	33	0.86**	W	F
2	IR58025A/Kanchan	426	65	201	75	0.75**	MP	F
3	IR58025A/Khumal-4	289	30	243	57	-0.84**	MP	F
4	IR58025A/Sabitri	440	70	202	84	0.97**	MP	F
5	IR58025A/Chaite-6	304	55	195	58	-0.06	W	F
6	IR58025A/IAR-97-34	451	56	256	49	0.03	MP	F
7	IR62829A/Deharadune	401	1	192	0	0.38**	W	F
8	IR62829A/Ratodhan	462	78	174	79	0.19	MP	F
9	IR62829A/Kature	352	56	198	76	-0.32*	MP	F
10	IR68888A/Radha-11	455	58	246	87	0.56**	MP	F
11	IR68888A/Bindesowri	288	1	181	0	0.36**	W	F
12	IR68888A/Khumal-7	193	1	177	0	-0.62**	W	S
13	IR68888A/Gogi	394	59	213	26	-0.99**	W	F
14	IR68888A/Chiunde	228	1	251	0	1	W	F
	Range	193-462	1-78	165-256	0-87	-0.99-1		
	Mean	361.21	41.43	206.71	44.57	0.16		
	SE	87.80	28.59	30.54	34.23	0.18		

**, **, Significant at 5% and 1% level respectively. † Simple correlation coefficient between fertile pollens and fertile spikelet. ‡ MP- male parent, W- weaker than MP. § F- plumpy yellow anthers, S-white shriveled anthers on visual basis.*

The higher percentage of SRS in hybrids: IR68888A/Bindesowri, IR68888A/Kumal, IR62829A/Dehardune and IR68888A/Chiunde was associated with on an average with 1% SRF. The hybrids having higher SRS were associated with high frequency of SRF as in IR68888A/Radha-11, IR58025A/Janaki, IR58025A/Kanchan, IR58025A/Khumal-4, IR58025A/Sabiri, IR58025A/Chaite-6, IR62829A/Ratodhan, IR62829A/Kature and IR58025A/IAR-97-34. Table 2 showed that hybrids with some SRF pollen had fewer filled grains in the panicles. It indicates that hybrids having higher UWS and USS will be better in developing new CMS lines from their sterile hybrids. The hybrids were classified as semi-sterile on the basis of spikelet fertility of 40-80%. The male parents of these hybrids were designated as partial restorer. In these hybrids, SRS had dominated the other pollen categories. The partial restorer, IAR-97-34 had more spikelet sterility than the other two partial restorers, Khumal-4 and Chaite-6. Spikelet fertility percentage varied widely among hybrids and many hybrids had lower spikelet fertility percentage than their parents. Therefore, it is of practical importance to understand the causes of high spikelet sterility in hybrids for possible increase in spikelet fertility.

IR58025A and IR68888A had Type I pollen abortion and IR58025A/Khumal-4, IR62829A/Deharadune, IR68888A/Bindesowari and IR68888A/Khumal-7 had Type III. Different pollen abortion pattern were found in hybrids, which indicate the different degree of interaction between nuclear and cytoplasm. Also this suggests that sterility in rice depends upon the genetic diversity between the cytoplasmic donor parent and the nuclear donor parent. Chaudhary et al (1981) suggested that morphology and staining pattern of pollen depend on the developmental sage at which it aborts. Further study on pollen staining system is necessary for better understanding the relationship between staining and viability of pollen. Data in some cases showed that pollen stainability with I-KI solution is not strong evidence to have generalization about spikelet fertility. Results of pollen test were underestimates of spikelet fertility. This may be due to the ability of single fertile pollen to fertilize a spikelet. Therefore even low number of fertile pollen counted in study can give higher seed set. In most of the cultivars and F_1 positive relationship was found between fertile pollen and filled grain. Hybrid having less fertile pollen showed the lowest percentage of filled grain. The sterilities of the inter-varietal rice hybrids are mainly the pollen sterilities and the spikelet sterilities are mainly caused by the pollen sterilities. Guiquen et al (1994)

reported that the sterility in the inter-varietal hybrids of cultivated rice is caused by the allelic interaction at the F_1 pollen sterility loci. Six loci of genes controlling F_1 pollen sterility in rice have been reported (Guiquen et al 1994). The finding of the more the F_1 hybrids had the heterozygote S^i/S^j at the six loci, the higher they showed the pollen sterility and the spikelet sterility (Guiquen et al 1994) is supported by this study.

Table 2. Pollen categories and types of male sterility in male sterile lines, hybrids and pollen parents, Rampur, Nepal, 1999

SN	Hybrid/parent	Total pollen examined, n	Frequency, %†				Type‡
			UWS	USS	SRS	SRF	
1	IR58025A	385.33	29.67	68.25	0.69	1.38	I
2	IR62829A	267.67	27.77	38.85	21.67	11.7	I
3	IR68888A	238.33	47.27	50.77	1.96	0	I
4	IR58025A/Janaki	373.67	12.13	18.11	20.79	48.97	
5	Janaki	520.67	3.78	2.18	14.08	79.96	
6	IR58025A/Kanchan	426.33	2.35	4.77	27.91	64.97	
7	Kanchan	427.33	0.86	0.86	14.74	83.54	
8	IR58025A/Khumal-4	289	3.92	15.11	51.1	29.87	III
9	Khumal-4	552.91	0	0	20.51	79.26	
10	IR58025A/Sabitri	440.33	1.29	7.65	21.05	70.02	
11	Sabitri	587.33	0.57	2.44	29.51	67.48	
12	IR58025A/Chaite-6	304	4.93	8.88	31.36	54.83	
13	Chaite-6	283.33	1.18	0.71	3.53	94.59	
14	IR58025A/IAR-97-34	451.33	6.35	13.52	23.71	56.43	
15	IAR-97-34	496.33	0.6	6.51	25.52	67.36	
16	IR62829A/Deharadune	401	4.91	21.36	73.15	1	III
17	Deharadune	338.33	4.14	17.24	9.07	69.56	
18	IR62829A/Ratodhan	462	0.22	2.74	19.48	77.56	
19	Ratodhan	454.67	0.37	3.59	12.68	83.36	
20	IR62829A/Kature	352	7.76	10.89	25	56.34	
21	Kature	547	0.43	4.2	32.66	62.71	
22	IR68888A/Radha-11	454.91	4.94	5.49	31.14	58.43	
23	Radha-11	492.67	0.14	1.49	24.36	74.02	
24	IR68888A/Bindesowri	288.33	12.49	38.27	48.25	1	III
25	Bindesoawri	468.33	0	2.99	0.36	96.66	
26	Masuli	265.67	0.63	16.44	54.96	27.98	III
27	IR68888A/Khumal-7	193	10.02	28.67	60.32	1	III
28	Khumal-7	457.33	0.22	1.31	21.79	76.68	
29	IR68888A/Gogi	394	4.82	22.51	13.96	58.71	
30	Gogi	439.67	0.3	2.2	16	81.5	
31	IR68888A/Chiunde	228.33	26.28	48.18	25.5	0.5	II
32	Chiunde	403	0.99	12.33	31.02	55.67	
	Range	193-587.33	0-47.27	0-68.25	0.36-73.15	0-96.66	
	Mean	396.38	6.92	14.95	25.24	52.91	
	SE	102.53	10.93	17.07	17.05	31.04	

† UWS, unstained withered sterile, USS, unstained spherical sterile, SRS, stained round sterile, SRF, stained round fertile, ‡ Type I- almost all pollen appears as UWS and USS, II-majority of pollen as USS (51%) followed by SRS (36%) and UWS (14%), III-majority of pollen SRS followed by USS and UWS.

With respect to maintaining ability, Bindesoawri, Khumal-7 and Deharadune, all appeared to be good source of maintaining the sterility. All F_1 of this pollen parents with CMS showed zeros spikelet fertility rate with 0.5 to 1% pollen fertility. Virmani (1996) found the frequency of restorer lines less among Northern China, Eastern Europe, Japan, and Korea. The restoring ability of rice cultivars has been found to be related to their origin to some extent (Govinda Raj and Virmani 1988). Among indica rice cultivars the frequency of R gene is higher in late maturing cultivars than in early maturing ones (Ahmed 1996). The restorer frequency is very low in typical japonica rice cultivars (Lin and Yuan 1980, Virmani et al 1981). It suggests that origin and pedigree of test lines are important characters to be considered in analyzing the pollen and spikelet in rice.

Highly significant positive correlation was found between SRF and spikelet fertility (Table 3). The regression coefficient of 0.96 indicates the strong evidence that SRF is the most important pollen to make spikelet fertile. These regression equations (Table 3) support the basis of pollen categorization system based on the shape and staining behavior. The positive value of correlation and regression coefficient on SRF and spikelet fertility were found between F_1 and mid parent, and F_1 and male parent. It is observed that spikelet fertility is significantly correlated between F_1 and their parents. Table 3 showed that SRF and spikelet fertility on F_1 could be predicted on the basis of pollen and spikelet of their parents. Many factors affect the pollen and spikelet development even though these characters are found inherited.

Table 3. Simple correlation (r), regression coefficient (b) and intercept (a) between F_1 and their parents for 12 characters, Rampur, Nepal, 1999

Pollen category	Spikelet fertility			Mid parent			Male parent		
	r	a	b	r	a	b	r	a	b
Unstained withered sterile	-0.77**	74.76	-2.43	0.56**	15.56	0.37	0.12	0.79	0.02
Unstained spherical sterile	-0.87**	84.0	-1.74	-0.04	30.23	-0.02	0.43**	1.36	0.16
Stained round sterile	-0.22*	09.14	-0.44	-0.12	13.49	-0.05	-0.31**	24.35	-0.18
Stained round fertile	0.86**	7.04	0.96	0.15*	38.61	0.03	0.09	75.0	0.04
Spikelet fertility	-	-	-	0.33**	39.0	0.03	0.33**	-84.85	1.59

**, **, Significant at 5% and 1% level respectively.*

Environmental factors eg stresses can induce nuclear abnormalities and then affect pollen development. Anther morphology, pollen dispersal and viability in rice (Satake 1991) are affected by chilling. Darkness causes pollen sterility in sorghum (Alami et al, cited by Demotes-Mainard et al 1996.). Tian et al (1998) reported that anomalies in the distribution of calcium accumulation correlate with the failure of pollen development and pollen abortion. Phytochrome also involve in regulation of pollen fertility (Oard and Hu 1995). Genetic male sterile system could be utilized in the production of backcross (Fujimaki 1975), interspecific and recurrent selection breeding population (Brim and Stuber 1973). It is assumed that genes at specific loci controlled both pollen and seed fertility. The thermo sensitive genic male sterility (TGMS) trait was controlled by a single recessive gene (Borkakati and Virmani 1996). At certain temperatures occurring after panicle initiation, the male sterility is altered into partial to complete fertility. Yabuno (1977) reported that spikelet sterility is caused by indehisced anthers in rice. Jennings (1966) surveyed pollen and spikelet fertility in F_1 in many crosses of Indica with japonica. He found that there were not any F_1s showing spikelet sterility with normal pollen fertility and spikelet fertility was correlated with pollen fertility. Variability for fertility restoration exists among these hybrids. The low pollen fertility of the inter varietal hybrids may be due to chromosome imbalance caused by incompatibility barriers. High fertility of cross may be due to the presence of a wide compatibility gene or restorer gene in the cultivar.

ACKNOWLEDGMENT

Department of Agriculture Botany, IAAS, Rampur provided the laboratory facility for pollen study.

REFERENCES

Ahmed HI. 1996. Outlines of heterosis breeding program in rice. **In**: MI Ahmad, BC Viraktamath, MS Ramesha and CHM Vijaya Kumar (eds). *Hybrid Rice Technology.* Hyderabad, ICAR, Directorate of Rice Research, pp. 55-65.

Borkakati RP and SS Virmani. 1996. Genetics of thermosensitive genic male sterility in rice. *Euphytica 88*:1-7.

Brim CA and CW Stuber. 1973. Applications of genetic male sterility to recurrent selection schemes in soybeans. *Crop Sci. 13*:528-530.

Chaudhary RC, SS Virmani and GS Khush. 1981. Patterns of pollen abortion in some cytoplasmic-genetic male sterile lines of rice. *Oryza 18:*140-142.

Dalmacio R, DS Brar, T Ishii, LA Sitch, SS Virmani and GS Khush. 1995. Identification and transfer of a new cytoplasmic male sterility source from *Oryza perennis* into indica rice (*O. sativa*). *Euphytica 82:* 221-225.

Demotes-Mainard S, G Doussinault and JM Meynard. 1996. Abnormalities in the male development programme of winter wheat induced by climatic stress at meiosis. *Agronimie 16:*505-515.

Erickson JR. 1970. Approach crossing of rice. *Crop Sci. 10:* 610-611.

Fujimaki H. 1975. Studies on the transfer of desirable genes by back crossing. II. Backcrossing breeding systems by the use of genetic male sterility in self-pollinated crops. *Japan J. Breed. 25:*275-282.

Govinda Raj K and SS Virmani. 1988. Genetics of fertility restoration of WA type cytoplasmic male sterility in rice. *Crop Sci. 28:* 787-792.

Guiquen Z, L Yonggen, Z Hau, Y Jinchang and L Guifu. 1994. Genetic studies on the hybrid sterility in cultivated rice (*Oryza sativa*) IV. Genotypes for F_1 pollen sterility. *Chinese J. Genet. 21:*35-42.

Ikehashi H. 1991. Genetics of hybrid sterility in wide hybridization in rice. **In**: YPS Bajaj (ed). *Biotechnology in Agriculture and forestry. Vol. 14. Rice.* Springer-Verlag, Berlin Heidelberg, pp. 113-127.

Ikehashi H and H Araki. 1984. Varietal screening of compatibility types revealed in F_1 fertility of distant crossed in rice. *Japanese Journal of Breeding 34:*304-313.

Ikehashi H and H Araki. 1986. Genetics of F_1 sterility in remote crosses of rice. **In**: Proceedings of the International Rice Genetics Symposium 27-31, May 1985. *Rice Genetics*, IRRI, Manila. pp. 119-130.

Jennings PR. 1966. Evaluation of partial sterility in Indica x Japonica rice hybrids. *Int. Rice Res, Inst. Tech. Bull. 5:*1-63.

Joshi BK. 2001. Assessment of the potential of Nepalese cultivars and landraces for hybrid production. *M Sc Thesis*, IAAS, Rampur, Nepal.

Kato S. 1930. On the affinity of the cultivated varieties of rice plants, *Oryza sativa* L. *J. Dept. Agric. Kyusyu Imp. Univ. 2:*241-276.

Lin SC and LP Yuan. 1980. Hybrid rice breeding in China. **In**: *Innovative Approaches to Rice Breeding*. IRRI, Manila, pp. 35-51

Mariani A and S Tavoletti. 1993. Pollen morphology as a tool for determining interspecific relationships in the genus *Medicago*. *J. Genet. and Breed. 47:*341-346.

Oard JH and J Hu. 1995. Inheritance and characterization of pollen fertility in photoperiodically sensitive rice mutants. *Euphytica 82:*17-23.

Oka HI. 1974. Analysis of genes controlling F_1 sterility in rice by the use of isogenic lines. *Genetics 77:*521-534.

Sano Y. 1983. A new gene controlling sterility in F1 hybrids of two cultivated rice species. *Journal of Heredity 74:*435-439.

Sano Y. 1990. The genic nature of gamete eliminator in rice. *Genetics 125:* 183-191.

Satake T. 1991. Male sterility caused by cooling treatment at the young microspore stage in rice plants. XXX. Relation between fertilization and the number of engorged pollen grains among spikelets cooled at different pollen developmental stages. *Jpn. J. Crop Sci. 60:*523-528.

Scoles GT and IN Kibirge-Sebunya. 1983. Preferential abortion of gametes in wheat induced by an *Agropyron* chromosome. *Canadian Journal of Genetics and Cytology, 25:*1-6.

Small E, LJ Basset and CW Crompion. 1981. Pollen variation in tribe *Trigonelleae* (Leguminosae) with special reference to *Medicago*. *Pollen et Sores 23:*295-320.

Sohu VS and PS Phul. 1995. Inheritance of fertility restoration of three sources of cytoplasmic male sterility in rice. *J. Genet. Breed. 49:*93-96.

Tian HQ, A Kumar, ME Musgrave and SD Russell. 1998. Calcium distribution in fertile an sterile anthers of a photoperiod-sensitive genic male-sterile rice. *Planta 204:*183-192.

Virmani SS. 1996. Hybrid rice. *Adv. Agron. 57:*328-462.

Virmani SS, BC Virakamath, CL Laral, RS Toledo, MT Lopez and JO Manalo. 1997. *Hybrid rice breeding manual*. IRRI, the Philippines. 151p.

Virmani SS, RC Chaudhary and GS Khush. 1981. Current outlook on hybrid rice. *Oryza 18:* 67-84.

Yabuno T. 1977. Genetic studies on the inter-specific cytoplasm substitution lines of japonica varieties of *Oryza sativa* L. and *O. glaberrima* Steud. *Euphytica 26:*451-463.

RESEARCH NOTE

Bulb Canker of Garlic Caused by *Embellisia allii*, Newly Found in Nepal

Ram D. Timila, Gyanu Manandhar and Sharada Joshi

Plant Pathology Division, Nepal Agricultural Research Council, Khumaltar, Lalitpur

Garlic (*Allium sativum* L.) is one of the mostly used spice crops of Nepal. Basically it is cultivated for bulbs as spice and young plants for leafy vegetables. The distinctive flavor of the genus, Allium is due to volatile sulfur compounds that are produced when plant tissues are bruished or cut. That sulfur compounds has the antimicrobial characteristics. It may also help to prevent cardiovascular and arteriosclerosis diseases (Schwartz and Mohan 1996).

Recently, a new disease, bulb canker or skin blotch of garlic has appeared in one of the farmer's fields at Bhaktapur (1400 masl) during March 2007. The variety of garlic was Chinese lasun. The local variety was found less affected in the field (as observed in the field). The bulb samples received from some locations of Sindhupalchowk district were also found to be infected.

Symptoms appeared as irregular lesions of dark layer underneath the epidermis which was as described by David (1991). The dark areas are developed due to powdery mycelia and conidia of the organism scattered on the outer scales of the developing garlic (Figure 1) or usually near the base of the bulb (Figure 2). Dark areas were also found on the backs and sides of the cloves within a head or on the edges of cloves (Figure 3). Field observations showed the yellowing of the plants (as observed recently in the field). The symptoms in the field appeared after watering followed by few days rain. The disease is reported to be a problem in wet years (Embellisia/OSU Plant Clinic 2007).

Infected plants and bulbs were collected and examined at Plant Pathology Division, Nepal Agricultural Research Council, Khumaltar, Nepal. Sign and symptoms were verified and studied with the help of cited literatures (Embellisia/OSU Plant Clinic 2007, David 1991) and under stereomicroscope as well as under compound microscope. The fungus was identified on the basis of conidial structure, secondary conidiophore (Ellis 1976). The conidia were ellipsoidal or subcylindrical, mid to dark brown smooth with 3-6 thick transverse septa with or without 1or 2 oblique or longitudinal septa (Figure 4). The colonies were effuse blackish brown to black on Potato dextrose agar medium. After symptomatology study and laboratory examination of characteristic conidial structures, the causal organism was found to be a fungus, *Embellisia allii* (Campanile) EG Simmons, (Synonym *Helminthosporium alli* Campanile).The fungus is reported to be overwintering in the plant debris, soil and infected bulbs (David 1991).

Figure 1. Infected garlic plants from the field showing dark patches on developing scales. 2. Canker on outer scale of bulbs. 3. Infected individual cloves. 4. Conidia of the fungus, *E. allii* with dark transverse septa.

On follow up in the field observations, according to the farmer, total loss had occurred due to bulb canker and failed to harvest bulbs. *E. allii* has been reported pathogenic to garlic in the USA also (Dugan and Lupien 2006). This fungus is of quarantine concern in India because of chances of affecting domestic and international trade of this particular crop. Several consignments from China were stuck at the Mumbai Port and in Chennai, over 20 containers each with 14 tonnes of garlic have been stopped (The Hindu Business Line 2005).

In Nepal, cultivation of Chinese garlic (lasun) is increasing among the farmers of Nepal. That exotic variety has been observed to be more susceptible than the local variety (as observed in the farmers' field and the bulb samples received in the Laboratory. However, mild symptoms have also been observed in the bulbs of local variety.

For the management of bulb canker of garlic, some tactics have been suggested such as peeling off the discoloured outer scales of garlic heads, maintaining low humidity in storage, avoiding visibly diseased garlic bulbs in storage and wrapping garlic in breathable materials for marketing (Embellisia/OSU Plant Clinic 2007). In field conditions, maintaining good drainage and burning crop stubbles might be helpful in managing this disease.

So far, at the moment the disease was found in one of the farmers' field only, it may be spread in congenial conditions. It may affect the trade regarding the requirements of World Trade Organization concerning the pests of garlic. Hence, survey of this disease in commercially garlic growing areas is essential to find out the status of this disease. This is the first report on the occurrence of garlic bulb canker in Nepal.

REFERENCES

David JC. 1991. *Embellisia allii*. IMI descriptions of fungi and bacteria, 1991(No.108) Sheet 1078. *CAB Abstract*.

Dugan FM and SL Lupien. *2006. Survey of pathogenic fungi in commercial seed garlic*. USDA-ARS Western Regional Plant Introduction Station, Washington State University, Pullman, WA 99164-6402.

Ellis MB. 1976. More dematiaceous hypomycetes. Common Wealth Mycological Institute CAB. The Cambrian News Ltd. Aberystwyth. 507p.

Embellisia/OSU Plant Clinic. 2007. Embellisia skin blotch on stored garlic. http://www.bcc.orst.edu/bpp/plant_Clinic/Garlic/embellisia.htm.

Schwartz HF and SK Mohan. 1996. *Compendium of onion and garlic diseases*. APS Press. The American Phytopathological Society. 54p.

The Hindu Business Line. 2005. Garlic imports from China stopped on fungal infection. http://www.thehindubusinessline.com/2005/09/14/stories/2005091401981200.htm.

RESEARCH NOTE

Reproductive Disorders and Seroprevalence of Brucellosis in Yak

SITARAM ARYAL AND KRISHNA P. PAUDEL

Animal Health Research Division, Nepal Agricultural Research Council, Khumaltar

ABSTRACT

A study on reproductive disorders among yaks in Mustang and Solukhumbu districts and seroprevalence of brucellosis among yaks in Mustang and Myagdi districts was conducted. Eleven farmers having 383 female and 72 male yaks in Mustang and 129 female and 27 male yaks in Solukhumbu districts were taken by purposive random sampling for the study on reproductive disorders. Likewise Sixty- seven serum samples were also collected from adult female yaks having history of abortion and retained placenta, and from male yaks used for breeding purpose from Mustang and Myagdi districts. An average of 12.63% of female yaks aborted, 7.26% had had retention of placenta and 6.6% repeat breeders in Mustang district. Similarly, 6.3% had abortion, 2.09% retained placenta and 6.5% repeat breeders in Solukhumbu district. However, all the 67 serum samples examined were found negative to the *Brucella abortu*s antibody on ELISA test suggesting that the yak population was free from brucellosis in these areas. Thus abortion and retention of placenta in Yak in Mustang district may not be due to brucellosis. A detail study should be done to find the specific cause of abortion and repeat breeding to recommend preventive and control measures.

KEY WORDS: ***BRUCELLA ABORTUS*, BRUCELLOSIS, REPRODUCTIVE DISORDERS, SEROPREVALENCE, YAK**

INTRODUCTION

Yaks are important high altitude dwelling animals of Nepal. They are hardy and strong, can thrive well under very cold (-50°C) climatic condition (Cheng 1984). It has a high economic value as pack animal, source of milk, meat and fiber in Himalayan region of Nepal. Unfortunately the population of yak is decreasing every year. Various reproductive problems may also be one of the reasons for the decline in population. Brucellosis is one of the important reproductive diseases of cattle, buffalo, sheep and goats, pig and horses caused by infection with *Brucella* spp. and characterized by abortion in the second half of pregnancy and a subsequent high rate of infertility in the female and varying degrees of sterility in male (Radostits et al 1994). It is a zoonotic disease and also causes economic losses to the animal industry (WHO 1971). This disease has been incriminated in various species of animals in Nepal such as cattle, sheep, goat, buffalo, yak, nak and chauri (Pyakurel and Mishra 1977). AHRD (1991) detected brucellosis serologically in cow, buffalo, goat, sheep and pig in Nepal. This disease is regarded as one of the occupational and public health hazards for veterinarians, animal attendants, dairy- man, slaughter- house workers, butchers and meat sellers. AHRD (1998) serologically detected 1.52 percent brucellosis in cattle. Pradhan (1996) found 3.37% brucella antibody positive in Chitwan. Khanal (1996) reported 4.62% abortion in cross-bred cattle of Kathmandu valley. In a study AHRD (1997) found 4.72% abortion and 9.26% repeat breeding in cattle and 2.07% abortion and 0.37% repeat breeding in buffalo. Thus this study was conducted to find the reproductive problems in general and to investigate if brucellosis is the cause of the problem in yak.

MATERIALS AND METHODS

Eleven farmers having a total of 72 male and 383 female yaks in Mustang district and eight farmers having a total of 27 male and 129 female yaks in Solukhumbu districts were interviewed individually for obtaining information on reproductive problems observed and felt by them.

Likewise a total of 67 serum samples from adult female, having history of abortion and retained placenta, and male yak used for breeding were collected from Mustang and adjacent places of Myagdi districts. Blood samples were collected from the jugular vein using a plain vacutainer. The serum was separated and collected in a sterile serum vial. The serum samples were given identification number and transported in ice to Animal Health Research Division, Kathmandu for examination. The serum samples were kept at –20°C until examination. The samples were examined using Enzyme Linked Immunosorbant Assay (ELISA) technique. Brucellisa –800 kit was obtained from Central Veterinary Laboratory (CVL), UK, for the detection of antibodies to *Brucella abortus*.

RESULTS AND DISCUSSION

Based on information obtained from the farmers 12.63% of pregnant female yaks had abortion, 7.26% had retention of placenta and 6.6% had repeat breeding in Mustang district. Likewise 6.3% of pregnant female yaks had abortion, 2.09% had retention of placenta and 8.65% had repeat breeding in Solukhumbu district (Table 1). The number of repeating time on an average was found 1.7 times in Mustang and 2.25 times in Solukhumbu districts.

Table 1. Reproductive disorders of Yak

Disorder	Percent disorder in Mustang/Myagdi	Percent disorder in Solukhumbu
Abortion	12.63	6.30
Retention of placenta	7.26	2.09
Repeat breeding	6.60	8.65
Brocelosis	0	not done

Fifty four percent of female came into estrus within 3 months of parturition and 46% came into estrus 3-12 months post calving in Mustang district. In Solukhumbu district, approximately 31.25% of yak came into estrus within 3 months after parturition and 68.75% came into estrus 3-12 months post calving. The reason of such variation between two districts was not known. The postpartum oestrus period in yak cows varies from 60 to 125 days and it has been reported to be influenced by a variety of factors including age, parity, nutritive environment and season of calving (Sarbagishev et al 1989). In India, Nivsarkar et al (1997) reported that the interval between calving and post partum estrus in yak cows varies from 85-120 days. However, in present study 46% yak cows in Mustang and 68.75% yak cows in Solukhumbu came into postpartum estrus longer than normal time suggesting problem of infertility in yak cows in these districts. No further studies on identifying the specific cause for reproductive problems were undertaken except serological examination for brucellosis. However, attempt was made to relate the cause for abortion and retention of placenta with respect to management practices. Transmigration temperature stress of yaks in lower river belt seems to be one of the influencing factors for abortion and retained placenta. According to the farmers, in Mustang district, the more- longer period the pregnant animal comes down to river belt from their normal high altitude pasture the cases of abortion are more. Under controlled heat stress, several studies have showed that progesterone concentrations were reduced during the luteal phase (Stott and Wiersma 1973, Rao and Pandey 1982, Bahga and Gangwar 1988) which is necessary for the maintenance of pregnancy. Nutritional deficiency, especially, mineral deficiency may also be one of the reasons for repeat breeding as none of the farmers were using mineral mixture in the feed.

All the 67 serum samples subjected to ELISA test in this study were found negative to the *Brucella abortus* antibody. Paykurel and Mishra (1977) have reported 16.66% and 8.33% of brucellosis in yak, nak and chauri in Jumla by plate test and tube test respectively. In Maiwa yaks the incidence was reported to be 12.6% (Yaun 1979 as cited by Nivsarkar et al 1997). Pan and Labaduoji (1984 as cited by Nivsarkar et al 1997) collected 59 *Brucella* isolates from aborted foetuses of yaks, sheep and goats in various parts of Tibet and China. This disease has been incriminated in various species of animals in Nepal such as cattle, sheep, goat, buffalo, yak, nak and chauri (Pyakurel and Mishra 1977). AHRD (1991) detected brucellosis serologically in cow, buffalo, goat, sheep and pig in Nepal. AHRD (1998) serologically detected 1.52 percent brucellosis in cattle. Pradhan (1996) found 3.37% brucella antibody positive in Chitwan. Khanal (1996) reported 4.62% abortion in cross- bred

cattle of Kathmandu valley. In a study AHRD (1997) found 4.72% abortion and 9.26% repeat breeding in cattle and 2.07% abortion and 0.37% repeat breeding in buffalo. The result of this study indicates that brucellosis does not appear as cause of abortion and retention of placenta among female yaks in Mustang district. It may be due to other causes such as temperature stress, nutritional deficiency or other bacterial diseases such as Campylobactor, however, this investigation was beyond the scope of the present study.

REFERENCES

AHRD. 1991. Epidemiological studies on various zoonotic diseases particularly in relation to the productivity of the animal specially brucellosis and tuberculosis. **In:** *Annual report-1991*. Animal Health Research Division (AHRD), Nepal Agricultural Research Council, Tripureswor, Kathmandu, Nepal. Pp. 7-12.

AHRD. 1997. A study on bovine abortion. **In:** *Annual report-1997*. Animal Health Research Division (AHRD), Nepal Agricultural Research Council, Tripureswor, Kathmandu, Nepal. Pp. 21-22.

AHRD. 1998. Study on bovine abortion and repeat breeding due to brucellosis in Chitwan and Western Nepal. **In:** *Annual report-1998*. Animal Health Research Division (AHRD), Nepal Agricultural Research Council, Tripureswor, Kathmandu, Nepal. Pp. 22-23.

Bahga CS and PC Gangwar. 1988. Seasonal variations in plasma hormones and reproductive efficiency in early post-partum buffalo. *Theriogenology* 30:1209-1223.

Cheng P. 1984. Livestock breeds of China. *FAO, Animal Production and Health Paper 46*, Pp. 96-102.

Khanal DR. 1996. Study on clinical cervicitis and metritis in cross bred cattle of Kathmandu valley. **In:** *Proceedings of First National Livestock and Fisheries Research Workshop*. Pp. 212-214.

Nivsarkar AE., SC Gupta and N Gupta. 1997. Diseases and health. *Yak Production*. Indian Council of Agricultural Research, New Delhi.

Pradhan A. 1996. Sero-surveillance of brucellosis in cattle and buffaloes in Chitwan. **In:** *Proceedings of First National Livestock and Fisheries Research Workshop*. Pp. 227-231.

Pyakural S and U Mishra. 1977. Seroepidemiological evidence of animal brucellosis in Nepal. *Bull. of Vety. Sc. and Ani.Husb,* 6:1-6. Nepal.

Radostits OM, DC Blood and CC Gay. 1994. Diseases caused by bacteria. *Vety Med.* 8th ELBS Edition. Bailliere tindall, Britain, 787 pp.

Rao LV.and RS Pandey. 1982. Seasonal changes in plasma progesterone concentrations in buffalo cows (*Bubalus bubalis*). *J. of Reprod. and Ferti.* 66:57-61.

Sarbagishev BS, VK Rabochev and AI Terebaev. 1989. Yaks in animal genetic resources of the USSR. *FAO Animal Production and Health Paper No. 65*. Pp. 357-364.

Stott GH and F Weirsma. 1973. Climatic thermal stress, a cause of hormonal depression and low fertility in bovines. *Int. J. of Biometeoro.* 17(2):115-122.

WHO. 1971. A report on brucellosis in dairy herd. Joint FAO/WHO expert committee on brucellosis. *Technical Report Series, No. 464.*

Author Index, Vol. 5, 2004

Author Index, Vol. 6, 2005

Author Index, Vol. 7, 2006

Author Index, Vol. 8, 2007

Subject Index, Vol. 5, 2004

Subject Index, Vol. 6, 2005

Subject Index, Vol. 7, 2006

Subject Index, Vol. 8, 2007