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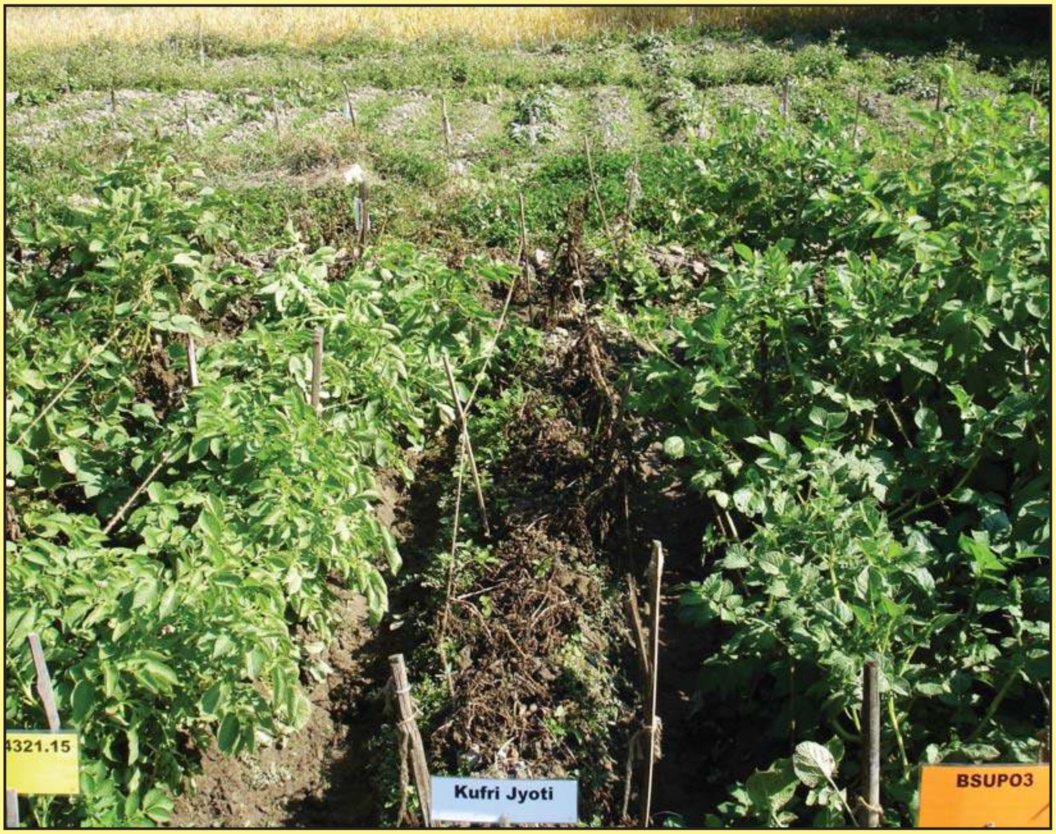

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Cover photo: Late blight resistant and susceptible potato genotypes.

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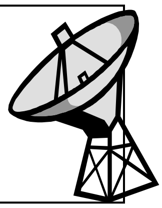

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Contents

RESEARCH NOTE

Alleviating Rural Poverty through Participatory Wheat Variety Selection

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ABSTRACT

Millions of resource poor farmers in Nepal and other South Asian countries derive their livelihood from wheat cultivation. Identification and delivery of high yielding cultivars to the poverty-stricken farmers living in the rural and remote areas of Nepal are the biggest challenges faced by breeders, extension workers and seed delivery system in the country. Participatory variety selection (PVS) offers opportunity to identify and deploy farmer preferred crop varieties among farming communities. PVS is particularly suitable in rural communities where the cultivar dissemination process is rather slow. It ensures that the cultivar selected is preferred and rapidly adopted by the farmers.

A farmer participatory wheat variety selection carried out in two villages (Mahilbar and Semarahana) of Rupandehi district of western Nepal during three crop seasons (2003 - 2005) has resulted in many positive impacts on varietal diversity, grain yield, seed availability, and food sufficiency level in the project area. Household survey revealed that in wheat is grown on 60 and 44 percent of rice areas in Semarahana, Mahilbar, respectively in the rice-wheat cropping sequence. The major constraints to wheat production in these villages were low seed replacement, slow rate of variety dissemination, and the use of poor quality of farmers' homegrown seed leading to low grain yield of wheat. A total of 14 wheat genotypes including two checks were evaluated in a farmer-managed participatory research during 3-year period to identify farmers' preferred varieties, speed up varietal adoption, increase varietal diversity and grain yield, and enhance food security in the area. The mother-baby system of varietal evaluation was adopted. Each year, four mother trials per village, consisting of 6 to 8 genotypes were planted using farmers input and management practices. In addition, each genotype was replicated in five farmers' fields as a baby set for making comparison with the farmers' local variety. The trials were evaluated jointly by farmers, researchers and extension workers. Large quantity of seeds of farmer-selected variety was produced and disseminated by participating farmers.

An impact assessment study after three season's successful implementation of the project activities revealed that farmers identified BL 1887 and BL 2217 as the most desirable genotypes. As a result, the national variety system has released BL 1887 as 'Gautam' while other genotypes are in the release process. There has been 31 percent net increase in wheat grain yield and 78 percent variety diffusion rate in the project area. The food sufficiency level has increased in the project area by 6 percent over 3-year period.

Keywords: Farmers empowerment, participatory varietal selection, poverty alleviation, wheat

INTRODUCTION

Participatory variety selection (PVS) offers ample opportunities to identify and deploy farmer-preferred crop varieties among farming communities. PVS is particularly suitable in rural communities where the cultivar dissemination process is rather slow. It ensures that the cultivar selected is preferred and rapidly adopted by the farmers. PVS has been very effective in identification of farmers-preferred and location specific varieties and faster dissemination of new cultivars among farming communities (Malhi et al 2001). This approach has revealed 30 percent increment in wheat grain yield over farmers' local varieties and there was as much as 80 percent variety replacement within three years of effective implementation of PVS work in a Kotounje village of Kathmandu valley, Nepal (Ferrara et al 2001). PVS can employ intensive system of participatory evaluation in farmer- managed participatory research that allows farmers to evaluate the suitability of wide range of new varieties (Joshi and Witcombe 2002). Farmer-managed participatory research is reported to be a rapid, cost effective and reliable way of identifying farmer- preferred cultivars (Joshi and Witcombe 1996, Witcombe et al 1996).

Rupandehi district of Nepal has the highest wheat area of over 30,000 hectares among other districts with the total production of over 63,000 metric tons. The average wheat productivity of the Rupandehi district was 2100 kg/ha during 2001 (base year). Mahilbar and Semarahana villages of Rupandehi district are the two extensively wheat growing area, covering more than 55 percent of rice fallow.

The major constraints to wheat production in these villages are: 1) low seed replacement, 2) slow rate of variety replacement, and 3) low grain yield, and 4) inadequate food availability.

National wheat research program with the financial support from DFID/CIMMYT, a farmer participatory variety selection program was carried out during 2003 to 2005 wheat seasons to address the above problems in the two villages.

MATERIALS AND METHODS

Site selection for PVS work was accomplished with the help of local extension personnel. A baseline household survey using rapid rural appraisal (RRA) was conducted to determine the farmers' wheat variety preferences, varieties grown, production constraints, present yield levels and also to have information on target population environments (TPEs) and target population traits (TPTs) of the villages. Based on TPEs and TPTs, National Wheat Research Program selected the wheat genotypes from its own breeding program for conducting mother/baby trials Figure 1. Four mother trials per village consisting of eight genotypes per year were evaluated under farmers' management conditions. A plot size of 50 square meter per genotype in the case of mother trial and 300 square meter for baby trial was fixed.

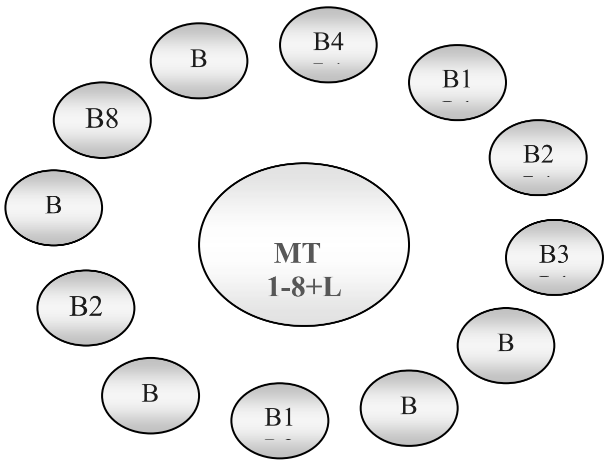


Figure 1. Mother-baby trials.

The mother and baby trials were evaluated relative to farmer's local varieties. A total of 14 wheat genotypes including two check varieties with different genetic back ground were evaluated during 2003-2005 wheat season. In baby trials, each entry from mother trial was replicated at least in five farmer's field for comparison with their local variety.

Farmers, extension personnel and researchers assessed genotypes jointly at physiological maturity (Figure 2). The analysis of variance for grain yield and other variables recorded in the mother trials was done. Also farmers' preferential ranking of genotypes in mother trials was given priority for final selection. Where as in the case of baby trials, farmers' perception as to whether the test cultivars are better, same or worse than their local counterpart was analyzed. Enough seeds of farmers' preferred variety(s) was provided to selected leader farmers for seed production and procurement at local level. Next season this seed was sold to farmers who could not participate in the project activities for faster dissemination.

Figure 2. Farmers selecting varieties.

Research partners: For the implementation of the participatory research activities, the partners responsible were; farmers of the two villages, National Wheat Research Programme (NWRP), District Agriculture Development Office (DADO) and Regional Seed Testing Laboratory (RSTL).

An impact assessment survey was carried out to measure the changes in farm level income, production, rural poverty, food sufficiency, variety diversity, farmers' knowledge and perceptions about PVS, and other changes due to PVS activities at the end of the project period.

RESULTS

Table 1 describes the target population environment (TPEs) and target population traits (TPTs) of the two villages. Both the villages are similar in agro-ecological variability except that Semarahana is dominated by Muslim community where as in Mahilbar is having mix population. Rice-wheat system is the dominant cropping pattern with partial to full irrigation facilities. Farmers of these villages used to grow very old varieties, seldom replace the seed that resulted low grain yield and rural poverty. It is revealed after baseline survey that they need short duration wheat varieties with high yield potential, shattering tolerant, bold seeded and good chapatti quality.

Table 1. Target population environments (TPEs) and target population traits (TPTs) of the two PVS villages

Site name	Target population of environments (TPEs)	Target population of traits (TPTs)
District Rupandehi (Mahilbar land Semarahana villages)	• R-W system, partially irrigated • R-W system, irrigated • Slow seed replacement rate • Poor varietal diversity • Post anthesis heat stress • Diseases (HLB and Leaf rust) • Drought • Poor soil fertility • High cost of cultivation	• High yielding, early maturing varieties • Resistant to LR and HLB • Drought tolerant • Good Chapatti quality • White and bold grains • Palatable quality of straw • Heat stress tolerant • Hard threshability (shattering tolerance)

The results of first year mother trials are shown in Table 2. Farmers' first choice was for BL 1887 although it is fourth in yield rank. Statistically top six to yielding genotypes are at par but there are numerical differences among them.

Table 2. Grain yield and other attributes of eight wheat genotypes in mother trials combined across Mahilbar and Semarahana (2002-03)

Genotype	DM, days	TGW, g	Grain yield, kg/ha			Preference ranks
			Mahilbar	Semarahana	Mean	Mean ranks
NL 792	119	40	3921	4076	3999	4.7
BHRIKUTI	118	36	4203	3576	3890	4.1
BL 1923	116	45	3437	3615	3526	4.1
BL 1965	118	49	3324	3648	3486	4.2
BL 1887	116	44	3406	3524	3465	4.9
BL 1998	115	41	2567	4198	3383	2.8
BL 1968	119	45	2679	3156	2918	3.9
BL 1473	114	46	3224	2524	2874	3.8
GM	116	44	3345	3549	3447	4.06
F-test	ns	HS	S	S	S	S
LSD		4.7	697	774.4	792	1.8

HS, Highly significant. S, Significant. ns, Not significant. TGW, Thousand grain weight. DM, Days to maturity.

BL 1887 is selected by majority of farmers because it has very attractive long spikes, bold grains, tolerant to shattering, early in maturity, and stay green characters that resist high temperature and hot winds during reproductive stage. NL 792 gave the highest grain yield but it has shattering tendency. BL 1887 was also evaluated during second year and farmers equally liked this variety.

The summary results of the second year mother trials conducted in the villages are given in Table 3. Three new entries, BL 2217, BL 2047 and BL 2195 added, BL 1887, BL 1923, BL 1968 and BL 1473 were retained and Bhrikuti was not included because many farmers are growing this variety in their fields. During the second year, farmers gave the highest rank to BL 2217 followed by BL 2195 and BL 1887. Again BL 2217 is similar to BL 1887 in major traits (early in maturity, bold gains, and shattering tolerant and good chapatti test) plus it has high yield potential compared to other variety. However, it is statistically at par with BL 2195 and BL 1887.

During 2004-05, three more genotypes, BL 2015, BL 2064 and BL 2067 were added and BL 2217, BL 2047 and BL 1968 were retained in the test cycle (Table 4). Framers' gave equal ranks to BL 2067 and BL 2047 and where as BL 2217 ranked second position.

Table 3. Means of grain yield and preferential ranking of genotypes across Semrahana and Mahilbar-2003-04

SN	Genotype	DM, days	TGW, g	Semrahana, kg/ha	Mahilbar, kg/ha	Mean, kg/ha	Preference ranks Mean ranks
1	BL 2217	114	44	3648	4066	3857	3.9 a
2	BL 2195	118	41	3644	3953	3799	3.6 ab
3	BL 1887	117	43	3655	3878	3767	3.5 ab
4	BL 2047	118	41	3727	3799	3763	3.3 b
5	BL 1923	116	45	3527	3955	3741	3.3 b
6	BL 1968	117	45	3548	3587	3568	3.3 b
7	LOCAL	115	43	3397	3270	3334	3.3 b
8	BL 1473	114	41	3059	3311	3185	1.0 c
	GM	116	43	3526	3727	3626	3.1
	F-test	ns	S	HS	HS	HS	HS
	LA					ns	ns
	LSD		2.3	358.2	349.7	495	6.3

HS, Highly significant. S, Significant. ns, Not significant. TGW, Thousand grain weight. DM, Days to maturity.

Table 4. Mother trial mean grain yield and preference ranks across Mahilbar and Semrahana (2004/05)

SN	Genotype	DM, days	TGW, g	Mahilbar, kg/ha	Semrahana, kg/ha	Mean, kg/ha	Preference ranks Mean ranks
1	BL 2217	114	38	3750	3500	3625	5.7
2	BL 2047	118	40	3625	3622	3624	5.8
3	BL 2015	116	45	3585	3611	3598	4.5
4	BL 2067	119	39	3667	3389	3528	5.8
5	BL 2064	116	39	3625	3056	3341	5.6
6	BL 1968	117	37	3292	3278	3285	5.7
7	LOCAL	116	39	3208	2889	3049	5.2
8	BL 1473	114	35	2583	3222	2903	5.2
	GM	116	38	3417	3321	3369	5.43
	F-test	HS	HS	ns	ns	ns	ns
	LSD	1.1	0.61				

HS, Highly significant. ns, Not significant. TGW, Thousand grain weight. DM, Days to maturity.

Impact assessment survey conducted at the end of three years of successful implementation of PVS activities in the two villages revealed many interesting impacts on wheat varietal diversity, enhanced farm level yields, increased availability of seeds and awareness among farmers regarding participatory research approaches. The summary of impact assessment is as follows:

There were altogether 936 farmers participated in different activities and benefited from the project (Table 5). Three hundred and twenty farmers participated in farm walk for the purpose of variety evaluation. Similarly, 266 farmers benefited by getting seeds of new varieties in the form of baby trials. Hundred and twenty farmers participated in seed production business to scale out farmers' selected varieties. More than 200 farmers participated in baseline and impact assessment survey.

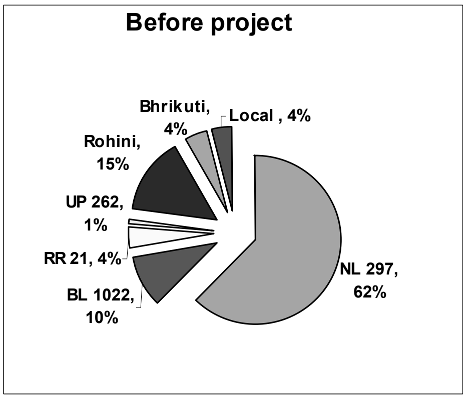
It is revealed after baseline survey that they need short duration wheat varieties with high yield potential, shattering tolerant, bold-seeded and good chapatti quality. Impact assessment survey conducted at the end of three years of successful implementation of PVS activities in the two villages revealed many interesting impacts on wheat variety diversity, enhanced farm level yields, increased availability of seeds, increased food sufficiency and awareness among farmers regarding participatory research approaches.

Table 5. Number of farmers directly benefited by the project activities

Activities	Mahilbar	Semarahana	Others	Total
Mother trials	12	12		24
Baby trials	135	131		266
Scaling up of variety	52	59	6	120
Farm walk	150	170	-	320
Base line survey	52	53	-	105
Impact assessment	53	54	-	107
Total	454	479	6	936

Impact on variety diversity: Figure 3 revealed that there is a big change in wheat variety diversity after three years of successful intervention of mother and baby trials and other variety scaling out activities. Nepal 297 that occupied 62 percent area earlier has come down to 22 percent and new farmers' preferred variety "Gautam" (BL 1887) has taken its place. Old varieties such as UP 262, RR 21, BL 1022 and Rohini occupying significant area have completely replaced from the project area. Within three years of PVS activities in the project area almost 78 percent of the wheat area has been replaced by new varieties.

Figure 4 revealed that 72 percent of Semarahana village and 94 percent of Mahilbar village farmers saved seeds of new varieties who received mother and baby trials (V1) compared to their local (V2) cultivars during the project period. This could have great impact in increased grain yield.



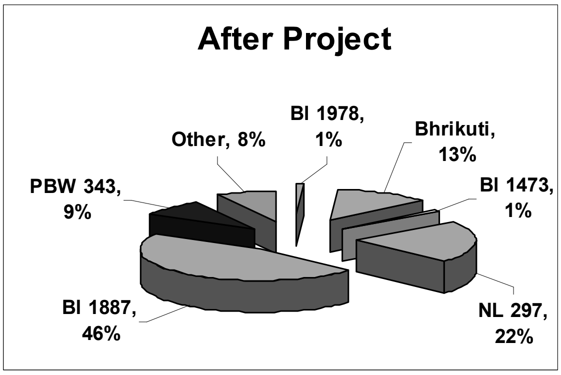


Figure 3. Change in wheat variety spectrum.

Wheat yield gains: There is a net wheat yield gain of 31 percent (Figure 5) compared to the base year (2002-03) in the project area due to PVS and variety scaling up activities. If this yield gain is translated into kilogram per hectare and rupees per hectare, it comes 557 kilogram and Rs 7241.00 (US$ 100.00) per hectare, respectively.

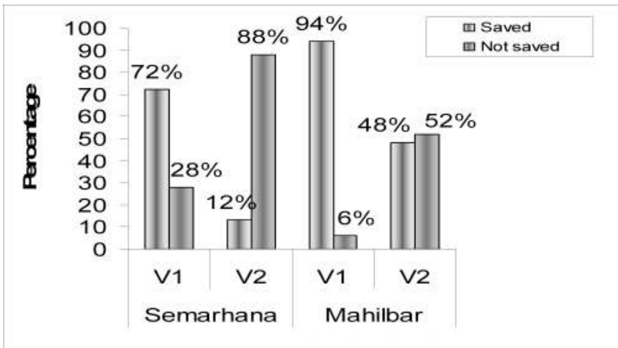


Figure 4. Seed saved by farmers.

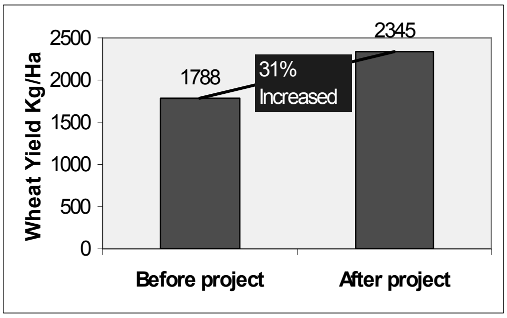


Figure 5. Average gain in wheat yield.

Empowering farmers with the new techniques: Farmers were asked whether they acquired the needed knowledge and skill to run such type of research work on their own in future. Their responses are presented in Figure 6. Seventeen percent of the farmers reported that they can do participatory variety selection (PVS) work independently, 23 percent responded that they can carryout such work in farming group and 60 percent need help from researchers and extension personnel.

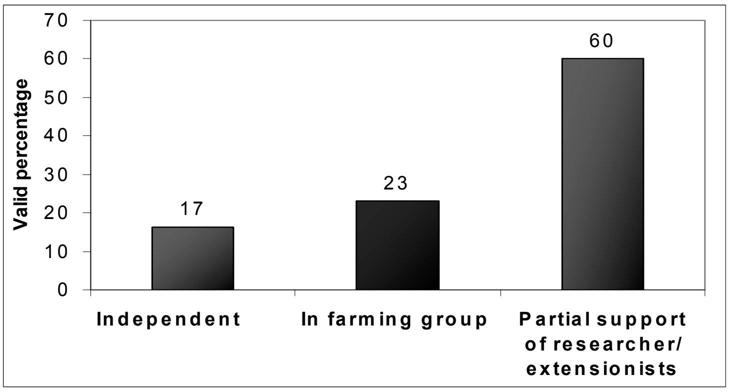


Figure 6. Farmers' knowledge and skill.

Enhanced food security: There is an increase of food availability by 6 percent among the villagers after three years of PVS intervention (Figure 7). Farmers' reported that due to spread of high yielding wheat varieties, there is a 31 percent increase in wheat grain yield that enhanced food sufficiency among villagers.

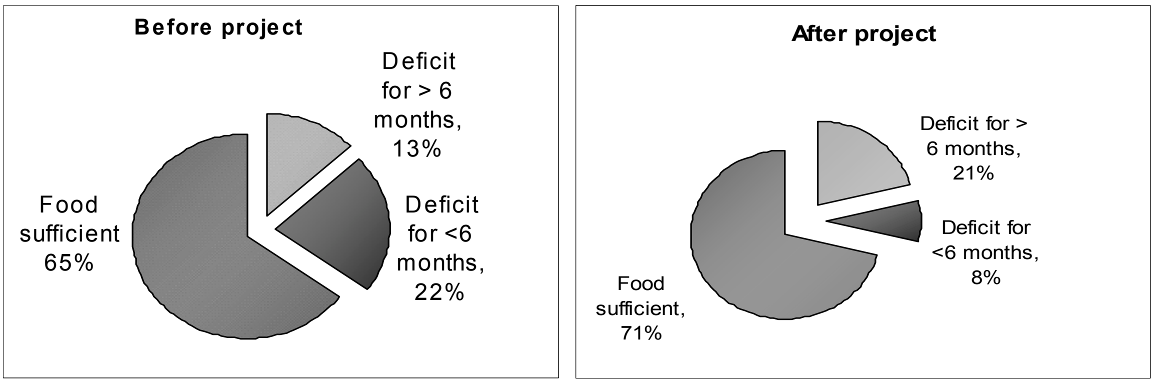


Figure 7. Enhanced food sufficiency.

DISCUSSION

There are altogether 28 wheat varieties released in Nepal since 1960. Of which 19 have been released for terai areas. Out of 19 varieties, five have been rated as very high adoption, six as medium and five as poorly adopted varieties (Bhatta et al 2001). This distinction was made based on area occupied by a variety during its productive life. There are several reasons for this low rate of adoption among released cultivars. The major reasons behind this could be inefficient seed supply system that leads to slow varietal and seed replacement rate, inadequate exposure of farmers to new cultivars during their testing phase, and the varieties released so far were not appropriate to meet the farmers' preference level. Farmer participatory research and development work has been reported to increase production, productivity, by selecting varieties of farmers' choice and also increasing the adoption rate in a shortest possible time (Joshi et al 1997), it also helps in identification of location specific varieties and enhances adoption rate (Malhi et al 2001). Witcombe et al (1996) has described the four important phases of a successful PVS program as assessing farmers' need in a cultivar; identification of suitable testing material with farmers; evaluation on its suitability in farmers' fields; and wider dissemination of farmer-preferred cultivars.

Participatory varietal selection work carried out into two villages of Rupandehi district over three years period has significant impact on varietal diversity, increased grain yield and enhanced food sufficiency level in the project area. There is as much as 78 percent varietal replacement with 31 percent gain in wheat productivity and 6 percent improvement in food sufficiency level compared to three years before. These findings are in agreement with (Ferrara et al 2001 and Joshi et al 1997). If we look at the wheat varieties grown by farmers into these villages before conducting PVS activities, Nepal 297, released in 1985 and the dominant one covering more than 62 percent of their wheat area, has come down to 22 percent after three years of successful PVS work. Similarly, other old varieties were completely replaced by new cultivars introduced as baby and mother trial test entries.

Seed production and availability of farmers-selected variety is of prime importance for faster adoption as well as increased production. There is common consensus that quality seed of improved variety can increase 15 to 20 percent production compared to farmers' homegrown seeds of old varieties. There is increased availability of seeds of farmers-selected varieties in the villages after three years of PVS activities. Farmers' tendency to buy seeds from local cooperatives, traders and seed corporation has gone down in the project area while farmer-to-farmer exchange of seeds remained unchanged. This is because neighboring farmers who did not receive seed from research station would have exchanged/purchased seeds of new varieties from those farmers who received new variety in the form of mother and baby trials or seed package. It has been found that more than 83 percent of the PVS participating farmers saved seeds for next season. This has greatly helped in enhancing varietal diversity as well as increased production in the villages. Farmers reported that there is 41.3 percent contribution from variety, 44.4 percent from crop management including fertilizers and 14.3 percent from irrigation in increased grain yield.

One of the objectives of the project was to empower farmers with the PVS techniques so that they could carry out such program in other crops too in the future. Interestingly, 61 percent of the participating farmers gained knowledge about varietal selection, 27.6 percent farmers knew about new crop management practices and 5 percent of the farmers gained knowledge about seed production technology.

Out of fourteen wheat genotypes experimented with the active participation of farmers, the majority of the farmers preferred BL 1887 and BL 2217. BL 1887 has released as "Gautam" in 2004 and has occupied 48 percent of wheat area in the two villages within three-crop seasons. This has become possible because the massive seed production of this variety was also carried out by participating farmers as well as in the research station. Therefore, seed production of farmers' preferred varieties is of prime importance for rapid diffusion among farmers.

CONCLUSIONS AND LESSONS LEARNED

- There is a gain of 31 percent in wheat grain yield at the end of three years successful completion of PVS activities in the project area. Food sufficiency level has changed in a positive direction. PVS has brought big shift in varietal diversity in the project area within a short period. There is 78 percent varietal replacement with new high yielding varieties compared to three years before. Farmers' knowledge and skill have improved due to this project.
- Farmers' preferred variety BL 1887 has been released as Gautam in 2004 for terai under timely-sown and late-sown irrigated conditions. Its seed is in high demand from all terai areas. Another variety preferred by farmers in the second year of PVS work was BL 2217 is in the process of release for terai areas. These varieties fulfill the farmers target population traits (TPTs) set by them during baseline survey. They are early in maturity, tolerant to shattering, have high yield potential, disease resistant and large grains.
- Seed production and availability of farmer-selected variety is of prime importance for faster adoption. PVS farmers from both the villages produced as much as 75 tons of seeds of selected varieties (BL 1887, BL 2217 and other baby entries) during three years of time. As a result, varietal diversity has enhanced in the project area and more than 20 tons of seeds of BL 1887 were sold to outside districts giving spill over effect.
- The low productivity in many crops in most of the developing countries is attributed to low seed and varietal replacement and that can be well addressed through participatory varietal selection approach.
- Location specific and farmers' preferred crop varieties could be identified within a short period with reasonable cost. The PVS system is cost effective and pro-farmer than the researchers-managed farmers' field trials in conventional plant breeding approach.
- The farmer participatory crop improvement approach need to be institutionalized at least in major crops such as rice, maize and wheat and gradually in other crops for enhancing varietal diversity, rapid diffusion of farmers' preferred varieties and increased grain yield within a short period. This would ultimately help in reducing rural poverty and improving food security in the country.

ACKNOWLEDGEMENTS

The authors would like to thank to all the participating farmers of two villages, Semarahana and Mahilbar, Rupandehi district, for their contribution in making this PVS program a successful piece of work. We would also like to acknowledge and thank to Department of International Development (DFID) and CAZS for funding and technical support to carryout PVS research in Rupandehi district.

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DNA Fingerprinting and Genetic Diversity of Improved and Wild Rice Populations Estimated by SSR Markers

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ABSTRACT

Maintaining genetic purity of a crop variety is the primary concern in seed production since there may encounter the process of outcrossing, introgression, mutation and admixtures in field and in post-harvest handlings. DNA fingerprinting is a genetic tool that has frequently been used to assess genetic variation and it can also be a quick way for plant breeders to select parents with widest range of genetic variability. The genetic diversity and DNA fingerprinting of 37 improved rice varieties (*Oryza sativa* L.) and four wild rice populations belonging to *O. rufipogan* Griff., *O. nivara* Sharma and Shastry, *O. officinalis* Wall ex Watt and *O. glumaepatula* Steud. was investigated using SSR markers occurring across the genome of rice. These all rice species belong to *Oryza sativa* complex in classification. Twenty eight SSR primers revealed a good amplification and showed a distinct polymorphism among cultivars and wild rice populations. Rice DNA fingerprinting based on SSR primers revealed a high level of polymorphism and clearly differentiated 33 rice varieties for 26 SSR primers. The results also revealed a clear grouping of coarse, medium and fine grain types and showed eco-geographical relationships among the varieties. This information can be used in distinguishing the varieties and also can be used in background selection during backcrossing in a breeding programme.

Key words: DNA fingerprinting, genetic diversity, improved rice varieties, polymorphism, simple sequence repeat

INTRODUCTION

Genetic purity is one of the seed quality attributes and maintaining genetic purity of a crop variety is the primary concern in seed production under seed certification programme. A list of varieties of rice (*Oryza sativa* L., 2n = 24) has been developed and released for cultivation in different production environments of Nepal (Annonymous 2011). The occurrence of mixture with other lines/varieties, out-crossing and mutation is frequently encountered in the field and threshing floor. Pure lines are often being maintained using the morphological characteristics. It requires a lot of time and effort, however this method does not give conclusive evidence due to mainly genotype and environment interaction.

Cultivated rice, belongs to the family poaceae is the major food crop and one of most important crops in context to country's food security and poverty reduction challenges in Nepal. It is grown under diverse cultural conditions and over wide range of geographical range from lowland (90 m) to high-hills (2300 m) and 1229680 ha (86%) of total land under rice cultivation is occupied by improved rice varieties (MoAC 2007). Further improvement in rice depends on the conserved use of genetic variability and diversity and use of new biotechnological tools. There exists a wide genetic variability among and between rice genotypes as landraces, modern cultivars, wild relatives in Nepal (Bajracharya 2003). The perennial wild rice (*Oryza rufipogan* Griffith.), known as ancestor of cultivated Asian rice, *O. Officinalis* Wall ex Watt, *O. nivara* Sharma and Shastry and *O. granulata* Nees et Am ex Watt. are the wild rice found in Nepal.

Diversity based on phenological and morphological characters usually varies with environments and socio-economic use values (Bajracharya et al 2010). The varietal identification based on these traits requires growing of the plants, wait till the full maturity and evaluate the variation on these traits. However, molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species and genuineness of varieties. Several molecular markers are presently available to assess the variability and diversity at molecular level (Joshi et al 2000). Microsatellites or simple sequence repeats (SSRs) are location specific and co-dominant, highly polymorphic PCR-based markers of short nucleotide repeats of 1-5 base pairs, abundantly distributed throughout rice genome (Wu and Tanksley 1993). This technique has extensively been used in studies of parentage, variety identification, genetic mapping and breeding, genetic diversity and population differentiation (McCouch et al 1998, Cho et al 2000), and gene flow (Innan et al 1997). Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique germplasm that compliments in existing cultivars. In case of rice and other species, the morphological traits like leaf sheath colour, inflorescence colour, awn colour, and seed types along with stress resistance traits are used to distinguish uniqueness of new cultivars (Chang and Bardens 1965, IRRI-IBPGR 1980). However, it becomes more difficult to distinguish the long list of new varieties. Moreover, since new cultivars normally are developed through hybridization between the members of an elite group of genetically similar parents, it has made even complicate distinguishing the new varieties with smaller variability. It is the molecular markers that provides the DNA profiles and protects the variety with true to the type at genetic level (Rahman et al 2009). The DNA fingerprinting is a quick method to the plant breeders in selecting the parents with wide genetic variability and has now applied in patenting the genes, variety registration and DUS test (Bhat 2008). In the present study, we therefore used SSR markers to assess genetic diversity of 42 rice genotypes and develop DNA fingerprints of improved rice varieties released in between 1979 and 2007.

MATERIAL AND METHOD

Rice genotypes for SSR study

Thirty-seven improved varieties of cultivated rice and one population of each of wild rice: *O. rufipogan* Griff., *O. nivara* Sharma and Shastry, *O. officinalis* Wall ex Watt (growing wild in natural environments in Eastern and Western Nepal) and *O. glumaepatula* Steud (IRRI, Philippines) were used for SSR survey. The details of rice varieties along with their parentage are listed in Table 1. Seeds of improved varieties were provided by variety release and registration subcommittee, National Seed Board (NSB), Nepal. The wild rice populations for the study were procured from Plant Genetic Resource (PGR) unit, NARC, Nepal. These improved rice varieties are released for cultivation for different agro-ecosystems of the country (Table 1).

DNA extraction

Seedlings were raised in plastic pots in a growth chamber for about 3 to 4 weeks in Seed Research Laboratory, Khumaltar, Nepal in 2007 and 100 to 200 mg of fresh green leaf tissue of 5 individual seedlings (equal amount) was used for genomic DNA extraction. The bulk DNA of 5 seedlings of each genotype was isolated by modified CTAB method (Ausubel et al 1994). The quality and quantity of isolated DNA was determined on 0.8% agarose mini-gels in 1 x TBE buffer (0.09 M Tris-borate and 0.5 M EDTA) at 80 V for 90 min with ethidium bromide staining by comparing bands with known concentrations of lambda DNA.

Table 1. Rice genotypes (cultivated and wild species) included in this study

Code	Genotype	Parentage	Origin	Year of release	Remarks
1	Himali	Cica 4/Kalu	IRRI	1982	Fine grain for warm temperate
2	Manjushree 2	Fuji 102/NR10157 (Jumli Marsi/IR 9129-159-3//kn-lb-361-1-8-6-3)	Nepal	2002	Medium for Kathmandu valley
3	Machhapuchhre 3	Fuji 102/Chhomroong Dhan	Nepal	1996	For cool temperate (1300-2000 m)
4	Pokhareli Jetobudho	Selection from Pokhara Local	Nepal	2006	Fine grain for Pokhara valley
5	Makwanpur 1	Ob678/IR20//H4	Sri Lanka	1987	Coarse grain for partial irrigated condition
6	Chandannath 1	Selection from Jingling 78-102	China	2002	Jumloa valley
7	Rampur masuli	Lalnakanda/IR30	India	1999	Foot hills, inner terai
8	Loktantra	Mahasuri/IR4547-6-2-2	Nepal	2006	River basins
9	Mithila	Fortuna//Miltor 6*2/Azucena	Philippines	2006	River basins
10	Taichung 176*	Tsai-Yuanchung/Dee-Geo-Woo-gen	Taiwan	1966	Coarse grain type for warm temperate condition
11	Bindeswori	TN 1/Co29	India	1981	Early rice
12	Chandannath 3	Selection from Yunlen-1	China	2002	Jumla valley
13	Chhummronmg	Selection from Ghandruk Local	Nepal	1991	Cool temperate (1300-2000 m)
14	Barkhe 3004	Kalinga-3/ IR 36	IRRI	2006	Inner terai
15	Janaki	Peta 3/TN1//Ramadja	Sri Lanka	1979	Irrigated land
16	Palung 2	BG 94-2/Pokhreli Masino	Nepal	1987	Cool temperate
17	Ghaiya 2	MTU/W.Kakaiku	India	1987	Upland
18	Kanchan	CR 126-42-5/IR 2061-21-3	IRRI	1982	Warm temperate
19	Sabitri	IR 1561-228-1/IR 1737//CR94-13	IRRI	1979	Irrigated land
20	Chainung 242	Hsingchio 4/Taichung 150//Taipe 17/T 45	Taiwan	1966	Warm temperate
21	Ram dhan	Mahasuri//IR30	India	2006	Inner terai
22	Khumal 2	Jarneli/Kn-LD-361-DLK-2-8	Nepal	1987	Warm temperate
23	Khumal 3	China 1039/IR580	India	1983	Warm temperate as chaite and barkhe dhan
24	Khumal 4**	IR 28/ Pokhreli Masino	Nepal	1987	Fine grain type for warm temperate condition
25	Khumal 5	Pokhreli Masino/KA-1B-361-BLK-2-8	Nepal	1990	Warm temperate
26	Khumal 6	IR 13146-45-2-3/IR7492-18-6-1-1-3-3	IRRI	1999	Kathmandu valley
27	Khumal 7	Chaina 1039 DEF MUT/Kn 18-361-1-8-6-10	IRRI	1990	Warm temperate
28	Khumal 8	Jumli Marsi/IR-36	Nepal	2007	Foot hills to mid hills
29	Khumal 9	K 28-76-D-1/Kn18-214-1-4-3	IRRI	1990	Warm temperate
30	Khumal 11	Akudaka/Barkat	Nepal	2002	Kathmandu valley
31	Chaite 2	BG34-8/IR2061-522-6-9	IRRI	1987	First early rice for double rice areas
32	Chaite 6	NR6-5-46-50/IR28	IRRI	1992	First early rice for double rice areas
33	Radha 4	BG 34-8/IR 2071-625-1	IRRI	1995	Lowland to midhills
34	Radha 7	Janaki/Masuli	Nepal	1992	Partially irrigated lands
35	Radha 11	Local selection in India	India	1995	Central terai
36	Radha 12	TN1/T141//Annapurna	IRRI	1995	Partially irrigated lands
37	Hardinath 1	BG 95///79-3348/H4//BW228-1-3	Sri Lanka	2004	Partially irrigated
39	*O. rufipogan*	Wild rice	E. Nepal		Wildly growing
40	*O. rufipogan*	Wild rice	W. Nepal		Wildly growing
41	*O. nivara*	Wild rice	W. Nepal		Wildly growing
45	*O. Officinalis*	Wild rice	W. Nepal		Wildly growing
47	*O glumaepatula*	Wild rice	IRRI		

**, Check variety for coarse grain type. **, Check variety for fine grain types.*

SSR analysis

A total of 73 genomic microsatellite markers (SSRs) of known map location and unmapped markers (MWG-Biotech AG, Paisley,UK) distributed throughout the genome of rice were assayed. Only 28 SSR rice primers were found to work with different sized amplification products with polymorphism in the studied rice samples and only these polymorphic markers were included in diversity analysis as well as in profiling the rice genotypes (Table 2).

Table 2. SSR primers and their linkage groups, repeat motifs, PIC values and sizes

Priner code	Primer sequence forward (5" to 3')	Linkage	SSR motif	PIC*	Size range*
RM3	ACACTGTAGCGGCCACTG	6	(GA)25	0.72	118-148
RM4	TTGACGAGGTCAGCACTGAC	12	(GA)16	0.63	141-155
RM5	TGCAACTTCTAGCTGCTCGA	1	(GA)15	0.77	106-130
RM9	GGTGCCTTGTCGTCCTC	1	(GA)15	0.83	124-194
RM11	TCTCCTCTTCCCCCGATC	7	(GA)17	0.83	123-147
RM16	CGCTAGGGCAGCATCTAAAA	3	(GA)15	0.77	168-230
RM17	TGCCCTGTTATTTTCTTCTCTC	12	(GA)21	0.63	162-184
RM21	ACAGTATTCCGTAGGCACGG	11	(GA)21	0.88	132-170
RM26	GAGTCGACGAGCGGCAGA	5	(GA)15	0.60	102-112
RM104	GGAAGAGGAGAGAAAGATGTGTGTCG	1	(GA)9	0.62	222-238
RM144	TGCCCTGGCGCAAATTTGATCC	11	(ATT)11	0.76	214-255
RM154	ACCCTCTCCGCCTCGCCTCCTC	2	(GA)21	0.80	165-199
RM164	TCTTGCCCGTCACTGCAGATATCC	5	(GT)16TT(GT)4GAG	0.87	246-304
RM166	GGTCCTGGGTCAATAATTGGGTTACC	2	(T)12	0.5	na
RM167	GATCCAGCGTGAGGAA CACGT	11	CGAA(GA)16GGGG	0.70	127-159
RM208	TCTGCAAGCCTTGTCTGATG	2	(GA)17	0.68	166-180
RM213	ATCTGTTTGCAGGGGACAAG	2	(GA)17	0.63	127-141
RM223	GAGTGAGCTTGGGCTGAAAC	8	(GA)25	0.77	139-163
RM224	ATCGATCGATCTTCACGAGG	11	(GA)13	0.88	124-158
RM226	AGCTAAGGTCTGGGAGAAACC	1	(AT)38	0.82	264-342
RM232	CCGGTATCCTTCGATATTGC	3	(GA)24	0.78	142-166
RM234	ACAGTATCCAAGGCCC T	7	(GA)25	0.83	133-163
RM247	TAGTGCCGATCGATGTAACG	12	(GA)16	0.84	130-176
RM346	CGAGAGAGCCCATAACTACG	7	(CTT)18	0.75	140-175
RM405	TCACACACTGACAGTCTGAC	5	(AC)14	na	110
RM411	ACACCAACTCTTGCCTGCAT	3	(CTT)17	na	110
RM412	CACTTGAGAAAGTTAGTGCAGC	6	(GA)22	na	na
RM413	GGCGATTCTTGGATGAAGAG	5	(AC)11	na	na

na, Not available.

** Source: Wang et al 1992, Paunad et al 1996, Cho et al 2000, Chen et al 1997, Temnykh et al 2000, Wu and Tanksley 1993.*

Polymerase chain reaction (PCR)

PCR reactions were performed in a total volume of 25µl containing 4-6 ng DNA, 0.25 µM of each primer, and Reddy Mix™ PCR Master Mix with 3.0 mM $MgCl_2$ (ABgene, Epsom, Surrey, UK) and processed for amplification in a MJ Research PTC-100™ programmable Thermal Controller with Hot Bonnet (MJ Research, Inc., Waltham, MA, USA) following a touchdown PCR programme. The touchdown thermal cycling comprised of 38 cycles of 94°C for 1 min denaturing and 72°C for 1 min extension. Annealing temperatures each for 30 seconds were progressively decreased by 1°C for every cycle from 64°C to 55°C. At 55°C annealing temperature, the cycles remained constant for final 30 cycles. The amplification ended with a final 5 min extension at 72°C and infinite hold at 4°C. PCR amplification products were detected on a 2% horizontal agarose gel electrophoresis using Midi ABgarose (ABgene, Epsom, Surrey,UK) stained with ethidium bromide for 4 hrs at 90 V. The products were visualized under UV illumination, photographed with a 'Gel Cam' and approximate product size (bp) was determined manually.

Data analysis
All the genotypes were scored for the presence and absence of the SSR bands. Data were entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of SSR band. The diversity parameters like number of alleles per locus, percentage of polymorphic loci, percentage of polymorphic alleles and diversity indices: Shannon and Simpson for allele richness and eveness were calculated. The Excel file containing the binary data was imported into NT Edit of NTSYS-pc 2.0 (Rohlf 1998). The 0/1 matrix was used to calculate Similarity as Jaccard's coefficient using SIMQUAL subroutine in SIMILARITY routine (Sneath and Sokal 1973). The resultant similarity matrix was employed to construct dendrograms using Sequential Agglomerative Hierarchical Nesting (SAHN) based Unweighted Pair Group Method with Arithmetic Means (UPGMA) to infer genetic relationships, and prepare genetic profile To measure of goodness of fit for the cluster analysis, a cophenetic correlation value between the original similarity matrix and the cophenetic matrix given by the UPGMA clustering process was calculated by Mantel test procedure (Mantel 1967).

RESULTS

Out of 73 primers analysed , 28 RM primers were found effective in detecting the genetic diversity among 42 genotypes of rice and generating their profiles at DNA level. . All 28 primers showed polymorphism between 42 rice varieties while two (RM 223 and RM5) out of 28 (93%) SSR markers detected no heterogenous population among 37 improved rice varieties and likewise four (RM104, RM166, RM17 and RM3) of 28 (84%) markers detected no polymorphism among 4 wild species (Table 3). A total of 81 alleles (bands) were scored and of which no bands were found to be monomorphic among these rice genotypes (Table 4). The number of alleles per locus ranged from 2 to 4 with an average value of 3 for cultivated cultivars and 2 for wild rice genotypes (Table 3 and 4). RM224, RM234, RM247, RM26, RM346, RM9 and RM226 revealed four alleles with high allelic richness (0.79 as Shannon index, Table 4). The PIC also called gene diversity (H') was calculated for each marker as a relative measure of informativeness and it ranged from 0.07 (RM5) to 0.68 (RM226) with an average value of 0.46 for 42 rice genotypes.

Table 3. Diversity parameters among the groups of rice varieties

Diversity parameters	Improved cultivar	Wild rice	Total genotypes
Total primers tested	73	73	73
Primers amplified	28	26	28
Polymorphic primers	26 (93%)	22 (84%)	28 (100%)
Total alleles/polyalleles	77/75	56/52	81/81
Alleles/locus	3	2	3
Alleles/poly locus	2.8	2.4	3
PIC value	0.44	0.40	0.46

Genetic similarity ratio revealed high degree of similarity to the extent of 84% corresponded to 'Khumal 8' – 'Khumal 5' pair whereas very low level of similarity of 7% corresponded to 'Mithila' – 'Taichung 176' pair. 'Khumal 8' (a fine grain type) and 'Khumal 5' (a coarse grain type) are the breed varieties developed for hill condition both having used a local landrace as one of parent (Table 1). They showed the highest similarity co-efficient indicating their similarity for genetic background. Whereas Taichung 176, a *japonica* type variety showed the least similarity with *indica* type variety, Manjushree 2. Likewise high similarity coefficients (72-78%) were observed among 'Manjushree 2' – 'Khumal 4'; 'Mithila' – 'Rampur Masuli' and 'Bindeswori' – 'Makawanpur 1'. However, the rice genotypes under study showed the average genetic similarity coefficient of 0.39 (39%) that the genotypes were different in their genetic background. Besides, all rice varieties were found identifiable and provided the distinct profile for each of 37 improved varieties when they were computed along with wild rice populations of Nepal and of India origin (Figure 1).

Table 4. Diversity indices among different groups of rice varieties

Primers	Total genotypes			Improved cultivar			Wild rice	
	PIC	Shannon index	Alleles per locus	PIC value	Shannon index	Alleles per locus	PIC value	Shannon index
RM104	0.191	0.340	2	0.227	0.387	2	0.000	0.000
RM11	0.349	0.533	2	0.339	0.522	2	0.444	0.637
RM144	0.395	0.714	3	0.395	0.714	3	na	na
RM154	0.653	1.078	3	0.650	1.075	3	0.375	0.562
RM16	0.375	0.562	2	0.375	0.562	2	na	na
RM164	0.507	0.936	3	0.430	0.755	3	0.720	1.332
RM166	0.322	0.567	2	0.349	0.604	2	0.000	0.000
RM167	0.407	0.699	2	0.368	0.555	2	0.480	0.673
RM17	0.662	1.160	3	0.678	1.189	3	0.000	0.000
RM208	0.608	0.999	3	0.581	0.950	2	0.640	1.055
RM21	0.517	0.794	2	0.518	0.798	2	0.500	0.693
RM213	0.285	0.460	2	0.211	0.367	3	0.500	0.693
RM223	0.113	0.271	3	0.000	0.000	1	0.560	0.950
RM224	0.668	1.217	4	0.665	1.097	3	0.666	1.099
RM232	0.539	0.900	3	0.510	0.829	3	0.500	0.693
RM234	0.488	0.990	4	0.476	0.760	4	0.444	0.637
RM247	0.639	1.143	4	0.634	1.152	4	0.320	0.500
RM26	0.445	0.771	4	0.453	0.783	3	0.444	0.637
RM3	0.648	1.136	3	0.670	1.173	3	0.000	0.000
RM346	0.636	1.199	4	0.638	1.033	4	0.320	0.500
RM4	0.322	0.594	2	0.283	0.546	2	0.480	0.673
RM405	0.322	0.594	2	0.234	0.396	2	0.640	1.055
RM411	0.497	0.690	2	0.500	0.693	2	0.320	0.500
RM412	0.304	0.583	3	0.297	0.567	3	0.320	0.500
RM413	0.638	1.058	3	0.639	1.059	3	0.500	0.693
RM5	0.065	0.146	3	0.000	0.000	1	0.375	0.563
RM9	0.506	0.388	4	0.460	0.772	3	0.625	1.040
RM226	0.680	1.321	4	0.632	1.221	4	0.375	0.562
H'	0.456	0.780	3	0.436	0.734	3	0.406	0.625

The UPGMA cluster analysis led 42 rice varieties in three major groups. A distinct group of rice cultivars for hill environment resulted out of analysis of pooled SSR marker data (Figure 1). This dendogram revealed that the genotypes within the group clustered more together and they comprised of Taichung 176, Chainung 242, Machhapuchhre 3, Chhommrong, Chandannath 1 and Chandannath 3. These cultivars are cold tolerant except Taichung 176. Wild rice species under study: *O. nivara, O. rufipogan, O. officinalis* and *O. glumapateula* clustered into a separate sub groups and they clustered at more than 50% similarity co-efficient compared to *indica* types in the study (similarity co-efficient less than 25%). In this study, the larger range of similarity values from 0.07 to 0.84 for cultivars revealed by micro satellite markers provided greater confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programs. A high cophenetic correlation (r = 0.84) between the original similarity matrix and those given by the clustering process was observed.

The 37 rice varieties released for different production environments were finger printed with 28 SSR markers and all markers (93%) except RM 5 and RM223 were polymorphic with 76 polymorphic fragments (Table 4). Among the experimental rice cultivars, 33 found with distinct DNA fingerprints differing at one to eight loci and they were identifiable with each other and possessed distinct genetic structure (Figure 2). The study revealed the practical approach of this SSR sets useful in DNA fingerprinting of rice cultivars. However, irrespective of the origin and cross parents of the rice cultivars, Khumal 8 – Radha 4 and Khumal 3 – Mithila found to be homogenous for these 26 SSR markers. We observed the set of SSR markers found effective for DNA fingerprinting of the rice varieties under study, they could be screened out and number of markers could be reduced.

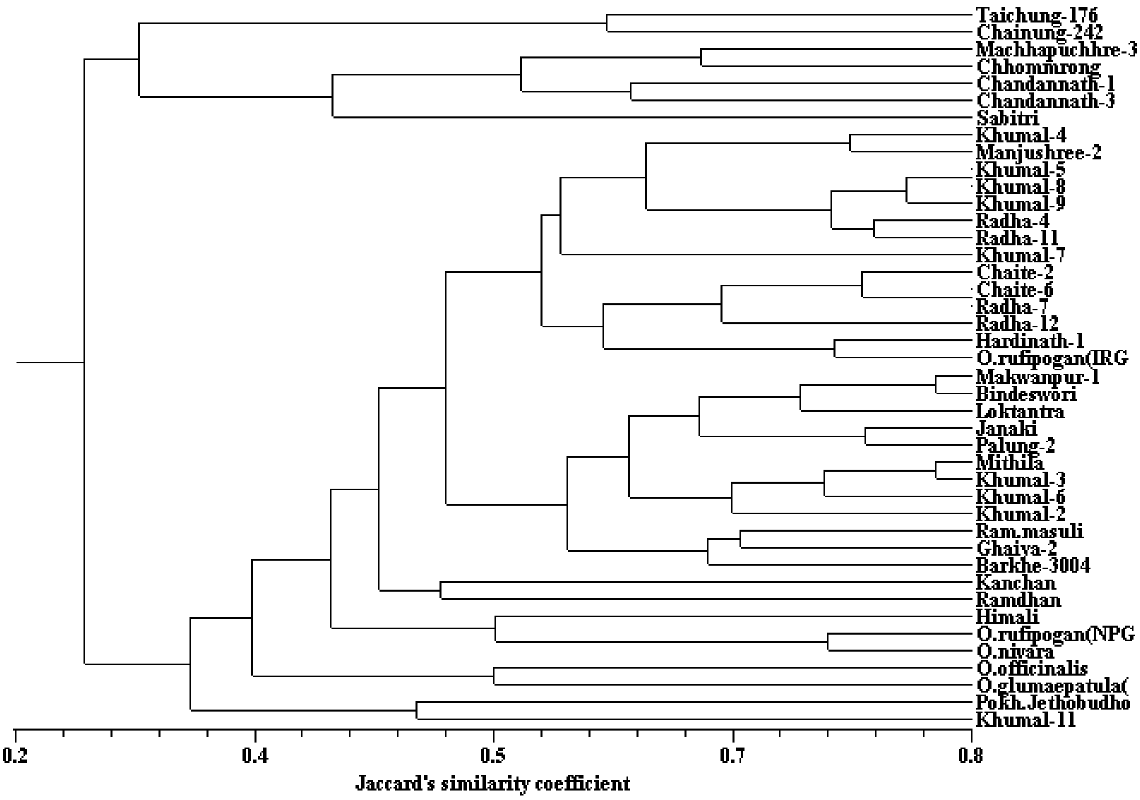

Figure 1. Dendrogram of 37 rice cultivars and 5 wild rice populations obtained UPGMA clustering based on microsatellite fragments of 28 SSR markers.

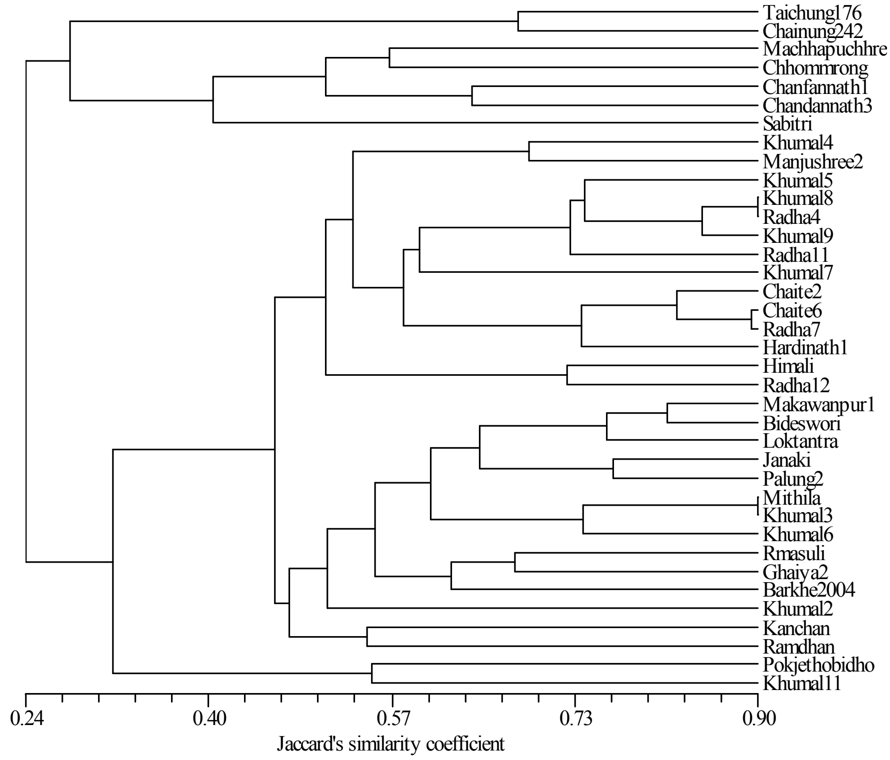

Figure 2. Dendrogram of 37 rice cultivars derived from a UPGMA cluster analysis using the Jaccard's similarity coefficient based on 26 SSR markers showing the distinct DNA profiles of 31 cultivars.

DISCUSSION

Assessment of genetic diversity is an essential component in germplasm characterization and conservation (Chakravarthi and Naravaneni 2006). It is the information on the genetic diversity within and among closely related crop varieties that is used rationally in selecting and monitoring the genetic resources in genebank and gcould greatly contribute in predicting the potential of the genotypes for the breeding programme. DNA marker is a new approach based on DNA polymorphism among tested genotypes and it is being intensively used to enhance the characterization and use of germplsam resources with lot advantages over morphological, cytological and biochemical markers. In the present study 28 microsatellite markers distributed across the rice genome except the chromosomes 4, 9 and 10 were used to characterize the 37 improved rice varieties and also to assess the genetic diversity in 42 rice genotypes including those of improved and wild rice. A total of 81 alleles were revealed and all loci found polymorphic for whole experimental rice varieties, however two alleles were found monomorphic among the improved rice varieties. Average number of alleles per locus was 3 with the range of 1-4. This value is comparatively low for the varieties under study. It showed these varieties are close in their genetic background. However, based on these allelic data we have been able in profiling the DNA fingerprints for 33 rice varieties and these varieties were found genetically identifiable for 2 to 6 loci. The UPGMA based dendogram deduced from the DNA profiles of rice genotypes also added a new dimension to the genetic relatedness among the rice varieties under study. Besides, the study also exhibited the genetic diversity and relatedness among the natural populations of wild rice species and these diversity could be used in breeding and developing the elite varieties with adapted desired traits. SSR markers have thus been effectively used to study the genetic diversity and DNA fingerprinting for genuineness of rice (McCouch et al 2002, Yu et al 2003, Zhu et al 2004, Garris et al 2005, Bajracharya et al 2006, Jayamani et al 2007, Rahman et al 2009, 2010). Panaud et al 1996 made similar studies and observed similar results among rice varieties of *indica* and *japonica* types using SSR markers. Moreover, DNA fingerprints of Basmati rice have been used in protecting the consumers and seed quality regulators in authenticating the process of labeling and inhibiting the adulteration of Basmati rice (Siwatch et al 2004). However, Fuentes et al (1999) and Qjan et al (1995) used RFLP and RAPD to study genetic diversity in cultivated rice.

In conclusion, the set of SSR markers used in the present study provided a positive assessment to the ability to develop unique profiles of 37 rice varieties and 4 wild rice species. The molecular data generated in the present study can be used in varietal identification, origin of the variety and moreover these database are useful to breeder in selecting the cross parents with genetic information of agronomic and quality traits of these rice varieties.

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Variability in Phase Transition and Contribution of Genetic Variance to Yield in Wheat at High Altitude

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ABSTRACT

Instability is a serious problem in wheat production in high hills. Relative susceptibility of three high yielding wheat cultivars were evaluated at high altitude by acquiring climate and field data on grain yield and its components over five years during 2003-04 to 2007-08. Environmental influence on plant was determined by vegetative to reproductive phase transition at minimal number of days (100 days) required for panicle initiation from date of planting. Climate at growing site was partitioned on the basis of environmental components that showed significant coefficient with yield and damage caused by stress. Variance component analysis revealed that yield was predominantly controlled by environment and genotype-environment interaction resulted in reproductive phase transition of crop. Diurnal temperature variation, frequency of rain fall, days to flowering and kernel numbers per spike appeared as essential adaptive traits of cultivar. Primary climatic limitation to crop production was water stress while increased differences in diurnal variation favoured grain production. Concomitant inheritance of multiple stresses would be required for the improvement of wheat yield on high hills.

Key words: High altitude, low temperature, water deficit stress, wheat cultivars

INTRODUCTION

India has achieved impressive progress in wheat production for the last few decades. The interaction between genotypes and cultivation technology under stress-free situation determined the diversity in yield potential among cultivars between two regions. On the contrary the state of decreasing variation in yield and lower dependence of cultivars on weather brings about stability in grain production under stressful situation of environment. Inability of high yielding cultivars to reduce yield variation (Rajaram et al 2006) at high altitude indicated that yield is more dependent on weather. Water shortage 30 days before and 10 days after flowering explained variation in wheat yield by 65 percent (Chloupek et al 2004), while the impact of negative effects of water stress has found less possibly due to elevated CO_2 concentration in the atmosphere (Harnos et al 2002) and development of more root and above ground biomass of crop (Bencze et al 2000). Peltonen et al (1990) described air temperature was the major determinant of crop production causing variability in yield. Precipitation and temperature during grain filling caused 55-69 percent of yield variation (Calvino and Sadras 2002). This implies that scarcities of information available to plant breeder and genetic modification of adaptive mechanisms (Halloran 1975, Pirasteh and Welsh 1980) perhaps involved in declining yield and constitute variability depending upon the genotype and severity of environment at growing site. The loss in yield could be severe when the periods of stress coincide with sensitive stage of plant growth (Herbek 1998). The data obtained from state or regional performance trials over many years and locations are often subjected to greater error while estimating the mean for cultivars and checks because this has ignored the confounding effect of environments. Interannual variation in yield grown at the same physical location using identical management practices assume to provide insight into the dynamic effect of climate on efficiency of grain production (Sharratt et al 2003) of wheat at high altitude. In this investigation, therefore, relative susceptibility in high yielding cultivars were examined to identify the climatic factors that are most important to the production of wheat and to determine the genetic variability on realization of total yield at high altitude environment.

MATERIALS AND METHODS

Yield-climate analysis was performed on three cultivars viz. HS 277, VL 616 and VL 829, which were used as standards of comparison in all trials conducted for high hills. The experiment was conducted at Hill Campus (78^o $24^/$ N, 30^o $18^/$ E) and the climatic conditions during experimental period was characterized by non-freezing cool temperature during winter (December to February) with having lowest minimum temperature (0.9 to -2.0^{o}C) between 02 and 05 meteorological weeks and experiencing snow (during 2003-04 and 2004-05) and/or rain fall during January and February. Exceptionally high minimum temperature and small fall of rain were recorded in 2005-06 (1.7^{o}C; on 04 meteorological weeks) and 2007-08, respectively. The seeds of cultivars were collected form All India Coordinated Wheat and Barley Improvement Project and were planted on 7, 8, 8, 9 and 10th day of October, respectively in 2003, 2004, 2005, 2006 and 2007. Experiment was laid out in randomized block design with three replications. Each plot was 3 m long with four rows spaced 23 cm apart. Recommended agronomic practices were employed for development of normal healthy and disease-free plant. Observations were recorded on days to flowering (DF, days), number seed per spike (S/Sp), thousand seed weight (SW, g), percent of damage (PD; estimated at the time of harvest) caused due to exposure of stress on plants and grain yield (GY, g/plot). Environmental influence on plant was determined by vegetative to reproductive phase transition, when tiny (> 0.5 mm) apex appeared with fuzzy tip to necked eyes after removing all tender leaves from seedling. Irrespective of difference of climatic variation among years the time limit for phase transition of cultivars was fixed on 100 days (lowest minimum number of days required for panicle initiation in 2003-04 and corresponds to 41st to 2nd meteorological weeks) from date of planting the seeds on field. Climatological data were collected daily from meteorological department of Hill Campus. Cumulative thermal values (expressed degree days) of maximum (Mxt), minimum (Mnt), average (Avt), diurnal (Dut) temperature, total volume of rain fall (Rf, mm), number of weeks experiencing rainfall (Wer) and experiencing no rain fall (Wenr) were estimated for individual genotypes on each year separately for the period of 100 days from date of planting. Data aggregated over the years on environmental components, yield and its attributing characters were subjected to correlation analysis. Significant association between two variables was examined by 't' test. All parameters with significant coefficients for grain yield and damage were identified and used for further analysis. Climatic conditions at growing site was partitioned into different combinations on the basis of variability in diurnal temperature and frequency of rain fall that had experienced during the period of phase transition (100 days) and established significant relation with both yield and damage. Variance component analysis was performed to describe the genetic response of cultivars on yield by considering cultivars as fixed effect and environmental components as random effect and to estimate heritability (Rasmusson and Glass 1967) for yield. Multiple regressions were employed to elucidate those factors exerting most influence on yield.

RESULTS AND DISCUSSION

For the entire trials conducted during 2003-04 to 2007-08 an apparent decrease in winter precipitation and increase in winter temperature (Table 1) was observed between the time intervals of 100 days from days to planting of seeds on field. Lowest minimum temperature with least differences in diurnal temperature, high average temperature and moderate rainfall was recorded in 2003-04. Dry and warm season was experienced in 2005-06 and 2007-08 while maximum rain fall had received in 2004-05.

Table 1. Cumulative values of different environment components[!!] on 100 days (ie minimum time required for phase transition) from date of planting recorded during experimental period from 2003-04 to 2007-08

	2003-04	2004-05	2005-06	2006-07	2007-08
Mnt (dagree days)	48.6	69.1	73.3	73.3	84.3
Mxt (dagree days)	226.8	220.7	245.6	231.8	257.5
Dut (dagree days)	92.7	99.9	114.4	107.7	125.9
Avt (dagree days)	178.3	151.7	172.3	158.1	173.2
Rf (mm)	77.7	115.0	37.6	100.9	44.1
Wer (weeks)	5.3	7.0	4.0	6.0	4.0
Wenr (weeks)	9.4	8.0	11.0	9.0	11.0
Snow fall (mm)	53.4	43.0	0.0	0.0	0.0

[!!]*Mnt_I, Minimum temperature. Mxt_I, Maximum temperature. Dut_I, Diurnal temperature variation. Avt_I, Average temperature. Rf, Total volume of rain fall. Wer_I, Number of week experienced rain fall. $Wenr_I$, Number of week not experience no rain fall (all from sowing date to date of panicle initiation).*

The presence or absence of a specific condition of environment thus explained relative variation in wheat production at high altitude and associated change in variability of different yield attributes (Table 2). Minimal time for flowering was registered on 118 days in 2003-04 while maximum number of days (174 days) was recorded in 2004-05 indicating that the expression of flowering- related genes in wheat acts in different path way (Shitsukawa et al 2007) to maintain vegetative phase. An acceleration of development under warmer conditions in presence of adequate moisture had led to reduce the time available for growth and induced flowering early in 2003-04. The process of physiological induction of flowering at high altitude, however, became delayed both under high and small fall of rains. The difference between maximum and minimum performance of yield over a range of environments and the difference in performance in the best and poorest environments were the good indicators of cultivar stability (Langer et al 1979). A perusal of data revealed that maximum severity of stress was experienced in 2007-08 as indicated by small fall of rain and low average performance of yield among cultivars while the best season for wheat production appeared on 2004-05. The best and poorest performances were recorded for HS 277 in 2004-05 and VL 616 in 2007-08, respectively. VL 616 had the highest yield in 2003-04. An exposure to moisture deficiency stress at post-flowering growth of crop, possibly due to commencement of early summers on high hills, reduced the efficiency of production capacity of this cultivar in 2004-05. In 2003-04 the lowest yields were recorded for early head emerging genotypes (HS 277 and VL 829). This suggested that for the crop with determinate growth habit like wheat early transition of reproductive phase produced low grain because it did not permit to built enough biomass to set and fill large number of seeds. Low productivity in 2003-04 was also associated with higher risk of injury due to spikelet sterility when the crops were exposed to cold stress during heading and anthesis in the month of January and February after the initiation early growth and advanced through its developmental stage quicker than normal (Richards 1991) in presence of higher than average temperature and adequate moisture in soil at growing site. Relative improvement of grain yield for VL 829 and HS 277 was noticed in 2004-05. This suggested that if there was enough water yield of wheat could be higher for early head emerging genotypes despite it exhibited increase in number of days for flowering. This also suggested that water deficiency at high altitude environment caused major damage on yield as compared to variation in air temperature. Alternatively it could be stated that the development of cultivars that flower close to the optimal time achieved good yield unless damaged by cold (Peltonen et al 1990) and water deficit stress (Nurminiemi and Rognli 1996, Mackown and Rao 1998) at post flowering growth. The flexibility in time of flowering thereby allows farmers to capitalize the opportunity (Anderson et al 1996) for good harvest from field. A perusal of this investigation revealed that HS 277 and VL 829 was prone to damage by cold (72 and 80%, respectively) than VL 616 while more susceptible to water deficit stress (60 and 65%, respectively recorded on 2007-08) was registered for VL 616 and VL 829.

Table 2. Mean performances of 5 characters# in wheat cultivars over five year at high altitude

Cultivar	Year	DF	SW	S/SP	PD	GY
VL 829	2003-04	118	32.90	10	80	216.66
	2004-05	164	36.40	18	0	600.00
	2005-06	142	39.02	15	0	413.66
	2006-07	143	35.00	15	0	383.33
	2007-08	148	41.14	8	65	216.66
	Mean	143.0	36.89	13.0	29.0	366.06
HS 277	2003-04	120	32.40	9	72	266.00
	2004-05	174	36.80	22	0	850.00
	2005-06	149	40.02	15	0	403.00
	2006-07	152	40.00	13	0	366.00
	2007-08	157	41.93	13	50	366.66
	Mean	150.4	38.23	14.4	24.4	450.33
VL 616	2003-04	128	40.40	18	25	600.00
	2004-05	174	38.20	15	0	433.00
	2005-06	147	40.20	13	0	366.00
	2006-07	167	42.00	11	0	317.00
	2007-08	157	40.00	7	60	196.66
	Mean	154.6	40.16	12.8	17.0	382.53

#DF, Days to flowering. SW, Thousand seeds weight, S/Sp, Number of seeds per spike. PD, Percent of damage. GY, Grain yield.

Grain yield exhibited significant negative correlation with percent of damage (Table 3) but constituted a strong positive association with days to flowering and kernel number per spike. Percent of damage established significant inverse relation both with days to flowering and kernel number per spike. This suggested that delayed initiation of flowering, increase in number of kernel per spike and minimal damage encouraged the grain production in high hills. Significant coefficients for both grain yield and damage determined that diurnal temperature variation and frequency of rain fall, days to flowering and numbers of seeds per spike were essential adaptive traits for wheat production at high altitude. The role of diurnal temperature variation and frequency of rain fall on governing the date of flowering, kernel number per spike was depicted by the presence of significant association. This suggested that presence and absence of specific component of environment experienced during reproductive phase transition from date of planting appeared as the most important criterion for the evaluation of wheat cultivars at high altitude. Insignificant positive (higher value) association between days to flowering and number of kernel per spike indicating that cultivars had a general tendency to increase the number of seeds per spike when physiological process of flowering delayed. Seed weight established insignificant relation with grain yield and percent of damage. It was presumed that reduction in leaf area, decrease in translocation efficiency and duration of transport of assimilates to the grain (Mackown and Rao 1998) possibly reduced seed growth. Favourable relations among these traits become imperative for the improvement of genetic potential on yield.

For the entire trials conducted during 2003-04 to 2007-08 significant differences on diurnal temperature, frequency of rain fall and their interaction effect existed for each characters (Table 4) suggesting that different level of stresses on high altitude environment could be effectively defined by these two components of environment experienced form date of planting to reproductive phase transition. Days to flowering showed significant variance for genotype and genotype × frequency of rain fall interaction effect. This indicated that different modification of flowering at high altitude were mostly generated by the interaction between genotype and frequency of rain fall effect in spite of showing significant differences among genotypes for flowering. The component of genetic variances appeared low as compared to error variance for grain yield and other traits.

Table 3. Correlation coefficient values of environment components[!!] and yield attributing traits[†] (i) with percent of damage and yield and (ii) among variables for significant coefficient with damage and yield

Environment and yield component[#]	Percent of damage (PD)	Grain yield (GY)	Among variable having significant relation with percent of damage and grain yield		
Mnt	-0.219	-0.157	Dut_1	Wer_1	-0.807**
Mxt	0.318	-0.537**		DF	-0.784**
Dut	0.683**	-0.525**		S/Sp	-0.520**
Avt	0.070	-0.392*	Wer_1	DF	0.386*
Rf	-0.352	0.486**		S/Sp	0.532**
Wer	-0.397*	0.560**	DF	S/Sp	0.347
Wenr	0.335	-0.544**			
DF	-0.563**	0.406*			
SW	-0.277	-0.027			
S/Sp	-0.686**	0.959**			
PD	1.000	-0.580**			

**, **, Significant at 5 and 1 percent level of probability. [#], As described in Table 1. [!!], As described in Table 2.*

The results revealed that the variances for first degree interaction components contributed less in all of characters except days to flowering while the contribution of second degree interaction components remained high in each trait. This indicated that combined interaction effect of diurnal temperature and frequency of rain fall variation with genotype would be required for the expression of characters at high altitude. The percent of genetic contribution for damaged appeared 'zero' possibly due to exposure of different kind of stress factors prevailed on high altitude. A perusal of data revealed that 9.00 percent of the total variability for grain yield was determined by genetic variance of wheat cultivars while 68.58 percent was contributed by the genotype × diurnal temperature × frequency of rain fall interaction effect and 22.40 percent to the random environmental factors and experimental error. This indicated that yield variation was predominantly governed by the interaction between the varieties and environment factors (both diurnal temperature and number of rainy days) then by the random and unidentified unexplained environmental factors and finally by differences in the genetic yield potential of varieties. Small differences among varieties suggested that cultivars might be possessed similar genetic yield potential. High environmental effect and low heritability for yield made it difficult to select superior yielding varieties. It was presumed that yield variation among varieties with equal genetic yield potential possibly associated with the differences in reproductive phase transition, duration and susceptibility reaction of cultivars to stress factors prevailed during sensitive stage of its growth and relative improvement of other morphological factors should be emphasized for the development of superior cultivars suitable for high hill condition. This implied that when unfavourable environmental conditions and not genetic potential *per se* limit yield substantial yield increases likely will require more emphasis on stress resistance. Selection for grain yield under stress conditions is the most practical way to select for tolerance to high altitude.

The genetic advance appeared to be low in proportion with that of heritability values and for the improvement wheat yield, new lines should be selected from HS 277 genotypes possessing high average yield among individual genotype. Multiple regression analysis (Table 5) showed that genetic improvement in wheat would be possible by combining different components of environment and yield attributing traits together. Wheat production on high altitude environment appeared to be favoured by increased differences in diurnal variation in temperature while primary climatic limitation to crop production was water stress associated with low frequency of rain fall. Diurnal temperature, frequency of rain fall, days to flowering and kernel number per spike explained 95.26 percent variability of yield in wheat. If it was additionally calculated with the percent of damage the results showed little or no influence on describing the variability of yield.

Table 4. Variance component analysis of wheat varieties on grain yield and other attributing traits# at high altitude

		Source of variation								
		Genotype (G)	Dut_1 (D)	Wer_1 (R)	G × D	D × R	G × R	G × D × R	Error	Total
	df	2	1	1	2	1	2	2	24	35
DF	MS	218.31**	27060.3**	40602.25**	0.0625	81510**	85.56**	54.81	18.75	150340
	C	15.62			0.00!!		5.12	12.02	18.75	51.51
	C (%)!	30.32			0.00		9.93	23.33	36.40	100
S/Sp	MS	1.93	162.6**	588.06**	23.81	518**	14.31	61.93**	12.00	1760
	C	2.14			0.00!!		0.00!!	16.64	12.00	30.78
	C (%)	6.96			0.00		0.00	54.05	38.98	100
PD	MS	685.14**	17755.6**	2023.50**	685.14**	2024**	691.67**	691.67**	13.55	27653
	C	0.00			0.00!!		0.00!!	226.04	13.55	239.59
	C (%)!	0.00			0.00		0.00	94.34	5.65	100
GY	MS	22220.47	119018**	702797.2**	44955.6**	404916**	17471.01	76373.6**	7499.9	1728772
	C	3013.95			0.00!!		0.00!!	22957.87	7499.9	33472
	C (%)!	9.00			0.00		0.00	68.58	22.40	100

**, **, Significant at 5 and 1 percent level of probability. #, As described in Table 2. MS, Mean square. # C, Components of variance. !!, The value was negative. df, Degree of freedom. Heritability (%) = 68.68, 29.31, 0.00, 32.13 respectively for DF, S/Sp, PD and GY. Genetic advance for yield under selection 64.06 g/plot or 17.23% of average yield.*

Table 5. Regression coefficient (b),intercept (a) and R^2 values of environment and yield components@ on yield in wheat

Dependable factors	Independable factors# (b values)					Intercept (a)	R^2
Yield components	Environmmental components		Yield components				
	Dut_1	Wer_1	DF	S/Sp	PD		
GY	-8.7313*					1855.330	0.2755
		80.7604*				-25.1580	0.3138
	-3.4684	56.4905				680.7636	0.3290
	6.3360	100.9344	4.3158			-1832.129	0.3688
	9.1585**	53.5280**	3.7269**	39.0132**		-2490.761	0.9526
	5.5769	34.1183	3.1085**	42.4497**	0.7968	-1764.180	0.9574

**, **, Significant at 5 and 1 percent level of probability. #, As described in Table 2.*

This suggested that where targeting environment is less favourable and initial yield is low relative improvement of other morphological factors should be emphasized more at higher priority (Evans, 1980). As it is nearly impossible to develop cultivars that counteract all limiting factors of the environment a concomitant inheritance of multiple stress factors would be required to improve effective production of grains in wheat under high altitude environments.

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Efficacy of Fungicides against *Phytophthora infestans* in Potato under Laboratory and Field Conditions

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ABSTRACT

Laboratory and field experiments were carried out in 2009 and 2010 at Khumaltar, Lalitpur, Nepal to evaluate the efficacy of fungicides and cost analysis of their applications for the management of potato late blight, *Phytophthora infestans* (Mont) de Bary. In both the years, field experiments were conducted in autumn season (September planting), which was conducive for late blight development. Efficacy of Acrobat (dimethomorph 50%), Fluazinam (fluazinam 50%), Agrifos-400 (phosphorus acid 40%), Sectin (phenomidon 10% + mancozeb 50%), Krinoxyl Gold (metalaxyl 8% + mancozeb 64%), Antracol (propineb 70%), Uthane M-45 (mancozeb 75%), Galaxy (metalaxyl 35%), Jatayu (chlorothalonil 70%) and '*Jeebatu*' (a mixture of micro organisms in liquid form) were evaluated along with their spray frequencies. Late blight susceptible potato variety 'Kufri Jyoti' was used in the field and its tubers for efficacy evaluation under laboratory conditions. Efficacy of Sectin at 3.6 kg a.i./ha and Acrobat at 3.0 kg a.i./ha/crop season under field conditions were found the most effective and both the fungicides were at par for field efficacy. Under laboratory conditions, Acrobat showed 100 percent colony growth inhibition on tuber slices even at the concentration of 50 ppm where as Sectin showed 100 percent inhibition at 250 ppm. Metalaxyl (ITIS Corporation, Kuala Lumpur, Malaysia) at 500 ppm concentration inhibited 100 percent colony growth of *P. infestans* (LPR-1 isolate), whereas Galaxy (Devidayal Ltd. Mumbai, India) showed 33.3 percent colony growth inhibition at 4000 ppm. Considering the efficacy, tuber yield and cost of fungicide application, alternate sprays of Sectin and Uthane M-45 for four times at 9 days intervals was found to be the best for late blight management under Khumaltar conditions.

Key words: Acrobat, Agrifos-400, costing of fungicide, efficacy of fungicides, late blight, Sectin

INTRODUCTION

Late blight, caused by the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, is a devastating disease of potato and tomato worldwide (Groves and Ristaino 2000). Potato is grown in all agro-ecological regions of Nepal because of its wider adaptability. Late blight is an important and widely spread disease throughout the potato growing areas of the country. However, this disease appears in epidemic proportion in high hills every year, but in the plains (terai) it occurs sporadically (Shrestha et al 1998). In Nepal, national economic losses due to late blight alone have been estimated 1.8 billion rupees (USD 25 million) per annum (Sharma and KC 2004). A recent study estimates that losses due to late blight in developing countries top USD 13.5 billion per year and a conservative estimate of fungicide use for the same region would be USD 2.5 billion, hence the total being USD 16 billion per year (Haverkort et al 2009), although actual losses in the field and storage are likely several folds higher (Groves and Restaino 2000). Nepalese farmers in major commercial potato growing periphery urban areas have been excessively using mancozeb and metalaxyl containing fungicides for more than 25 years. Presently, several new fungicides ie dimethomorph (Acrobat), metiram, chlorothalonil, metalaxyl, Sectin (fenamidon 10% + mancozeb 50%) and many brands of mancozeb are being used by the farmers. However, Indofil M-45 and Dithane M-45 (mancozeb 75% WP) and Krinoxyl Gold (metalaxyl 8% + mancozeb 64%) are the most common ones. Farmers of Katmandu valley apply fungicides 10 - 15 times (Sharma and KC 2004) to control late blight depending on the weather conditions and the potato varieties grown. To provide farmers with safer alternatives to late blight management, the International Potato Center (CIP) and partners are investigating the efficacy of phosphonate fungicides for the control of potato late blight, generally applied as salts of phosphorous acid, which are often referred to as phosphites. Phosphonate is an alternative that appears to be appropriate for developing countries primarily because it represents a low risk to human health and environment (Guest and Grant 1991).

Despite the fact that fungicide use increases production cost and adds extra health and environmental risk, fungicide use is the most widely used measure for late blight control in the developing countries. In rain-fed areas around the world, it is practically impossible to produce potato without fungicides because of this disease (Hijmans et al 2000, Kirk et al 2001). Despite the little efficacy to late blight control, application of essential micro-organisms (EM), extracts of *Justicia adhatoda* increased the tuber yield by 20.4 percent (Sharma 2007). The Manufacturer of *Jeebatu* (a mixture of micro-organisms) claims its efficacy on stimulating overall plant growth and helps in suppressing late blight disease of potato and tomato (KB Poudel, personal communication).

Krinoxyl Gold (metalaxyl 8% + mancozeb 64%), manufactured by Nepal Krishi Rasayan Products, Banjaria, Bara, is the most commonly used fungicide for the control of late blight disease in Nepal. Its efficacy is getting reduced, either because of poor quality of product or prevalence of metalaxyl insensitive strain of *Phytophthora infestans*. There was prevalence of different strains of *P. infestans*, mating type type A1 and A2 in Nepal (Ghimire et al 2002, Shrestha 2005 and Shrestha et al 1998). During the previous 5 years, farmers are complaining about the poor efficacy of fungicides and reporting severe symptoms as stem blight rather than typical leaf blight in some commercial areas. In other countries, isolates of *P. infestans*, have been detected as metalaxyl insensitive, intermediately insensitive, and sensitive (Reis et al 2005). In Nepal, there is possibility of having metalaxyl insensitive isolates that could be the cause of poor efficacy of metalaxyl fungicides. The present study was done to test the efficacy of fungicide formulations commonly used in Nepal along with some new products. A preliminary economic assessment of the different products was also done.

MATERIALS AND METHODS

Pathogen isolation and inoculum production

Phytophthora infestans-infected single lesion leaf samples were washed with distilled water, put into the individual leaflet per moist Petri dishes and incubated for 24 h at 16 ± 1°C, RH 85 ± 5% with 12 h light cycle using seed germinator. Tubers of susceptible variety 'Kufri Jyoti' were washed, surface sterilized by dipping in 2 percent sodium hypo-chloride (NaOCl) solution for 5 min and washed again with distilled water 3 times. Tubers were cut into slices of 0.5 cm thick and placed two slices per Petri dish. After sporulation of lesions on incubated leaflets, a small piece from the edge of each growing lesion (5 mm^2) was transferred onto a tuber slice. After 5 days of incubation at 16±1°C the mycelium grew through the tuber slice. Without touching potato tissue, a little plug of mycelium was transferred onto Petri dishes containing rye agar-A medium. Petri dishes were incubated at 16 ± 1°C for 5-6 days. Cultures were again transferred to rye agar slants incubated for 16 ± 1°C for 15 days and placed in a refrigerator at 4^{o}C for up to 3 months.

Medium size, healthy tubers (without rotting or green coloration) of Kufri Jyoti were washed and surface sterilized as described earlier. Around 5-10 mm thick slices were made using sterilized sharp stainless steel knife. Transparent medium size (8 × 10 × 5 cubic inch) plastic boxes were made moist by lining with water saturated two layers of blotting papers at 1.5 cm below and 3.0 cm above the slices.

Slices were inoculated with the isolate of *P. infestans* (LPR-1) at the center of slices with 50 µl inoculum suspension having sporangial density of 6×10^3/ml using micropipette. Sporangia were washed from the upper side of a sporulating lesion with distilled water using plastic atomizer avoiding bacterial rots. The suspension was passed through four layers of cheese cloth to separate large clots of mycelia. Millipore filter was used to separate sporangia using 30 µm nylon filters to remove mycelium and other debris. The filtrate then passed though a 10 µm filter, which traps the sporangia. These sporangia were washed 3-4 times with distilled water and then collected from the filtering device with small amount of distilled water. Desired sporangial concentration (6×10^3) was obtained using hemacytometer.

***In-vitro* evaluation of fungicides**

A 100 ml buffer stock solution of 8000 ppm (active ingredient basis) for each of 9 fungicide formulations except *Jeebatu* and Krinoxyl Gold (Table 1) was prepared and diluted in 1000, 2000, 3000 and 4000 ppm. Highly effective fungicides were further tested for lower concentrations as 750, 500, 250, 200,150 100, 50, 40, 30 and 20 ppm.

Table 1. Trade name, common name and manufacturers of test fungicides

Trade name	Common name	Manufacturers
Galaxy	Metalaxyl	Devidayal (sales) Ltd., Mumbai
Krinoxyl Gold	Metalaxyl + Mancozeb	Nepal Krishi Rasayan, Bara
Uthane M45	Mancozeb	Phosphorous India Limited
Acrobat	Dimethomorph	BASF India Limited
Sectin	Phenomidon +Mancozeb	Bayer Crop Science India
Agrifos-400	Phosphite	Agrichem Mfd Ind.Qld, Australia
Jatayu	Chlorothalonil	Coromandel Fertilizer Ltd, India
Fluazinam	Fluazinam	Ishihara Sangyo Kaisha Ltd., Japan
Antracol	Propineb	Bayer Crop Science Ltd., India
Metalaxyl	Metalaxyl	ITIS Corporation Kuala Lumpur, Malaysia
Jeebatu (a mixture of microorganisms)	Jeebatu	Nepalese Natural Bio-Products Pvt. Ltd., Danchi, Kathmandu, Nepal

Three tuber slices per replicate, prepared as described earlier, were dipped into 50 ml of each fungicide concentration for 20 sec and the treatments were replicated three times. Slices were inoculated with *P. infestans* isolate (LPR-1) and incubated as described earlier. After 6 days of incubation, percent tuber slice area covered by fungal colony was recorded by visual estimation.

Percent inhibition of colony growth was calculated as follows:

$$\text{Inhibition growth (\%)} = \frac{C - T}{C} \text{x } 100$$

Where,

C = Percent area covered by colony in untreated control

T = Percent area covered by colony in fungicide-applied slice

Efficacy of fungicides under field conditions

Field experiments were carried out during autumn seasons of two successive years, 2009 and 2010 at Hattiban, Lalitpur, respectively. The late blight susceptible potato variety Kufri Jyoti was used both years. For 2009, eight fungicides were tested; three of these were retested in 2010 (Tables 2 and 3).

For both years, planting distance was 60 cm between and 25 cm with rows. Plant nutrients in the form of N, P_2O_5 and K_2O @ 100:100:60 kg/ha, respectively, through urea, di-ammonium phosphate and muriate of potash, were applied on the line demarcated for planting just prior to planting. Farm yard manure @ 20 t/ha was also applied on furrows before planting. Sprouted tubers of approximately similar physiological age were planted at 5-6 cm depth in ridges. Two flood irrigations at 30 and 45 days after planting (DAP) were given both years.

In 2009, chemical fungicides were sprayed six times at an interval of seven days, beginning with the appearance of symptoms at 26 days after planting. The spray volume was 800 l/ha for the first three sprays and 1000 l/ha for two sprays and 1200 l/ha the last one sprays with the same amount of active ingredient per unit area. *Jeebatu* @ 50ml/ l was sprayed ten times at 5 day intervals started 7 days prior to the start of chemical spray schedule.

In 2010, the fungicides Acrobat, Sectin and Agrifos-400, found effective in the 2009 experiment were further evaluated either alone or in alternation with mancozeb comparing with Krinoxyl Gold (metalaxyl 8% + mancozeb 64% -1.13 kg a.i./spray/ha) as standard check. Spray timing (Table 3) was chosen to cover crucial crop stages for tuberization and tuber bulking periods, as late blight infection starts from 30 DAP under Khumaltar conditions and Kufri Jyoti matures in 90 DAP during this autumn season. For both years fungicides were applied using hand compression plastic sprayers (2.5 l) until foliage was completely wet; an adjuvant 'APSA 80' @ 0.3 ml/l was also used.

Late blight severity was recorded as percent leaf area damaged for the two central rows of each plot at 7–14 day intervals starting from the initiation of disease symptoms. Disease assessments ended when the non-sprayed treatment reached 100% percent severity. In 2009, observations were taken at 26, 40, 46, 52, 59, 68, 74 DAP. Whereas, in 2010 observations were taken at 30, 40, 50, 60, 70 and 80 DAP. Tuber yield, foliage-biomass (vine mass) and late blight incidence in tubers was recorded at harvest.

Data analysis

For each plot and assessment date, the area under the disease progress curve (AUDPC) was estimated using the following formula (Campbell 1990, Madden and Hughes 1995).

$$AUDPC = (T_{i+1} + T_i) * \left[\frac{(D_{i+1} + D_i)}{2}\right]$$

Where,

T is the time in days since planting and D was the estimated percentage of area with blighted foliage.

The relative AUDPC (RAUDPC) for the entire season was estimated using the following formula (Baker et al 2000).

$$RAUDPC = \frac{\sum (T_{i+1} - T_i) * \left[\frac{D_{i+1} + D_i}{2}\right]}{T_{Total} * 100}$$

Where,

T_{total} was the period in days from the first to the last evaluation. Values of the RAUDPC range from 0 to 1. Percent disease control is given as the treatment RAUDPC divided by the RAUDPC of the unsprayed control.

Genstat 5 second edition was used for analysis of variance of all the variables measured and MSTATC was used for Duncan's multiple range test for treatment differences as required.

RESULTS

Efficacy of fungicides under laboratory conditions

Colony growth of *P. infestans* (Isolate LPR-1) was inhibited by 100 percent on tuber slices treated with Acrobat at 50 ppm, Sectin at 250 ppm, metalaxyl at 500 ppm and Agrifos-400 at 4000 ppm. Jatayu, Galaxy and Uthane-45 caused poor inhibition of colony growth (18.3-33.3%) even at 4000 ppm concentrations. Fluazinam and Antracol also did not inhibit 100 percent colony growth at 4000 ppm. A mixture of microorganisms '*Jeebatu*' commercial product even at 1:5 dilutions did not inhibit colony growth and was found comparable with untreated check. Among the fungicides tested, Acrobat was found to be the most effective in controlling *P. infestans* under laboratory conditions (Figure 1), inhibiting growth by 50 percent even at 20 ppm.

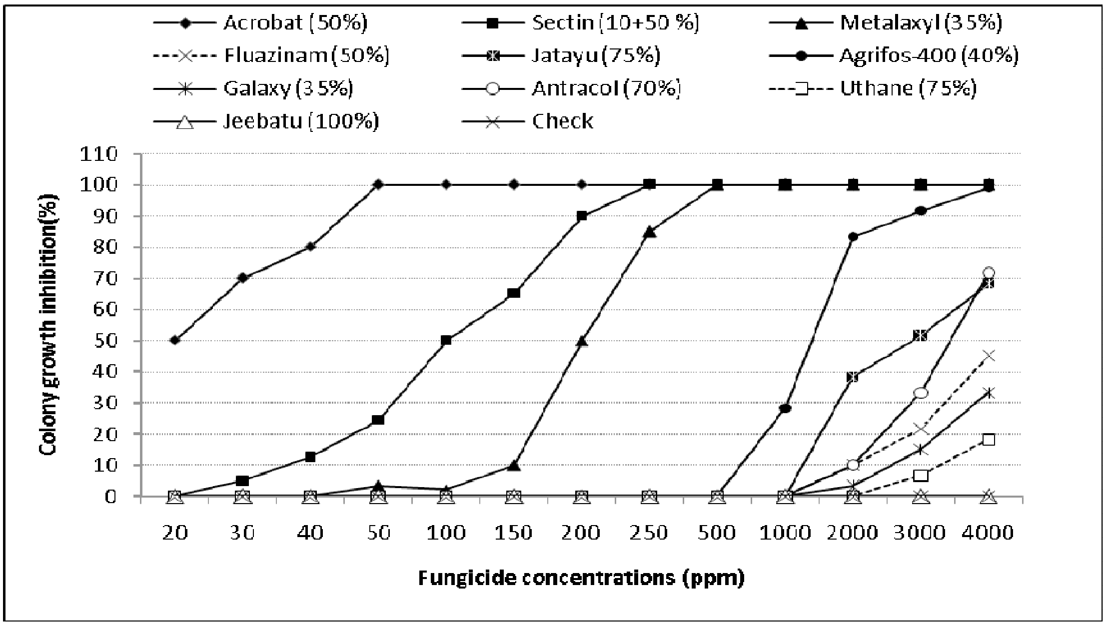

Figure 1. Effect of fungicide concentration on colony growth of *Phytophthora infestans.* Data presented as percent inhibition relative to an untreated control.

Effects on fungicide treatments on late blight severity in the field

The highest disease control in 2009 (93.8%) was observed in Sectin-sprayed plots followed by Acrobat (89.8%) and Agrifos-400 (81.9%) (Table 2). All other compounds gave reduced control, with Galaxy providing the lowest control among chemical compounds (17.1%). *Jeebatu* was not statistically different from the control and thus provided no measureable control.

Relative area under disease curve (RAUDPC) was minimum (0.028) in Sectin-sprayed plots, followed by Acrobat (0.047) and Agrifos-400 (0.083) and as compared to unsprayed control plots (0.459). Efficacy of Sectin and Agrifos-400 was comparable with Acrobat but Agrifos-400 showed significantly ($p < .001$) poor efficacy than Sectin (Table 2). Galaxy containing 35% metalaxyl showed significantly poor efficacy showing high RAUDPC (0.380) compared with the other seven test fungicides (Table 2). Antracol performed better than Jatayu (chlorothalonil) in controlling *P. infestans*. '*Jeebatu,*' a mixture of micro-organism product, did not show any effect on controlling the disease. There was no significant difference between *Jeebatu*-sprayed and unsprayed check plots (Table 2). Among the high efficacious fungicides, Sectin and Acrobat were at par in controlling the disease.

Table 2. Effects of fungicides application on tuber number, yield and vine mass (kg/7.2 m^2 plot) at Khumaltar in 2009

Treatments (Fungicides)	Vine mass, kg/plot	RAUDPC	Disease control, %	Tuber yield, kg/plot	Yield increase over untreated control, %
Acrobat 50%WP	6.00 a	0.047 fg	89.8	16.33 ab	127.8
Fluazinam 50%WP	3.73 bc	0.187 e	59.3	13.26 abc	84.9
Jatayu 75%WP	2.53 cde	0.323 c	29.6	7.97 de	11.2
Agrifos-400 (40%)	5.40 ab	0.083 f	81.9	14.13 abc	97.1
Galaxy 35% WS	1.70 de	0.380 b	17.1	11.57 cd	61.4
Uthane M-45: 75% WP	3.30 cd	0.313 c	31.9	13.17 abc	83.7
Sectin (10+50 %WP)	7.10 a	0.028 g	93.8	16.63 a	131.9
Antracol 75% WP	4.10 bc	0.245 d	46.7	12.2 bc	70.2
Jeebatu (5% v/v)	0.87 e	0.443 a	3.6	7.07 e	-1.4
Untreated control	0.90 e	0.459 a	0.0	7.17 e	0.0
F-test (Level of significance)	< .001	< .001		< .001	
LSD (0.05)	1.732	0.036		0.036	
CV, %	28.3	12.9		12.9	

Values within the column followed by same letter do not differ significantly ($p < 0.05$).

In 2010, there was a clear effect of fungicide spray frequency (2, 4 and 6 times) in most of the fungicide applied plots. The best control occurred with Acrobat (92.2%), followed by Sectin (84.2%) and Agro-fos 400 (74.6%). Alternation of two sprays of these fungicides with two sprays of mancozeb provided levels of control between 47 and 63 percent. Six sprays of Krinoxyl Gold provided only 25.6 percent control (Table 3).

Table 3. Effects of fungicides and spray frequencies on relative area under disease progress curve (RAUDPC) and late blight control at 80 DAP in 2010

Treatments	RAUDPC	Disease control (%)
Acrobat, 2 sprays	0.274 de	42.44
Acrobat, 4 sprays	0.107 kl	77.52
Acrobat, 6 sprays	0.037 m	92.23
Agrifos-400, 2 sprays	0.306 de	35.71
Agrifos-400, 4 sprays	0.221 fg	53.57
Agrifos-400, 6 sprays	0.121 jk	74.58
Sectin 2, sprays	0.293 de	38.45
Sectin 4, sprays	0.155 ij	67.44
Sectin 6, sprays	0.075 l	84.24
Krinoxyl Gold, 2 sprays	0.420 b	11.76
Krinoxyl Gold, 4 sprays	0.394 b	17.23
Krinoxyl Gold, 6 sprays	0.354 c	25.63
Acrobat, 2 sprays + Uthane M45, 2 sprays	0.174 hi	63.45
Agrifos, 2 sprays + Uthane M45, 2 sprays	0.252 ef	47.06
Sectin 2, sprays + Uthane M45, 2 sprays	0.191 gh	59.87
Untreated control (water spray)	0.476 a	0.00
F-test (level of significance)	< 0.001	
LSD (0.05)	0.035	
CV, %	8.8	

Values within the column followed by same letter do not differ significantly ($p < 0.05$). Two sprays: 18 days intervals; four sprays: 9 days intervals and six sprays: 6 days intervals.

In 2010, late blight disease progress was slow up to 40 DAP in all the fungicide-treated plots. After 60 DAP disease progress became faster in water-sprayed plots and Krinoxyl Gold sprayed plots. Whereas, disease progress was significantly minimum up to 80 DAP in Acrobat, Sectin and Agrifos-400 six time-sprayed plots (Figure 2).

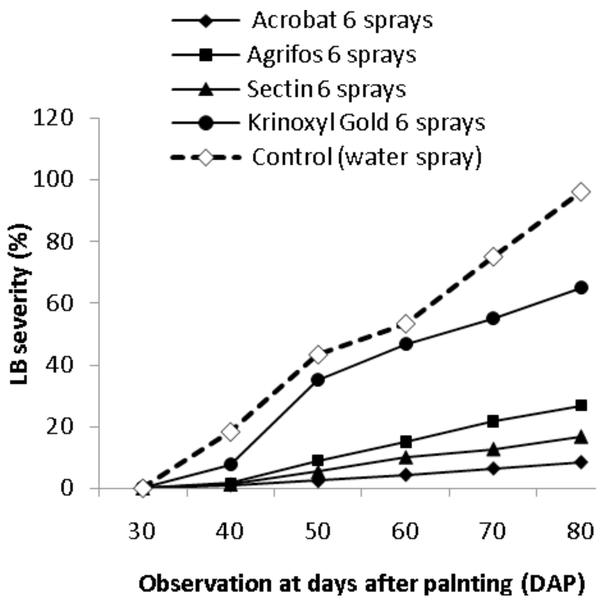

Figure 2. Effect of some selected fungicide spray frequencies on late blight severity at 30-80 DAP.

Effects of fungicide on vine mass and tuber yield

At harvest, amount of vine mass production was the highest (7.10 kg) in Sectin-sprayed plot followed by Acrobat (6.0 kg), Agrifos-400 (5.4 kg) and Antracol (4.10 kg). All the fungicide-treated plots had significantly higher vine mass at harvest than the water-sprayed and *Jeebatu*- sprayed plots (Table 2). The tuber yield increase was the highest in Sectin-sprayed plots (131.9%) followed by Acrobat (127.8%), Agrifos-400 (97.1%) and Fluazinam (84.9%) over the yield of untreated plots (Table 2). Tuber yield differences between the five fungicides other than Jatayu and Galaxy were insignificant (Table 2).

Potato vine mass production and tuber yield were found highly correlated (r = 0.91) under field conditions (Figure 3). *Jeebatu* had no effect on vine mass production and tuber yield as compared to untreated check.

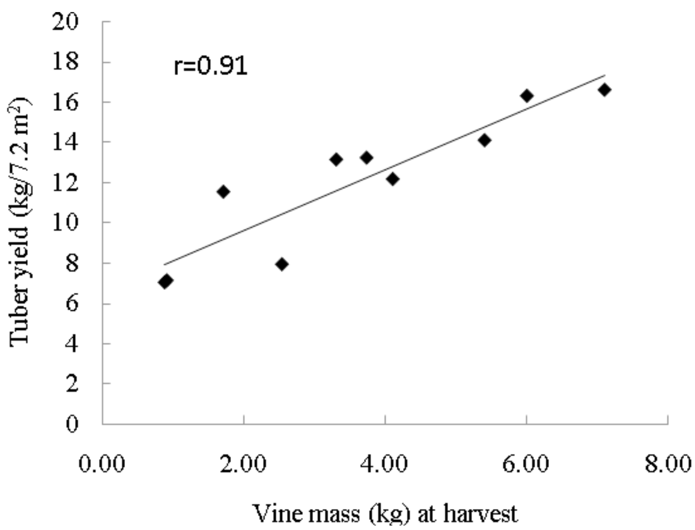

Figure 3. Correlation between vine mass and tuber yield in 2009.

Tuber yield increased by Sectin followed by Acrobat, Agrifos-400 and Fluazinam were significantly higher than rest of the treatments. All the treatments, except Krinoxyl Gold two sprays, had significant effect on vine mass production when compared with untreated control. Vine mass production was the highest in the plots sprayed six-times with Acrobat (0.90 kg), Sectin (0.81 kg) and Agrifos-400 (0.70 kg) per 3 m^2 plot at harvest (Table 4). Similarly, six-times sprays of either Sectin or Acrobat gave significantly higher tuber yield which were not significantly different (Table 4).

Table 4. Effects of fungicides on vine mass, tuber blight and tuber yield under field conditions at Khumaltar, 2010

Treatment	Vine mass, kg/3m^2plot	Tuber blight incidence, %	Tuber yield, t/ha
Acrobat, 2 sprays	0.29 hi	1.12 cde	12.58 de
Acrobat, 4 sprays	0.56 cd	0.59 de	14.83 bcd
Acrobat, 6 sprays	0.90 a	0.14 e	18.65 a
Agrifos-400, 2 sprays	0.39 fgh	0.00 e	9.90 ef
Agrifos-400, 4 sprays	0.61 bc	0.00 e	14.58 bcd
Agrifos-400, 6 sprays	0.70 b	0.00 e	18.10 ab
Sectin, 2 sprays	0.44 efg	0.71 de	11.74 de
Sectin, 4 sprays	0.58 cd	0.74 de	16.07 abc
Sectin, 6 sprays	0.81 a	0.43 de	18.90 a
Krinoxyl Gold, 2 sprays	0.26 ij	2.99 b	7.12 f
Krinoxyl Gold, 4 sprays	0.36 ghi	2.89 b	8.71 ef
Krinoxyl Gold, 6 sprays	0.31 hi	2.15 bc	9.22 ef
Acrobat, 2 sprays+ Uthane M-45, 2 sprays	0.65 bc	1.12 cde	15.10 cd
Agrifos-400, 2 sprays+Uthane M-45, 2 sprays	0.49 def	0.95 de	11.49 de
Sectin, 2 sprays + Uthane M-45, 2 sprays	0.54 cde	1.57 de	15.20 cd
Untreated control (water spray)	0.17 j	6.71 a	7.06 f
F-test (Level of significance)	< .001	< .001	< .001
LSD $_{(0.05)}$	0.10	1.05	3.23
CV, %	12.1	45.4	14.8

Values within the column followed by same letter do not differ significantly (p < 0.05)

There was no tuber blight incidence at harvest on Agrifos-400-sprayed plots whereas water-sprayed plots had 6.71 percent. Other fungicides also had significant effects on minimizing tuber blight infection (Table 4).

Cost-benefit analysis of fungicide applications

Benefit per treatment was the highest (Rs 87200) in six-times Sectin-sprayed plots, followed by Agrifos-400 (Rs 74850) and Acrobat (Rs 72700). Regarding with the cost benefit ratio of these fungicides ie Sectin, Agrifos-400 and Acrobat applications were 2.79, 2.11 and 1.68, respectively. The highest cost: benefit ratio (4.58) was obtained from four alternate sprays of Sectin and Uthane M-45 followed by Sectin two-times sprayed plot (3.5) and Sectin four-times sprayed plot (3.3) with net return of Rs 69300/ha (Table 5).

The cost benefit analysis demonstrated that the greatest return on investment was not necessarily from the treatment that gave the greatest disease control. All the treatments involving alternation with Uthane M-45 had high rates of return, as did the treatments involving Sectin, only. Treatments with Agrifos-400 and Acrobat generally had lower rates due to the high cost of the products, even though disease control was relatively higher.

Table 5. Cost-benefit analysis of fungicide applications under Khumaltar conditions, 2010

Treatment	Cost of fungicide application* /trt/ha (Rs)	Tuber yield, ton/ha	Yield increase due to trt, ton	Value of increased yield, Rs*	Benefit /trt, Rs	C:B ratio
Acrobat, 2 sprays	14400	12.58	5.52	55200	40800	2.83
Acrobat, 4 sprays	28800	14.83	7.77	77700	48900	1.70
Acrobat, 6 sprays	43200	18.65	11.59	115900	72700	1.68
Agrifos-400, 2 sprays	11850	9.9	2.84	28400	16550	1.40
Agrifos-400, 4 sprays	23700	14.58	7.52	75200	51500	2.17
Agrifos-400, 6 sprays	35550	18.1	11.04	110400	74850	2.11
Sectin, 2 sprays	10400	11.74	4.68	46800	36400	3.50
Sectin, 4 sprays	20800	16.07	9.01	90100	69300	3.33
Sectin, 6 sprays	31200	18.9	11.84	118400	87200	2.79
Krinoxyl Gold, 2 sprays	6000	7.12	0.06	600	-5400	-0.90
Krinoxyl Gold, 4 sprays	8400	8.71	1.65	16500	8100	0.96
Krinoxyl Gold, 6 sprays	10800	9.22	2.16	21600	10800	1.00
Acrobat+Uthane , 2+2 sprays	18600	15.1	8.04	80400	61800	3.32
Agrifos+Uthane, 2+2 sprays	10380	11.49	4.43	44300	33920	3.27
Sectin+Uthane, 2+2 sprays	14600	15.2	8.14	81400	66800	4.58
Untreated control (water spray)		7.06	0	0	0	

*Acrobat@ Rs 6000/kg. Sectin @ Rs 4000/kg. Agrifos-400 @ Rs 600/L. Krinoxyl Gold @ Rs 1500/kg. Uthane M-45 @ Rs 360/kg. Potato selling price at farm gate @ Rs 10/kg. Labour cost Rs 200/head/day. 6 labours/ha/spray (Rs. 1200/spray/ha). *(cost of fungicide + cost of labor at local level).*

DISCUSSION

Mancozeb is one of the oldest fungicides that have been in use since 1948 (www.epa.gov/oppsrrd1/REDs/factsheets/mancozeb_fact.pdf). In Nepal, mancozeb under different trade names are widely available and has been used extensively for many years. In the present study, the results showed that the fungicide has significant effect on late blight control and increase in tuber yield under field conditions when compared with untreated control. However, the efficacy of the fungicide (Uthane M-45) under laboratory test gave only 18 percent colony growth inhibition even at 4000 ppm concentration, which was far below than the inhibition shown by Acrobat, Sectin, Agrifos-400, Metalaxyl, Fluazinam and Antracol. In general, efficacy of mancozeb for the control of late blight has decreased over the years, and new fungicides have come up but new systemic fungicides are costly. Thus, rational application schedule should be identified and followed to make the application cost effective. Results showed that among the fungicide tested, Sectin, Agrifos and Acrobat were effective against late blight under laboratory and field conditions. Mancozeb (Uthane M-45) didn't do well in laboratory but appears to work fairly well in field as compared to untreated control.

Metalaxyl was first registered as a pesticide in the U.S. in 1979 (www.epa.gov/oppsrrd1/REDs/factsheets/0081fact.pdf), and has been the most effective therapeutic fungicide for the control of late blight (Groves and Ristaino 2000). Fungicides containing metalaxyl (8%) and mancozeb (64%) are also available in different trade names ie Krinoxyl Gold, Matco and Krilaxyl. Sole metalaxyl (35% a.i.), are also available under different trade names eg Galaxy (Devidayal Ltd, Mumbai, India) and Metalaxyl (ITIS Corporation Kuala Lumpur, Malaysia). In our studies, Metalaxyl (35% a.i.) showed the 100 percent colony growth inhibition on tuber slice at 500 ppm concentration whereas, Galaxy inhibited colony growth only 33 percent even at 4000 ppm concentration. Field efficacy of Galaxy was also poor when compared with Uthane M-45, Fluazinam, Jatayu and Antracol. Efficacy of Krinoxyl Gold was quite low (35% disease control) even with six sprays compared with Sectin, Acrobat and Agrifos-400 (91.6 to 83.3%). In our present studies, we found good efficacy of Metalaxyl (manufactured in Malaysia) against late blight, which indicates that *P. infestans* isolates prevailing in Khumaltar conditions 'LPR-1' are sensitive to metalaxyl. The cause of poor efficacy of Galaxy or Krinoxyl Gold might be due to the low content of metalaxyl active ingredient in the products, suggesting for the need of quality control by the concerned authorities.

In Nepal, Acrobat, Sectin, Agrifos-400, Fluazinam and Antracol are newly introduced fungicides for testing purpose. Of them, Acrobat and Sectin were found superior in controlling the disease and increasing in tuber yield under field conditions when compared with Uthane M-45, Krinoxyl Gold and other test fungicides. Even the two sprays of Acrobat or Sectin were equally or more effective than six sprays of Krinoxyl Gold. The field efficacies of these fungicides were comparable with the in-vitro tests as Sectin gave 100 present colony growth inhibition at 200 ppm and Acrobat at 50 ppm. No other fungicides gave 100 percent colony growth inhibition even at 4000 ppm, except Agrifos-400. Though dimethomorph (Acrobat) is highly effective there is possibility of developing *P. infestans* resistance to it (Stein and Kirk 2004). In Europe, phenomidon (Sectin) has been registered for late blight control (Bain 2002). Both fungicides are systemic in nature, residual effect may be higher and that may create resistance in the targeted pathogen.

In the present study, Agrifos-400 (Phosphorous acid) significantly reduced both the foliar and tuber blight incidence. Also, 100 percent colony growth inhibition was obtained with Agrifos-400 at 4000 ppm under laboratory conditions. Johnson et al. (2004) have also reported the efficacy of phosphorous acid (@7.49 kg a.i./ha), reducing the tuber rot incidence and severity significantly when applied at the initial tuber bulking stage. Phosphorous acid is also known to stimulate the plant's natural defense against the pathogen attack (Kadish et al 1990). It has been reported that phosphorous acid is comparatively environment friendly and low health hazardous (LD_{50} >5000 mg/kg) compared with rest of the tested fungicides (http://www.fao.org/ag/AGP/AGPP/Pesticid/ Code/Download/label.pdf). Agrifos-400 does work pretty well in field but is somewhat expensive, however, has many qualities for health and environment and also very good for tuber blight as data show. Minimization of tuber blight may lead to less blight in subsequent season.

Though the fungicides, Sectin, Acrobat and Agrifos-400 were found highly effective, the cost involved was quite high and the cost-benefit ratio was low. These fungicides when sprayed alternately with Uthane M-45 for four sprays at an interval of nine days cost of fungicides application decreased, field efficacy increased and cost-benefit ratio also increased. For obtaining high cost-benefit ratio alternate sprays of Sectin and Uthane M-45 or Acrobat and Uthane M-45 could be adopted for autumn season potato crop to manage late blight under Kathmandu valley conditions.

Cost benefit ratio depends on price of potatoes as more expensive fungicides gave more yields but didn't justify price. Cost benefit analysis should be interpreted with caution. It may vary depending on cost of fungicide, yields and price of potatoes in the local markets. If potatoes paid more cost benefit ratio would change. Additional sprays in alternation (6 instead of 4) may have given even better results and this should be researched more.

Alternate sprays of systemic and contact fungicides also minimize adverse effect on environment and risk of developing resistance in the pathogen population. In the previous studies, Sharma (2007) found three sprays of Krilaxyl @1.5 kg a.i./spray/ha most economical than six sprays for autumn season potato crop in Kathmandu valley. It should be clear that Krilaxyl and Krinoxyl Gold are two different products of different manufacturers though both products claim to have metalaxyl as their active ingredient.

In addition to foliar blight control, phosphorous acid significantly reduced the tuber blight incidence than rest of the fungicides used as foliar spray. The phosphonate salts are metabolized to phosphonic acid inside plants (Fenn and Coffey 1984). The phosphonate anion (HPO_3^{2-}), also named phosphite, is the hydrolysis product of phosphonic acid, also called phosphorous acid (H_3PO_3); in plants phosphorous acid is exceptionally mobile in the phloem of plants, moving both up and down stems (Cohen and Coffey 1986). In our studies, the dose of phosphorous acid was low (3.15 kg a.i./ha/spray) as compared to 7.49 kg a.i./ha (Kadish et al 1990) and was sprayed six times as foliar spray. Phosphorous acid has both a direct and an indirect effect on oomycetes. Phosphorous acid has an indirect effect by stimulating the plant's natural defense response against pathogen attack (Kadish et al 1990). It has been reported that phosphorous acid is comparatively environment friendly and low health hazardous (LD_{50} >5000 mg/kg) than rest of the tested fungicides (www.fao.org /ag/AGP/AGPP/Pesticid/Code/Download/ label.pdf).

Regarding with bio-control of late blight, manufacturer of *Jeebatu* (mixture of microorganisms) has suggested for the use of this product as late blight disease suppresser. In our studies, this product was not found effective in controlling late blight (*P. infestans*) under field conditions when sprayed ten times at two to five days intervals during the crop season and it had no effects on colony growth inhibition on tuber slice test under laboratory conditions. In the present study, we did the bioassay on tuber slices using simple techniques, which can be applied anywhere without sophisticated arrangements. We found the techniques reliable and the results reproducible. Porter et al (2006) also found tuber disk method more sensitive in determining the efficacy of a fungicide in inhibiting infection and spore viability when *Phytophthora*-infested soil inoculated on to tuber disk.

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Effect of Nitrogen and Potassium on Yield, Storability and Post Harvest Processing Qualities of Potato for Chips

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ABSTRACT

Field and storage experiments were conducted at Hattiban Farm, Khumaltar and Dark Storage House of National Potato Research program (NPRP) at 28 ± 0.6°C and 88-89% R.H. This study explored the main effects and interactions effects between nitrogen (50, 100, 150 and 200 kg/ha) and potassium (30, 60, 90 and 120 kg/ha) on processing grade tuber yield, total yield and their storability and better processing chips quality of potato on var. Kufri Jyoti. Field and storage experiments were conducted in randomized complete block design and complete randomized design with factorial arrangement of treatments in 3 replications, respectively. After harvesting and curing of potato, 5 kg of tubers weighing more than 60 g were kept in plastic trays and placed on cemented floor in two layers for 90 days. Observations were recorded on yield, storage losses, sprouting percentage, chips quality parameters like specific gravity (SG), dry matter (DM) and reducing sugar (RS). The storage loss and sprouting percentage was recorded at 15 days intervals and quality parameters were determined before and after storage of potato. The results showed a significant effect of nitrogen, potassium and their interaction on yield, sprouting, weight loss percentage and quality parameters. Based on the yield and quality parameters the combination of 150:100:60 kg $N:P_2O_5:K_2O$ along with 20 t compost/ha is found suitable for higher yield (30.22 t/ha) and processing qualities for chips at Lalitpur and similar soil and environment conditions of Nepal.

Key words: Nitrogen, potassium, potato, processing qualities for chips, yield

INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important crop in Nepal. It occupies the fifth position in area coverage and second in total production. Potato plays an important role in the country's food security and poverty alleviation because of its short vegetative cycle and high cash and food value as compared to major cereals (Adhikari et al 2008). The gradual and steady increase in potato production in last few years has created post-harvest problems because of inadequate storage and processing facilities. The storability, post harvest handling and processing qualities of potato are important parameters for its better utilization. In the developing countries of the tropics and sub-tropics, post harvest handling and storage losses of seed and ware potatoes have been estimated to be 20-30 percent (Prasad et al 1989, Satter et al 2002). It is reported that, the reduction of post harvest losses is the better option for increasing the same amount of food availability than by increasing the same amount of yield (Gautam and Bhattarai 2006, Karki 2002).

Potato is a heavy feeder crop and requires a large amount of nutrients. The cultivation of high yielding potato varieties in intensive cropping system without proper combination of manure and fertilizer causes the soil mining and decline the soil fertility and productivity. Nutrient deficiencies may limit the leaf canopy growth and its duration, resulting in reduced carbohydrate production and tuber growth. However, excessive nutrients application may cause nutrient imbalance or over stimulate vegetative production at the expense of tuber production. Khatri and Shrestha (1998) reported that, the production of 20 ton crop extract 140:140:190 kg N:P:K, while the average application of 15-30 t/ha FYM would supply maximum of 35-50:50-100 and 35-70 kg of N:P:K, not enough to support the potential higher yield. Nitrogen is the most important nutrient which increases the vegetative growth, crop duration, tubers quality and decides the yield level, but excessive N supply will suppress tuber initiation, reduce yield and decrease specific gravity in some cultivar (Mikkelsen 2006). Its excessive application resulted tuber with lower reducing sugar at harvest and accumulated less reducing sugar during storage (Iritani and Weller 1978). Potassium is another most important nutrient for potato production. It has crucial role to metabolic function such as the movement of sugar from the leaves to the tubers, transformation of sugar into potato starch. Potassium helps on photosynthesis, maintains cell turgid, enhances shipping quality, extends shelf life and improves chips colour and decreases storage losses (Marschner 1995). The application of both nitrogen and potassium can increase dry matter if there is a yield response from their application and their specific response is partly dependent upon variety and field environment (Schippers 1976). However, the effect of potassium and nitrogen was found more outstanding on potato production. Potato tubers remove 1.5 times much potassium than nitrogen and 4-5 times than phosphorus (Perrenoud 1993). National Potato Research Programme (NPRP) recommended 80:80:60 kg NPK/ha along with 20 tons FYM for on farm and 100:100:60 kg NPK/ha along with 20 tons FYM for on-station experiment based on research review and trials (Khatri et al 1999) before a decay ago for ware potato production, which could not be same for long term storage and chips grade potato production.

Potato chips are the most popular processed products in Nepal. The high demand of potato chips has been fulfilled by importing large quantity of chips especially from India. In Nepal, commercial production of chips started by CG Foods Pvt Ltd, Nawalparashi in 2003 by using sophisticated plant and produced 5 brand of potato chips. It has capacity of producing 200 kg chips/hours and total potato used for chips making was 1500 t/year and the capacity has been increasing by 10-15 percent annually (Sah 2010). Besides this, there are a large numbers of cottage industries for preparation of chips in different part of country to fulfill the high demand of chips, but the research on this aspect is lacking.

For processing potato into chips, potato should have round or oval tuber shape and fleet eyes, high dry-matter and specific gravity and low reducing sugars. The dry matter content of about 18-20 percent and reducing sugar content up to 250 mg/100 g fresh weight is considered acceptable for chips making (Ezekiel and Shekhawat 1999). The dry matter content determines the yield of chips, oil uptake and crispness of fried product where as reducing sugars determines the colour of chip.

Storage of potato for long period is very difficult. There is always sacrifice between processing quality and weight loss during storage. At low temperature, storage potato shows accumulation of reducing sugar due to increased membrane permeability and starch breakdown where as high temperature increases high weight loss. The brown colour of chips which is undesirable is caused due to reaction of reducing sugars and amino acids while frying at high temperature. Many researches conducted in India suggested to store processing potato at 12^{o}C by applying sprouting inhibitors to inhibit increase in reducing sugars and decrease weight loss percentage. However, such separate storage rooms or chambers were still not constructed and practiced in Nepal. So, the only alternative method is storage of potato at prevailing ambient temperature by manipulating different cultural practices to reduce reducing sugars and weight loss percentage. Therefore, present study was conducted to investigate the appropriate dose of nitrogen, potassium and their interaction effects on yield, storability and better processing qualities of potato for chips in the mid hill conditions.

MATERIALS AND METHODS

Field experiment was conducted at Hattiban Farm, Lalitpur during summer season of 2010 at an elevation of 1340 m asl, clay type of soil with below 6.0 soil pH. The experiment was laid out in RCB design with factorial arrangement of treatments and three replications. Total of 16 treatments involving combinations of four levels of nitrogen (50, 100, 150 and 200 kg/ha) and potash (30, 60, 90 and 120 kg/ha) were included. The recommended variety Kufri Jyoti was planted on 25th January and harvested on 17th May 2010 (103 days after planting). The plot size was 10.5 m^2 (3 × 3.5 m) and planted at 60 cm row to row and 25 cm plant to plant distance. Compost (20 t/ha) and phosphorus (100 kg/ha) were applied in all treatments at the time of planting. Other cultural practices were carried out as NPRP recommendation. Observations were recorded on numbers and weight of different size tubers and total yields.

The storage experiment was conducted at the Dark Storage House of NPRP, Khumaltar under ordinary dark room condition at 28 ± 0.6°C and 88-89% RH. After harvesting and curing of potato for 15 days, five kilogram of potatoes of each treatment was stored in plastic trays under dark room for 90 days (21 May to 18 August 2010). Trays were kept in two layers and altered at 15 days intervals as upside down order.

Observations were recorded on yield, storage losses, sprouting percentage and chips quality parameters like specific gravity (SG), dry matter (DM) and reducing sugar (RS) before and after storage. Storage loss and sprouting percentage were recorded at fortnight intervals. DM was determined by chopping and mixing of tubers in to small pieces and oven drying 100 g sample at 80°C for 6 hours and then at 65°C till constant weight. SG was determined by using potato hydrometer (developed by Snack Food Association) by weighting 8 pound of potato before storage and by water displacement methods after storage. Reducing sugars was determined by di-nitrosalicyclic colorimetric method (Miller 1959) by recording the absorbance in spectrophotometer at 575 nm. The observed data were analyzed by using Gen-stat 532-2 programme and DMRT of MSTATC was used for mean comparison.

RESULTS AND DISCUSSION

Tuber size distribution and total yield

The main effect of N was highly significant for production of 30-60 and more than 60 g tubers and total yield of potato. The application of 150 kg nitrogen produced the highest yield (13.71 t/ha) of 30-60 g tubers and more than 60 gram (13.46 t/ha) tubers and total yield (29.62 t/ha). These parameters increased linearly up to 150 kg N/ha and deceased thereafter but not at significant level (Figure 1).

The main effect of K showed significant and highly significant differences only for the production of more than 60 g tuber and total tuber yield. There was linear increased of more than 60 g tubers and yield of potato due to higher dose of K (Figure 2).

The combined effect of N and K was significant on production of 30-60 g tubers and total yield of potato. The highest (15.0 t/ha) 30-60 g tuber yield was produced with 150 kg N and 90 kg K/ha and it was at par with 200 kg N and 90 kg K; 150 kg N and 60 kg K and 150 kg N and 30 kg K. The total highest yield (30.69 t/ha) was recorded on 200 kg N and 120 kg K and it was found at par with 200 kg N and 90 kg K (30.59 t/ha), 150 kg N and 60 kg K (30.22 t/ha) and 150 and 90 kg K (29.84 t/ha) where as the lowest yield (21.43 t/ha) was recorded on 50 kg N and 30 kg K and it was at a par with 50 kg N and 60 kg K (22.81 t/ha). The detail main effect of N, K and their interaction effect is presented in Table 1.

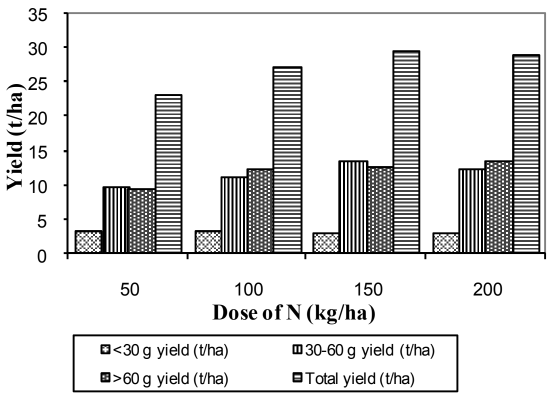

Figure 1. Effect of N on different size and total yield of potato (t/ha).

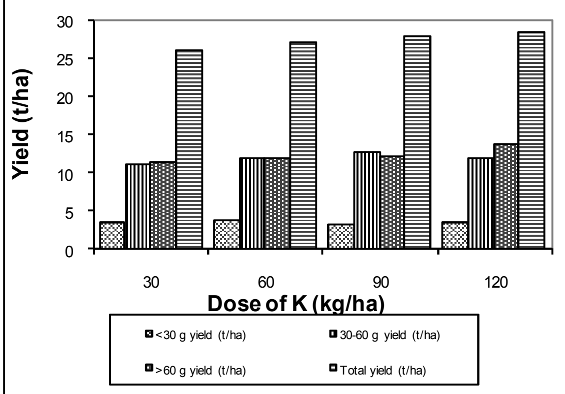

Figure 2. Effect of K on different size and total yield of potato (t/ha)

Table 1. Interaction effect of nitrogen and potash on tuber distribution and total yield of potato (t/ha) at Khumaltar, 2010

Dose of N:K_2O, kg/ha	Tuber yield (< 30 g)	Tuber yield (30-60 g)		Tuber yield (> 60 g)	Total tuber yield (t/ha)	
50 : 30	3.43	8.85	e	9.14	21.43	h
50 : 60	3.42	9.55	ef	9.84	22.81	gh
50 : 90	3.30	11.21	cdef	9.35	23.86	fg
50 :120	3.83	9.72	ef	10.86	24.41	f
100 :30	3.43	11.77	bcde	11.47	26.67	e
100 : 60	3.89	11.48	bcdef	11.52	26.89	e
100 :90	3.28	9.72	ef	13.52	26.52	e
100 :120	3.27	12.15	bcde	13.11	28.53	cd
150 :30	3.39	13.83	abc	11.54	28.76	bcd
150 : 60	3.48	13.29	abc	12.79	30.22	ab
150 :90	2.72	15.00	a	12.12	29.84	abc
150 :120	2.71	12.05	bcde	14.90	29.67	abcd
200 :30	2.73	10.84	def	13.04	26.61	e
200 : 60	3.18	11.78	bcde	13.16	28.12	de
200 :90	3.32	14.16	ab	13.11	30.59	a
200 :120	3.36	12.79	abcd	14.53	30.69	a
Mean	3.30	11.80		12.13	24.23	
F-test	Ns	*		ns	*	
LSD (± 0.05)	-	2.412		-	1.483	
CV, %	19.6	12.3		13.0	3.3	

*ns, *,**, Non significant and significant differences at 0.05 and 0.01 levels, respectively.*
Common letters in a column are not significantly different by DMRT at 0.05 levels.

These findings are in agreement with the report of Khatri and Shrestha (1998). They reviewed fertilizer trials conducted in different part of country and reported that potassium and nitrogen had the greatest effect on tuber yield both in the mid and high hills. Khairgoli (1977) reported, based on three years trial conducted in Kathmandu valley, that the potato yield was increased with increased dose of nitrogen up to 160 kg along with 25 ton FYM/ha. In Chitwan conditions, the application of 200 kg K/ ha was found prominent and significant only in accumulation of dry matter in leaves but the highest yield was recorded on application of 100 kg K/ha (Basnet et al 2001). Adhikari and Sharma (2002) reported the maximum bulking rate (18.75 g/hill/day) in the combination of 100:75:100 kg NPK/ha in Kufri Sindhuri variety under Chitwan conditions.

Weight loss percentage

The effect of nitrogen on weight loss percentage was significantly different only at 15 days after storage. The minimum percentage (3.65%) was on application of 100 kg N and it was at par with 50 kg N (4.09%). Since there was no significant effect of N on weight loss percentage on different dates after storage, but 50 kg N/ha had minimum weight loss (11.61%) than higher doses of nitrogen. The effect of potash on weight loss percentage was insignificant up to 15 days storage and then showed significant and highly significant differences at 30 and 45, and 60, 75, 90 days, respectively. However, the application of 60 kg and higher dose of potassium showed similar results. The interaction effect of N and K showed significant effect on weight loss percentage at 15 days storage and thereafter did no show any difference on weight loss percentage. The detail effect of N and K on weight loss percentage at different dates of storage is presented in Table 2.

Table 2. Effect of N, K and their interaction on weight loss percentage of potato tubers at different days after storage in ambient temperature (28 ± 0.6 ºC) under dark room at Khumaltar, 2010

Treatments	Percent weight loss at different dates											
	15 days		30 days		45 days		60 days		75 days		90 days	
Nitrogen (kg/ha)												
50	4.09	ab	6.06		7.46		9.20		10.36		11.61	
100	3.65	b	6.02		6.82		9.53		10.82		12.63	
150	3.57	b	6.20		7.37		8.93		10.83		12.53	
200	4.62	b	6.12		7.80		9.77		10.90		12.58	
F-test	*		ns		ns		ns		ns		ns	
LSD (± 0.05)	0.795		-		-		-		-		-	
Potash (kg/ha)												
30	4.48		6.85	a	8.05	a	10.59	a	12.38	a	14. 22	a
60	3.70		6.14	ab	8.01	a	9.13	b	10.26	b	12.46	b
90	3.50		5.33	b	6.57	b	8.33	b	9.87	b	11.44	b
120	4.23		6.07	ab	6.82	ab	9.37	ab	10.40	b	11.23	b
F-test	ns		*		*		**		**		**	
LSD (± 0.05)	-		1.034		1.315		1.241		1.245		1.466	
Interaction (N:K kg/ha)												
50:30	3.60	bc	6.67		7.73		9.60		10.80		12.47	
50:60	3.55	bc	5.11		*7.89*		8.67		*9.78*		11.78	
50:90	3.87	bc	6.00		6.93		8.93		10.07		11.0	
50:120	5.33	ab	6.47		7.27		9.60		10.8		11.20	
100:30	3.67	bc	7.13		8.40		12.20		13.60		15.73	
100:60	3.60	bc	5.60		6.40		8.27		9.47		11.53	
100:90	3.73	bc	5.20		5.87		8.13		9.87		11.47	
100:120	3.60	bc	6.13		6.60		9.53		10.33		11.80	
150:30	4.27	bc	5.73		6.40		8.90		11.60		12.93	
150:60	2.93	c	7.33		9.53		10.33		11.53		14.47	
150:90	2.93	c	5.60		6.73		7.73		10.0		11.97	
150:120	4.13	bc	6.13		6.80		8.73		10.20		10.73	
200:30	6.40	a	7.87		9.67		11.67		13.53		15.73	
200:60	4.73	abc	6.53		8.20		9.27		10.27		12.07	
200:90	3.47	bc	4.53		6.73		8.53		9.53		11.33	
200:120	3.87	bc	5.53		6.60		9.60		10.27		11.20	
Mean	3.98		6.10		7.36		9.36		10.73		12.34	
F-test	*		ns		ns		ns		ns		ns	
LSD (± 0.05)	1.590		-		-		-		-		-	
CV, %	24.0		20.4		21.5		15.9		14.0		14.3	

*ns, *,**, Non significant and significant differences at 0.05 and 0.01 levels, respectively.*
Common letters in a column are not significantly different by DMRT at 0.05 levels.

Dry matter content

Dry matter (DM) is an important parameter for processing quality of potatoes. It is directly proportional to the weight of chips and indirectly proportional to oil consumption of chips. In this experiment, the application of different dose of nitrogen and potassium alone and their combined effect showed the significant effect on DM percentage of potato before storage. The application of 50 kg N/ha and 100 kg N/ha produced 17.39 and 16.99 percent DM, respectively, which were not significantly different. The lowest DM (16.58%), which is not desirable for processing, was observed in treatment of 150 kg N/ha and it was at par with 200 kg N/ha (16.48%). This might be due to accumulation of more water and delay in maturity of tubers. Beukema and Van der Zaag (1979) reported that haulm weight during growth was relevant to the effect of N on DM content, as N increases the haulm weight. They reported high N application caused haulm weight above optimal for photosynthesis and DM content in tubers may be lower, even the crop gets the chance to mature. William and Smith (1967) reported that omission of N from the fertilizer greatly reduced the absorption of P. Low phosphorus and low calcium in potatoes as a result of high N applications tend to further reduce DM of potatoes. The application of K showed mixed results and did no show any increasing or decreasing trends on DM percentage.

The storage of potato for 90 days in ambient room temperature (28.1 ± 0.6ºC) had no effect on DM percentage among treatments due to effect of N or K alone and their interactions (Table 3). However, there was increment of on the dry matter percentage after 90 days (Table 3 and Figure 3).This might be de to loss of water while storing of potato in higher temperature. Various researchers reported that the DM content of potato was declined with heavy application of K alone or complete fertilizer (Hill 1953, Sheard and Johntson 1958, Gausman et al 1960, Lujan and Smith 1964, Teich and Menzies 1964). This decrease in DM content of potatoes is largely due to the chloride ion in the muriate of potash rather than potassium ion. Latzko (1955) reported that the hydrolytic activity of carbohydrates, such as invertase, amylase and B glycosidase is inhibited by the chlorine ion. Khatri and Shrestha (1998) reported that the increasing amount of nitrogen and potassium reduced the dry matter content under Jumla conditions. It has also been reported that the K has very important effect on DM content, which is normally inversely proportional to K content of soil (White et al 1974, Beukena and Van der Zaag 1979). They reported that the decrease in DM content was mainly caused by an accumulation of water by tubers in relation to K supply and plant age.

Reducing sugars

Reducing sugars is an important parameter for chips quality. Its content up to 150 mg/100 g fresh weight is consider good and up to 250 g/100 g fresh weight is consider acceptable for chips making. In this experiment, the application of higher dose of N and K significantly decreased the reducing sugars of potato. However, the content of reducing sugars was acceptable level due to treatment effect. It could be due to varietal characters. The interaction of N and K also showed significant effect on reducing sugar content of potato before storage.

There is decreasing trend of reducing sugars with increasing dose of N and K (Figure 4). It might be due to increment in water uptake and turgidity of cells. This finding is not in agreement with the finding of Banu et al (2007), but with the findings of Herlihy and Carroll (1969), Sharma and Arora (1988) and Chapman et al (1992). The effect of N was significant after 90 days storage in ambient temperature, but the effect K and its interaction with N showed insignificant results (Table 4).

Specific gravity

It is reported that high specific gravity has positive role on the processing quality of tuber. As a rule, high specific gravity means high dry matter content and high recovery percentage of chips.

The specific gravity of potato was significantly the highest with 50 kg N (1.067) and it was at par with 100 kg N/ha (1.065). The lowest specific gravity was at 150 and 200 kg N/ha (1.062). However, after 90 days storage there was insignificant effect of N on specific gravity (Table 4). The effect of K also showed significant effect on specific gravity. The application of 30 kg and 120 kg K/ha produced same specific gravity (1.065) and which was higher than 60 kg (1.062) and 90 kg (1.063) K/ha. There was increment in specific gravity after 90 days storage in ambient room temperature in all treatments but did no show any difference among the treatments before storage.

Table 3. Effect of N, K and their interaction on dry matter and reducing sugars of potato before and after 90 days storage in ambient temperature (28 ± 0.6 ºC) under dark room at Khumaltar, 2010

Treatments	Dry matter, %			Reducing sugars (mg/100 g fresh weight)			
	Before storage		After 90 days	Before storage		After 90 days	
Nitrogen (kg/ha)							
50	17.39	a	18.56	182.86	a	156.8	a
100	16.99	a	18.03	169.27	b	143.7	ab
150	16.26	b	18.06	158.45	c	144.1	ab
200	16.48	b	17.95	157.68	c	136.3	b
F-test	**		ns	**		*	
LSD (± 0.05)	0.4028		-	4.258		13.69	
Potash (kg/ha)							
30	17.12	a	17.94	170.94	a	149.1	
60	16.43	b	18.58	167.81	ab	146.8	
90	16.58	b	17.87	165.70	b	146.0	
120	17.01	c	18.22	163.80	b	139.0	
F-test	**		ns	*		ns	
LSD (± 0.05))	0.4028		-	4.258		-	
Interaction (N × K kg/ha)							
50 : 30	17.80	a	18.35	197.46	a	154.8	
50: 60	17.50	ab	19.20	185.14	b	162.7	
50: 90	16.70	bcde	17.73	173.25	c	160.0	
50: 120	17.57	ab	18.96	175.57	c	149.8	
100: 30	17.30	abcd	18.04	170.34	cd	154.6	
100: 60	16.43	de	18.41	166.44	cde	140.1	
100: 90	17.37	abc	17.97	169.71	cd	147.3	
100: 120	16.87	bcd	17.72	170.58	cd	132.7	
150 : 30	16.97	abcd	17.69	159.17	ef	149.9	
150: 60	15.87	e	18.68	163.14	def	148.0	
150: 90	15.80	e	17.91	157.84	ef	141.4	
150: 120	16.47	cde	17.97	153.65	f	137.1	
200: 30	16.40	de	17.68	156.79	ef	137.2	
200 : 60	15.93	e	18.02	156.50	e	136.3	
200: 90	16.47	cde	17.87	162.0	def	135.5	
200: 120	17.13	abcd	18.22	155.42	f	136.3	
Mean	16.785		18.15	167.06		145.2	
F-test	*		ns	**		ns	
LSD (± 0.05)	0.8055		-	8.518		-	
CV, %	2.9		4.7	3.1		27.37	

*ns, *,**, Non significant and significant differences at 0.05 and 0.01 levels, respectively.*
Common letters in a column are not significantly different by DMRT at 0.05 levels.

Sprouting percentage

The sprouting of potato causes relatively the high weight losses. First of all, the sprout itself is a direct weight of fresh potato and secondly, it losses through intensive evaporation of water from the sprout surface, which causes shrunken of tubers and peeling losses while processing. The effect of N showed highly significant differences on sprouting after 75 and 90 days storage in ambient room temperature. The application of 100 kg N/ha recorded the maximum sprouting percentage (43%) and the minimum (19.5%) was in 200 kg N/ha at 75 days after storage. Similarly, the minimum sprouting percentage (85.64%) was observed at 90 days after storage in application of 200 kg N/ha. The application of 50, 100 and 150 kg N/ha showed similar results on sprouting after 90 days storage. The application of different dose of potash showed a highly significant effect on sprouting at 60 and 75 days and insignificant thereafter. The minimum sprouting percentages, 8.9 and 24.9, were in application of 90 kg K/ha at 60 and 75 days, respectively. The detail effect of N and K on sprouting percentage is given in Table 5.

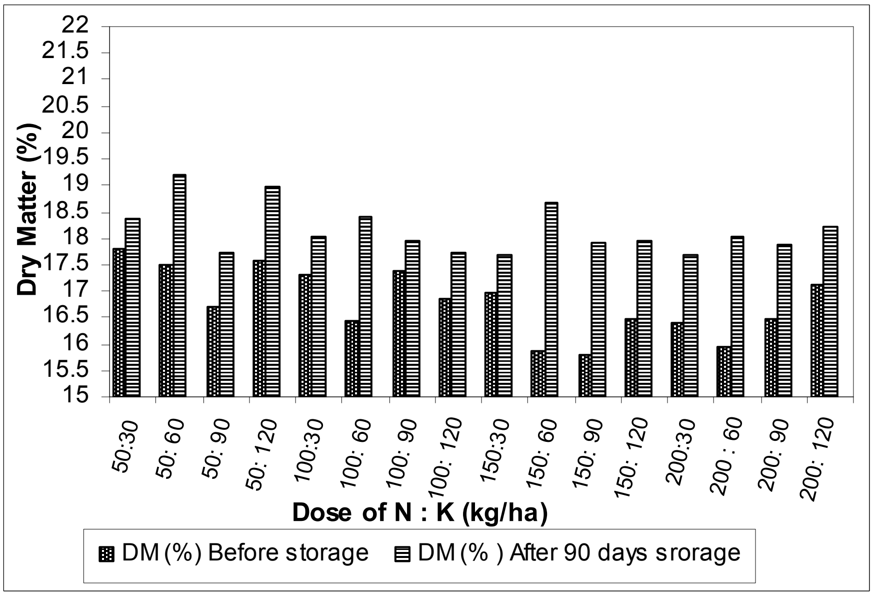

Figure 3. Interaction effect of N and K on dry matter (%) of potato before and after 90 days storage in ambient room temperature at Khumaltar, 2010.

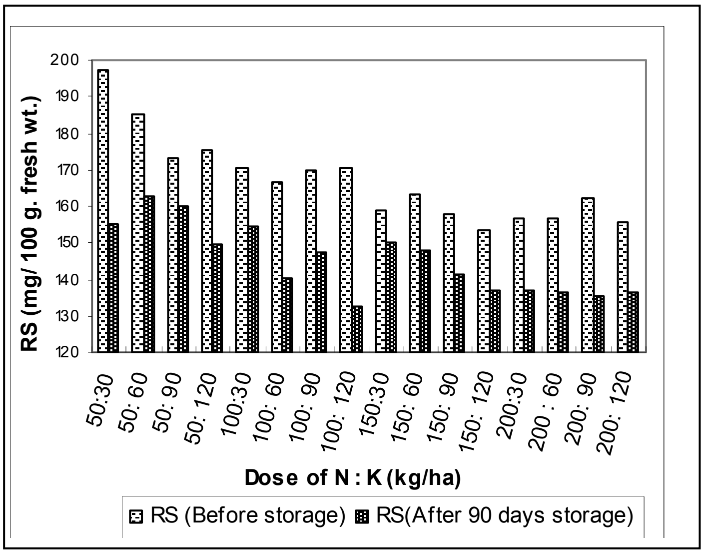

Figure 4. Interaction effect of N and K on reducing sugars of potato before & after 90 days storage in ambient room temperature at Khumaltar, 2010.

The interaction effect of N and K showed insignificant differences on specific gravity before storage of potato but significant after 90 days storage in ambient room temperature (Figure 5). The application of 200 kg N and 30 kg K/ha showed the highest specific gravity (1.0885) and it was at par with 100:90 (1.0786) and 100:60 (1.0758) kg N and K/ha, respectively.

The interaction effect of N and K showed a significant effect of sprouting only at 75 days after storage. However, it was the maximum (100%) with the application of 100 kg N and 120 kg K/ha at 90 days after storage (Figure 6).

Table 4. Effect of N, K and their interaction on specific gravity of potato tubers before and at 90 days after storage in ambient temperature (28 ± 0.6 ºC) under dark room at Khumaltar, 2010

Treatments	Before storage		At 90 days after storage
Nitrogen (kg/ha)			
50	1.067	a	1.0730
100	1.065	a	1.0731
150	1.062	b	1.0667
200	1.062	b	1.0742
F-test	**		ns
LSD (0.05)	0.00197		-
Potash (kg/ha)			
30	1.065	a	1.0732
60	1.062	b	1.0701
90	1.063	b	1.0713
120	1.065	a	1.0724
F-test	**		ns
LSD (0.05)	0.00197		-
CV, %	0.2		0.7

*ns, *,**, Non significant and significant differences at 0.05 and 0.01 levels, respectively.*
Common letters in a column are not significantly different by DMRT at 0.05 levels.

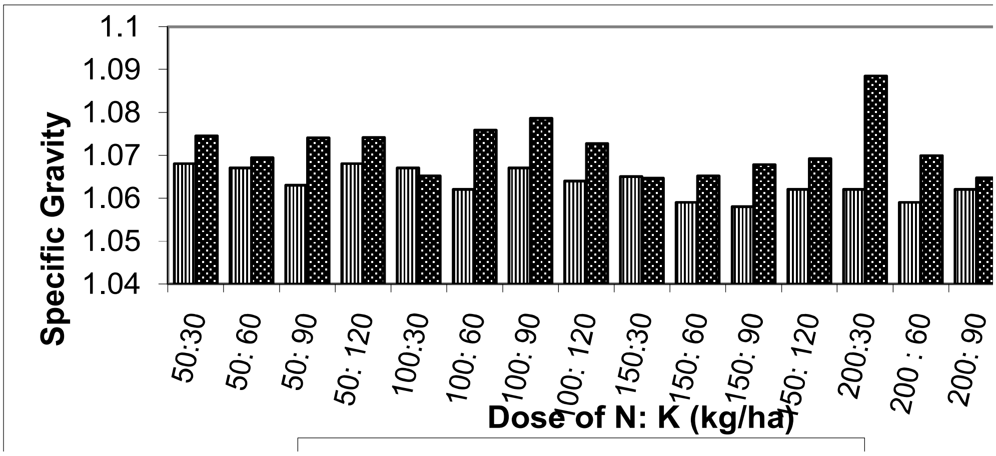

Figure 5. Combined effect of N and K on specific gravity of potato before storage and after 90 storage in ambient room temperature at Khumaltar, 2010.

CONCLUSIONS

The effect of nitrogen and potassium have found positive role for production of processing grade tuber and total yield of potato. The interaction effect of N and K was significant on dry matter percentage, reducing sugar and insignificant effect on weight loss percentage at 90 days storage in ambient room temperature. The weight loss percentage ranges from 10.73 to15.73 percent at 90 days storage at ambient room temperature. The dry matter content increased from 16.78 to 18.15 percent, reducing sugars decreased from 167.06 to 145.2 mg/100 fresh weight and specific gravity increased from 1.0637 to 1.0717 after 90 days storage in higher temperature. Based on the results following conclusions can be made:

- Application of 150 kg nitrogen and 60 kg potassium along with 100 kg phosphorus and 20 t FYM/ha seemed to be appropriate for higher yield, processing grade potato tubers for Khumaltar and similar soil and environment conditions.

- Potato can be safely stored under ambient room conditions (28 ± 0.6°C temperature and 88-89% RH) under dark with minimum storage losses (15%) up to 90 days. These potatoes were found suitable for chips making due to increase in dry matter, specific gravity and decrease in reducing sugars.
- There was 7 percent weight loss of cold stored potato for 120 days and it was increased up to 9.63 percent after 15 days reconditioning under ordinary conditions.
- For conformation of results further experiment is needed.

Table 5. Effect of N and K on sprouting percentage of potato at 60, 75 and 90 days of storage in ambient temperature(28.1 ± 0.6 °C) under dark room at Khumaltar, 2010

Treatments	Sprouting percentage at different days after storage					
	60 days		75 days		90 days	
A. Nitrogen (kg/ha)						
50	12.7		36.9	b	92.9	a
100	17.4		43.0	a	96.0	a
150	14.2		31.9	c	91.1	a
200	10.7		19.5	d	85.6	b
F-test	Ns		**		**	
LSD (0.05)	-		8.51		5.093	
B. Potash (kg/ha)						
30	15.1	ab	41.6	a	93.0	a
60	11.4	b	27.8	b	88.6	b
90	8.9	b	24.9	b	90.2	ab
120	19.6	a	37.1	a	83.9	a
F-test	*		**		ns	
LSD (0.05)	6.99		8.51		7.84	
CV, %	61.1		31.2		6.7	

*ns, *,**, Not significant and significant differences at 0.05 and 0.01 levels, respectively.*
Common small letters in a column are not significantly different by DMRT at 0.05 levels.

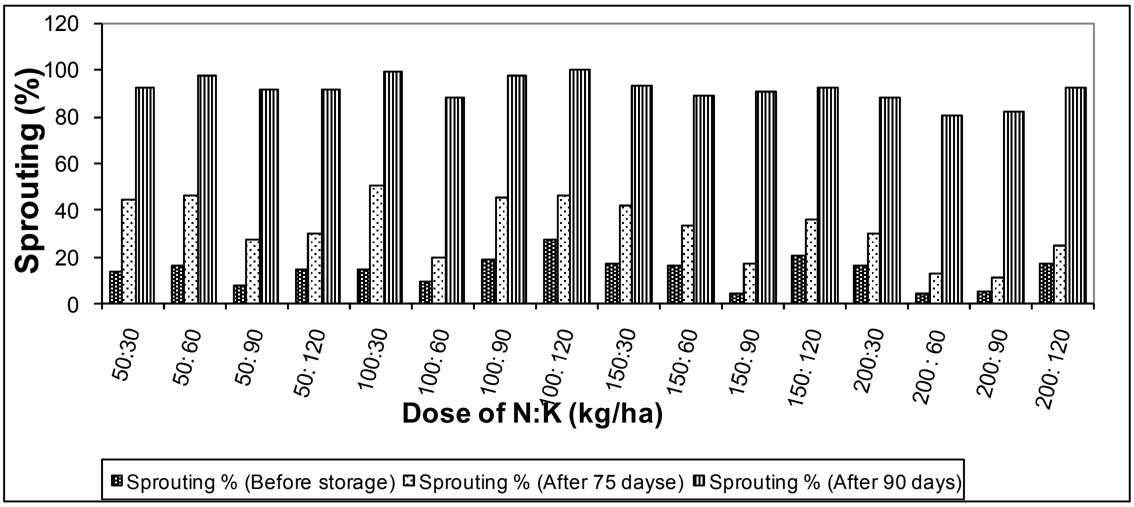

Figure 6. Combined effect of N and K on sprouting percentage of potato before storage and after 75 and 90 days storage in ambient room temperature at Khumaltar, 2010.

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Agro-meteorological Indices in Relation to Phenology of Promising Rice Cultivars in Chitwan, Nepal

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ABSTRACT

Agro-meteorological parameters are the major yield governing factors in rainfed farming and could be managed safely by proper selection of crop varieties and planting dates. The major agro-meteorological indices (phenology, and heat and radiation use efficiencies) of rice were evaluated from the data sets of the rainfed field experiment (rainy season of 2008) at the Agronomy Farm of Institute of Agriculture and Animal Sciences, Rampur, Chitwan (27°37' N, 84°25'E and 256 masl). Four rice cultivars of different grain qualities [Ram, Prithvi (hybrid), Masuli and Sunaulo Sugandha] with early, mid and late maturity days were sown in nursery at three different dates (5 June, 20 June and 5 July) coinciding the enough access of the normal rainfall for assured transplanting, and 25-day-old seedlings were transplanted for each variety and sowing date. The experiment was conducted in RCBD with three replications. The average of total rainfall received during the monsoon season of 15 years (1994-2008) showed that the enough access of rainfall for rice transplanting was from last week of June under rainfed conditions of the central terai region. The agro-meteorological results further revealed that the number of days required to attain different phenological stages were comparatively longer for the early plantings than the late plantings. None of the rice cultivars could show stable yield in late planting conditions suggesting their planting could be better for early planting, however, the short duration cultivars could be sown in nursery up to 20 June and transplanted by 15th of July. The higher heat, helio-thermal and radiation use efficiencies for all promising rice cultivars were noted on 5 June sowing followed by 20 June and 5 July. Physiological maturity can be predicted using AGDD and HTU which accounted for 96, 99, 98 and 92 percent, respectively for Ram, Prithvi, Masuli and Sunaulo Sugandha. However, the greater validity of such studies could be obtained if long-term studies could be followed.

Key words: AGDD, agro-meteorological indices, HTU, HUE, phenology, rice, RUE

INTRODUCION

About 80 percent of Nepalese agriculture is rainfed and is encountered with several risks like adverse agro-meteorological records viz. extreme hot and cold temperatures, the lesser brighter sunny days and irregular and unequal distributions of rains. Weather variability is thus considered one of the major factors of inter-annual variability of crop growth and yield especially under rainfed conditions. The amount, intensity and distribution pattern of rainfall is vital in rainfed agriculture because the growth and yields of any crops are dependent on it. In rainfed farming, farmers should have to plan their sowing time, either based on their past experiences about normal trend of onset of rainfall or wait for some precise agro-meteorological advices from experts. This change in sowing time governs the changes in growth phases of crop variety because these are also determined basically with growing season. Among so many causal factors, the atmospheric ambient temperature and solar radiation are the major governing factors for thermo and photoperiod response (Sastry et al 2000). This in consequence has a great bearing on the phasic development and partitioning of the dry matter. Quantification of these effects may help in the choice of the sowing time and match phenology of crop in specific environment to achieve higher heat and radiation use efficiencies.

Temperature based agro-meteorological indices such as growing degree days (GDD) and helio-thermal units (HTU) have been reported quite useful in predicting the growth and yield of crops (Doraiswamy and Thompsosn 1982, Jones et al 2003). Growing degree days is based on the concept that the real time to attain a phenological stage is linearly related to the temperature range between base temperatures (Tb) and optimum temperature (Monteith 1958). On this line, several researchers have shown the influence of temperature on phenology and yield of crops and expressed it under field conditions through accumulated heat unit system (Sikder 2009, Rao et al 1999, Rao and Singh 2007). Heat and radiation use efficiencies in terms of dry matter or yields are important aspects which have great practical applications. The efficiency of conversion of heat and radiant energy into the dry matter depends upon the genetic factors, sowing time and crop type (Rao et al 1999). Hence, the knowledge on the calculation of the heat summation unit (HSU), mostly called the growing degree days and their further mathematical derivations like helio-thermal unit (HTU), and heat and radiation use efficiencies (HUE and RUE) will be the basic pre-requisite to understand the phenology and follow the proper sowing times for different crop varieties over the spatial and temporal variations (Sreenivas et al 2010).

Rice is the first staple crops mainly grown in rice-wheat sequence in the central terai region of Nepal. Nepalese agriculture being rainfed, majority of farmer's seldomly grow rice on time by which there is marked reduction in yield (NARC 2009). The late onset of monsoon mostly delaying the rice transplanting not only hampering the rice yield but is also causing delay in its maturity and forced to late wheat sowing in the major provident of rice-wheat system (NWRP 2010). Growing spring maize in a single piece of lowland field is common practice in Chitwan and the adjoining areas which also caused delayed rice transplanting. The shorter growing duration between sowing to harvesting hence was resulting for lesser growing degree days and unstable and low heat and radiant use efficiencies (Sreenivas et al 2010, Reddy et al 2004). There are several reports documented on sowing time and crop yield on rice and wheat (Giri 1998) and winter maize (Amgain 2011) but very few have analyzed and documented the specific reasons of lower yields under late planting conditions in Nepal which is basically an agro-meteorology and physiology based factors. By keeping the above facts in view, an attempt was made to study the phenological behavior, and heat and radiation use efficiencies in prominent rice cultivars to obtain higher and stable grain yield.

MATERIALS AND METHODS

The major agro-meteorological indices (phenology, and heat and radiation use efficiencies) of rice were evaluated from the data sets of the rainfed field experiment (rainy season of 2008) at the Agronomy Farm of Institute of Agriculture and Animal Sciences, Rampur, Chitwan (27^{o}37' N, 84^{o}25'E and 256 masl.). Rice cultivars of different grain qualities [Ram, Prithvi (hybrid), Masuli and Sunaulo Sugandha] with early, mid and late maturity days were sown in nursery at three different dates (5 June, 20 June and 5 July) coinciding the enough access of the normal rainfall for assured transplanting, and 25 days old seedlings were transplanted for each variety and sowing date. The experiment was conducted in randomized complete block design with three replications in an experimental plot having the soil of sandy loam texture, 28.39 percent soil moisture at field capacity, slightly acidic reaction (pH-6.4), low organic carbon (0.4%) and total soil N (0.18% N) and medium phosphorus (21.2 kg/ha) and potassium (153.0 kg/ha). The records on the major phenological stages (emergence, panicle initiation, flowering, grain filling and physiological maturity), and yield and dry matter was taken as standard international protocol given for rice by IRRI, where ten fixed hills in each plot were randomly selected from emergence and fixed up to the physiological maturity stages to visualize their different pheno-phases and 50 percent developmental stages have been marked. Crop growth was divided into different phases, as emergence to panicle initiation (PI), PI to flowering,

flowering to physiological maturity and in total emergence to physiological maturity. The standard package of practices was followed to grow rice (Reddy 2005).

The daily weather data records were collected from the Meteorological Observatory of National Maize Research Program, Rampur, Chitwan and the rainfall data of last 15 years (1994-2008) were compared to fix the normal sowing of rice nursery (Table 1). The average mean temperature and total sunshine hours attained by the various rice cultivars from sowing to physiological maturity has been depicted in Figure 1 and 2, respectively. Similarly, the average temperature and mean sunshine hours from one developmental stage to the other stage for a particular planting date and cultivar was also taken for the further mathematical expressions (Rao et al 2000, Singh et al 1998, Ritchie and Nesmik 1991).

1. Growing degree days (GDD) = {(T max + T min) ÷ 2} - Tb
   (Tb = Base temperature =10 ^{0}C)
2. Helio-thermal unit (HTU) = GDD × Duration of sunshine hours
3. Heat use efficiency (HUI) = Biomass yield (kg/ha) ÷ GDD
4. Helio-thermal unit use efficiency (HTUE) = Biomass yield (kg/ha) ÷ HTU
5. Radiation use efficiency (RUE) = Biomass yield (kg/ha) ÷ Radiation hours

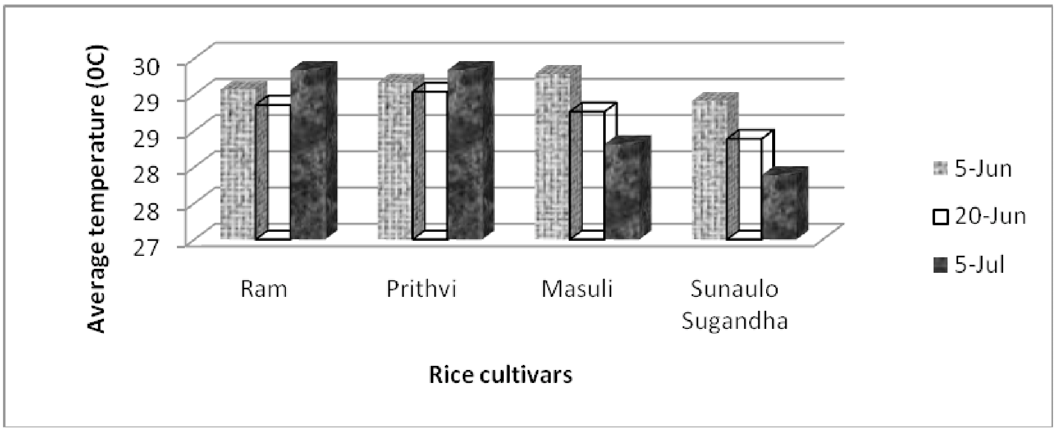

Figure 1. Average temperature received by various rice cultivars under different sowing dates.

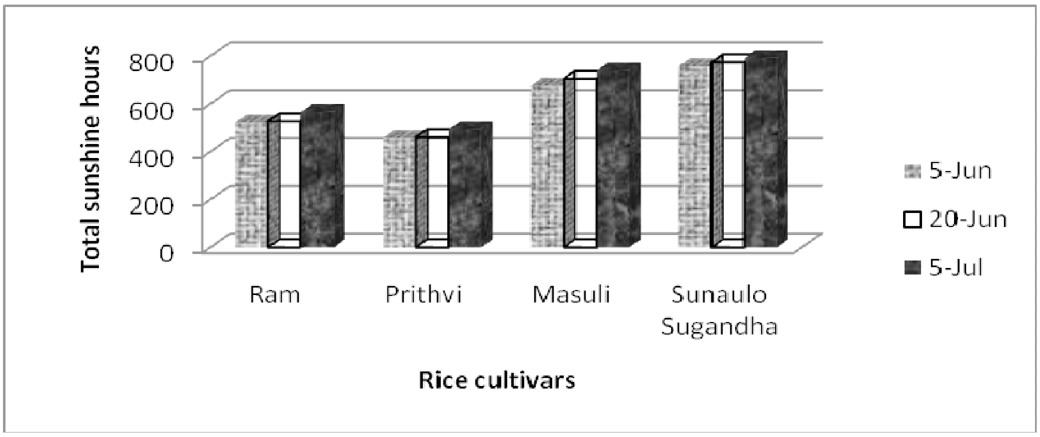

Figure 2. Total sunshine hours received by various rice cultivars under different sowing dates.

RESULTS AND DISCUSSION

Rainfall pattern and history

The historical data of total monthly rainfall for the last 15 years (1994-2008) showed that the total monthly rainfall (133.6 mm) in May, 2008 was slightly lesser than the 14 years of mean values of total rainfall (191.0 mm). There was a record of 2004.4 mm rainfall at Rampur during the experimental year, of which about 80 percent was from June to November (Table 1). The raising of rice nursery during May is common practice only under assured irrigation in Chitwan, but under the rainfed conditions it is commonly practiced in the month of June predicting there will be enough access of rainfall for transplanting rice after third week of June. About 80 percent rice grown in the central terai region of Nepal is still rainfall dependent.

Table 1. Historical records of total monthly rainfall (mm) at NMRP, Rampur Chitwan

Year/ Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1994	42.7	37.2	9.3	19.0	158.2	406.4	419.7	415.7	469.1	3.6	0.0	0.0	1980.9
1995	1.5	22.9	5.9	36.0	152.9	507.4	469.0	448.3	183.8	32.5	1.7	3.5	1865.4
1996	55.7	40.4	0.0	6.0	113.6	358.2	588.5	341.8	259.8	117.3	0.0	0.0	1881.3
1997	13.4	1.5	2.9	144.6	83.4	321.5	579.7	481.3	290.4	50.3	7.6	146.0	2122.5
1998	4.4	13.2	87.2	88.3	150.1	332.3	563.7	1047.0	253.8	92.5	8.2	1.0	2641.7
1999	0.4	0.0	0.0	10.1	334.2	384.5	611.4	686.6	312.4	202.1	0.2	0.0	2541.9
2000	0.8	9.8	24.9	7.6	315.8	520.8	617.6	333.2	206.9	0.0	6.4	0.0	2043.8
2001	1.6	18.6	0.8	67.4	238.0	386.3	644.8	548.9	376.8	28.3	20.4	0.0	2331.9
2002	31.9	28.3	45.6	57.7	391.9	600.9	883.3	303.3	254.7	22.7	44.6	0.0	2664.9
2003	35.1	59.4	62.0	58.7	99.9	473.2	930.0	548.9	292.2	81.1	79.8	10.7	2731.0
2004	62.7	0.0	0.0	180.2	91.7	492.2	457.9	214.3	417.7	75.7	12.0	0.0	2004.4
2005	38.1	6.4	38.9	28.8	133.5	139.9	349.2	671.1	148.6	183.5	0.0	0.0	1738.0
2006	0.0	0.0	3.0	125.9	279.7	387.1	352.3	405.4	362.0	60.6	2.1	19.0	1997.1
2007	0.0	80.3	47.6	100.9	131.0	406.7	497.2	427.4	926.7	120.2	4.6	0.0	2742.6
Mean	20.6	22.7	23.4	66.5	191.0	408.4	568.9	512.2	333.6	76.5	13.5	13.9	2252.5
2008*	17.1	1.7	33.8	40.4	133.6	378.6	431.4	457.9	218.7	87.3	0.0	0.0	1800.5

* *Experimental year.* (Source: National Maize Research Program, Rampur, Chitwan)

Phenology, growing degree days and helio-thermal units

The phenology, growing degree days, and helio-thermal unit results showed that day for the attainment of different phenological stages differed significantly from cultivar to cultivar and sowing dates (Table 2). The stable temperature and sunshine hours at the early vegetative phases of rice was resulting in increased number of days for attaining different phenological stages under normal planting. The similar results have been noted in winter maize (Amgain 2011) and in wheat (Ghosh et al 2000, Paul and Sarker 2000, Sandhu et al 1999). Late planting decreased the duration of phenology as compared to normal planting due to fluctuated unfavorable temperature and sunshine hours associated with late rainfall, especially during latter phenological stages. Sandhu et al (1999) and Paul and Sarker (2000) also reported that requirement of heat units decreased for different phenological stages with delay in sowing of wheat. Helio-thermal unit of a definite phenology is the product of the length of sunshine hours of a day and accumulated heat units of those particular pheno-phases shown by plants. The requirements of HTU for normal planting conditions were significantly higher than the late planting conditions with few exceptions with some cultivars and planting dates (Table 2). The variation of sunshine hours recorded at different developmental stages of rice has affected the magnitudes of the HTU though there were records of higher GDD at advanced growth stages. The random trend of HTU for different phenological stages was also found to be for late sown wheat (Rajput et al 1987, Paul and Sarker 2000).

Heat and radiation use efficiencies and grain yields of rice

From the results (Table 3) it was observed that all the rice cultivars were more efficient to show more heat and radiation use efficiencies at normal growing conditions than the late growing conditions. Hybrid Prithvi showed higher HUE (5.62 kg/^{o}C day) under 20 June sowing. However, the radiation and helio-thermal efficiencies too are also higher with earlier sowing. The late sowing reduced the duration of vegetative phase and resulted in the decreased AGDD and HTU which correspondingly decreased the values of HTTU and RUE. The results are in accordance to Amgain (2011) in winter maize, as evidence with Paul and Sarker (2000) in wheat, as with Rao et al (2000) in clusterbean and in pearlmillet as with Singh et al (1998).

Table 2. Calendar days, accumulated growing degree days (GDD) and helio-thermal units (HTU) during different pheno-phases of rice in Chitwan

Treatment	Emergence to panicle emergence			Panicle emergence to flowering			Flowering to physiological maturity			Emergence to physiological maturity		
	Cal days	GDD	HTU	Cal days	GDD	HTU	Cal days	GDD	HTU	Cal days	GDD	HTU
Ram												
June 1	63	1236	5522	24	453	3531	32	580	3189	118	2269	12242
June 20	60	1184	4946	26	488	3063	29	522	2870	115	2194	10880
July 5	55	1080	4567	26	490	2672	27	539	2615	108	2108	9854
Prithvi												
June 1	56	1098	4284	20	389	2325	32	602	3417	108	2089	10026
June 20	53	1049	3819	22	413	2897	30	562	2988	105	2023	9703
July 5	50	991	3654	22	419	2466	28	493	3584	100	1903	9466
Masuli												
June 1	80	1564	11392	26	488	4503	32	629	4937	138	2681	20831
June 20	78	1518	8542	27	505	3890	31	555	4235	136	2578	16677
July 5	78	1516	9898	23	435	2126	29	448	3223	130	2399	15246
S Sugandha												
June 1	90	1766	12446	26	488	4710	32	584	3227	148	2818	20383
June 20	88	1710	11570	27	484	3966	30	498	2834	145	2692	18370
July 5	86	1661	12127	24	530	3436	28	395	4591	138	2586	20154
LSD	15	245	2325	2	78	364	2	123	487	11	386	3465
CV, %	4	5	13	4	6	11	5	7	12	6	8	10

Table 3. Radiation, dry matter, heat and radiation use efficiency of rice cultivars in Chitwan

Treatment	Radiation, $MJ/m^2/day$	Dry matter, kg/ha	HUE, kg/°C day	HTUE, kg/°C hour	RUE, kg/MJ
Ram					
June 1	1878	9348	4.12	0.76	4.98
June 20	1897	8847	4.03	0.81	4.66
July 5	2036	8125	3.85	0.82	3.99
Prithvi					
June 1	1648	13292	5.36	1.33	8.06
June 20	1658	11383	5.62	1.17	6.86
July 5	1774	9161	4.81	0.97	5.16
Masuli					
June 1	2433	11948	4.46	0.57	4.91
June 20	2538	10041	3.90	0.60	3.96
July 5	2663	9481	3.52	0.52	3.56
S Sugandha					
June 1	2727	12526	4.40	0.61	4.59
June 20	2786	12208	4.53	0.66	4.38
July 5	2849	9861	3.81	0.49	3.46

S, Sunaulo.

The date of planting is major governing factor in crop production and it is considered to be low-cost high monetary-returned technology under best management conditions. The rice varieties sown on 5 June produced higher yield than their subsequent late plantings almost for all rice cultivars. The percentage reduction in yield was more for 5 June vs 20 June planting than the June 20 vs 5 July planting for early varieties Ram Dhan and Hybrid Prithvi and the lower for longer duration varieties Masuli and Sunaulo Sugandha suggesting that early planting is a must for long duration varieties

(Table 4). This might be due to the late planting of rice resulted in less dry matter and metabolized less photosynthates as resultant of less growing degree days and helio-thermal units. The reduction in yield was more for 5 June vs 5 July sown rice varieties. Rao and Singh (2007) have also found the lesser yield of pearlmillet when planted delay in Rajsthan, India.

Table 4. Grain yield (kg/ha) and yield reduction (%) due to delayed planting in different rice and wheat cultivars

Rice cultivars	Grain yield, kg/ha			Yield reduction (%) due to late sowing		
	5 June	20 June	5 July	5 June vs 20 June	20 June vs 5 July	5 June vs 5 July
Ram Dhan	3885	3415	3125	12.10	8.49	19.56
Prithvi	5754	4732	4164	17.76	12.0	27.63
Masuli	4435	3892	3214	12.24	17.42	27.53
S Sugandha	4468	4108	3547	8.06	13.66	20.61

Correlation and regression

Correlation between the calendar days and AGDD and HTU indicated that significant relationship between calendar days and AGDD and HTU were obtained during emergence to panicle initiation stage for all promising rice cultivars while calendar days and HTU during PI to flowering was found to be insignificant (-0.891) for Ram Dhan and hybrid Prithvi (-0.271) during flowering to physiological maturity stages. The correlation was found variable and inconsistent on Sunaulo Sugandha, especially during panicle initation to flowering and especially at the latter stages (Table 5).

Table 5. Correlation coefficients between calendar days and AGDD and HTU during different phenophases of rice under different planting dates

Pheno-phases	Ram		Prithvi		Masuli		Sunaulo Sugandha	
	AGDD	HTU	AGDD	HTU	AGDD	HTU	AGDD	HTU
E – PI	0.999	0.966	0.998	0.964	0.999	0.999	0.997	0.982
PI- F	0.999	-0.891	0.980	0.690	0.999	0.858	-0.969	-0.973
F- PM	0.776	0.999	0.989	-0.271	0.996	0.996	0.998	-0.739
E- PM	0.982	0.953	0.999	0.973	0.992	0.851	0.962	-0.123

E, Emergence. PI, Panicle initiation. F, Flowering. PM, Physiological maturity.

This might be due to the several ups and downs of temperature and sunshine hours during the longer vegetative phase of this variety. The average temperature records as depicted in Figure 1 clearly mentions the accumulation of less temperature for Sunaulo Sugandha cultivars of rice.

Further regression equations were developed to predict the phenology of rice using AGDD and HTU in all rice cultivars and reported as under:

Ram Dhan: Y= 71.23 + 0.1650 AGDD - 0.0070 HTU $R^2 = 0.964$
Prithvi: Y= 12.71 + 0.0385 AGDD + 0.0015 HTU $R^2 = 0.999$
Masuli: Y= 47.72 + 0.0371 AGDD - 0.0001 HTU $R^2 = 0.984$
Sunaulo Sugandha: Y = 49.21 + 0.0444 AGDD - 0.0014 HTU $R^2 = 0.926$

Physiological maturity can be predicted using AGDD and HTU which accounted for 96, 99, 98 and 92 percent, respectively for Ram Dhan, Prithvi, Masuli and Sunaulo Sugandha.

SUMMARY AND CONCLUSIONS

Hybrid Prithvi and Masuli were better to show greater stability to solar energy, growing degree days (GDD) and heat use efficiency than the Ram Dhan and Sunaulo Sugandha cultivars by which their popularity amongst the farmers of terai and inner terai was higher. By raising the nursery on 5 June and transplanting the 25-day-old seedling on 1 July, the higher and stable heat, radiation and helio-thermal indices were achieved. Physiological maturity can be predicted using AGDD and HTU for rice cultivars of diverse genotypes if agro-meteorological indices are calculated precisely. However, at least two years of multi-location research is needed to validate this result further.

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Investment in Crops Research in Nepal: Empirical Evidence on Adequacy and Balance

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ABSTRACT

Resource allocation in agricultural research was analyzed for the past ten years in context of national budget and eco-zonal distribution. The study was focused into research intensity in major cereal crops on full time equivalent (FTE) basis of researchers with survey data collected in 2010. The scale and growth of investment in agricultural research was small and slow as compared to other development sectors. The FTE of researchers in rice, wheat, maize and finger millet was 35, 28, 18 and 2, respectively that indicated a large variation of investment among those crops. Congruency analysis based on actual production share showed that there was underinvestment in rice and overinvestment in wheat. The mismatch between resource allocation and production share was small in maize and finger millet as compared to rice and wheat. When the production share was adjusted with efficiency and equity criteria, magnitude of underinvestment in rice was amplified. Investment in rice research needs to be raised with increased number of researchers to have more efficient and equitable production.

Key words: Congruency, full time equivalent (FTE), overinvestment, production share, underinvestment

INTRODUCTION

Agricultural research is an investment in the production of knowledge and innovations that essentially competes with other activities for scarce resources. Resource allocation in agricultural research is important investment on food production, however, it may consume from short to long time period to show the impact. Evaluation of past investment as well as estimation of ex-ante returns of future investment in agricultural research are the matter of interest to all concerned in agricultural sector, particularly planners and policy makers. Evaluating past investments, assessing alternatives, and setting priorities for future investments in agricultural research are all the subject of economics (Alston et al 1995).

For agricultural growth, any country undoubtedly requires research investment for technology generation. Technical change has accounted for half to more than four-fifth of agricultural growth since the sixties in developed countries (Hayami and Ruttan 1985). Empirical studies show that agricultural research has been the primary source of total factor productivity growth in India (Kumar and Rosegrant 1995, Kumar and Mruthunjaya 1992, Evenson and Mckinsey 1991). Regarding the issue on how much to invest on agricultural research, use of conventional investment criteria to determine appropriate research investment levels is fraught with many conceptual and operational difficulties (ICAR, 1996). For agricultural research and development in Nepal, investment policy has not been formulated despite the higher priorities in agriculture set by the government. An aggressive research investment strategy is absolutely important for agricultural growth in an internationally competitive environment. World Bank (1981) suggested a norm of 2 percent of Agricultural GDP

(AGDP) to invest in agricultural research for sustainable agricultural growth. This investment ratio has been around 0.39 percent in India, 0.42 percent in China and 2.64 percent in developed countries in 1995. In recent years, the average investment ratio has been increased up to 0.60 percent in developing countries as against 0.26 percent in Nepal.

Trend analysis of research investment in developed countries from 1965 to 1985 showed there was positive growth in real expenditures per scientist, implying distinct improvement in research support to scientists (Pardey et al 1991). Agricultural research and development (R&D) investment in the low- and middle-income countries grew by an average of about 3 percent per year during 1981-2000. During 1990s, the growth further slowed, with most of the growth occurring in Asia-Pacific region. There was a high growth in agricultural R&D spending in the two largest countries China and India with annually 5.3 and 5.8 percent, respectively, during last two-decade period (Beintema et al 2010). Thapa (1994) found the agricultural sector was underfunded in Nepal relative to other countries of south Asia since the expenditure on this sector in terms of percentage of total expenditure, US dollars per cultivated hectare, and US dollars in relation to agricultural population was significantly lower in Nepal than in other agrarian countries of the region like Bangladesh, Bhutan and Sri Lanka.

In Nepal, the ratio of financial investment in agricultural research to AGDP is quite smaller as compared to other developing countries. Public sector organization, Nepal Agricultural Research Council (NARC) and non-governmental organizations such as LIBIRD and FORWARD have been involved in agricultural research and development (R&D). National Agricultural Research and Development Fund (NARDF), a separate government entity, provides competitive grants for promotion of agricultural R&D.

Government of Nepal has raised the budget allocation to NARC from Rs 345 million in 2006/07 to Rs 560 million in 2009/10 at current price for agricultural research. Government investment through NARDF was about Rs 75 million at current price for agricultural R&D in 2009/10. Regarding the research investment, staff cost was much higher than the operational cost in NARC resulting serious constraints in research work. The issue of inadequate budget to agricultural research is important but at the same time efficient use of allocated budget in output-oriented research is even more important. World experience shows that technology generation through meaningful investment in agricultural research has largely contributed towards poverty alleviation and food security. Numerous studies have proved that investments in agricultural research typically rank first or second in terms of returns to growth and poverty reduction as compared to investments in infrastructures and education. Investment in wheat breeding in Nepal had generated an internal rate of return of about 84 percent during the past three decades from 1960 to 1990. This attractive rate of return was explained by the relatively modest cost of the Nepalese wheat research program in relation to the substantial benefits it generated.

The general objective of this study was to analyze research intensities in Nepalese agriculture with focus in major cereal crops and eco-zones over the years. The focused cereal crops of this study were rice, maize, wheat and finger millet, which collectively account two-third of AGDP (MoAC 2009) and have significant contribution in food security. This study also investigates the research investment by eco-zones (hills, mountain and terai plain) for each crop.

METHODOLOGY

Data on agricultural research expenditure and full time equivalent (FTE) of researchers was collected from concerned agencies, including NARC, IAAS and non-governmental organizations (NGOs) such as LIBIRD and FORWARD which were involved in research and development of agriculture. There might be other organizations and individuals which were occasionally involved in agricultural research, especially in cereal crops however, analysis on their investment was beyond the scope of this study. Only the well known organizations which were consistently involved in agricultural R&D were included in the study. Structured questionnaire was used to collect researchers' information on their FTE involvement in major crops, thematic area, environments and eco-zones. Research investment in particular crop was estimated on the basis of FTE researchers involved in the same crop. It is because researchers usually spend their time in different crops and activities but we need their net time spending in a particular crop. Unit cost of researchers was derived on the basis of total research spending and total FTE researchers. Total research spending was derived by adding annual expenses of research agencies that comprise staff cost, operational cost, administration cost and capital cost. The output value of each crop was derived on the basis of its total production and average market price. Simple congruence analysis was carried out to compare the research investment with output value in each crop and eco-zones.

Data on research costs, FTE of researchers as well as output value were derived from secondary and primary sources for specified crops viz. rice, wheat, maize and finger millet. Output value of each crop was calculated by multiplying total production with their market price. Output value included the both main products (food grains) as well as by products (straw and stalk) of the crop. The production share of each crop was weighted according to the estimated poverty ratio of farmers and anticipated research progress in that crop. Production share would be more meaningful if it was weighted according to poverty ratio because each crop has its own contribution in local food security and poverty alleviation. The maize and finger millet has much role in food security and poverty alleviation of marginal farmers in the hills. Similarly, future research progress that differs from one crop to another needs to be considered to derive normative production share. For instance, rice has good research progress as scientists have developed suitable varieties that can resist drought and submergence for considerably longer period. Such weighting or adjusting the production share with various economic factors is much justified in economic surplus approach (Alston et al 1995, Pandey and Pal 2007, Fugli 2007). Index of research progress and poverty according to crops and eco-zones was estimated through interaction with experienced scientists.

Congruency model

Congruency model is a simple and commonly used approach which generally implies that the share of a commodity (or environment) in agricultural research budget should be proportional to its production share in the national economy. This rule maintains that research resource to any commodity should be allocated in proportion to its contribution to the value of production across environments, production regions, commodities and disciplines. Following the rule adopted by Pandey and Pal (2007) and Byerlee and Morris (1993), congruency is measured as:

$$C = [1 - \Sigma (Ri - Vi)^2] - \quad (1)$$

Where,

$0 \leq C \leq 1$, with C = 0 indicating no congruency between the allocation of research resources and the output value of particular commodity. Congruency increases as the value of C approaches towards unity. Ri is the share of the research resources allocated to commodity i and Vi is the share of the output value of the commodity i.

The share of the output value Vi is calculated as:

$$Vi = PiWi / (\Sigma PiWi) - \quad (2)$$

Where,

Pi = the price of particular commodity i

Wi = the production quantity of particular commodity i

The above congruency approach can be modified to incorporate elements of the scoring approach described by Anderson and Parton (1983). The index of the output value in each crop or environment was justified by weighting the value of production based on two factors viz. research progress and poverty ratio. The first factor consisted of efficiency criteria relating the expected returns to commodity research expenditures which is termed as expected research progress. The second factor consisted of an equity criterion relating to the expected distributional effects of technical change which is termed as poverty incidence. Some more explanation on these factors is given below.

Rate of research progress: Standard congruency analysis assumes that the rate of future research progress will be equal in all environments and crops. Since the rate of progress differs between environments and crops, differences in expected future rates of progress should be explicitly considered. For instance, research progress in rice for marginal area is considerably good since suitable varieties for stress condition has been developed and up-scaling programs for mass adoption of these varieties has been carried out in the marginal area.

This progress rate was based on subjective judgments made by knowledgeable concerned scientists and real productivity growth of the particular commodity. The productivity growth of rice, wheat, maize and finger millet in Nepal during last 24 years was 47.7, 63.7, 55.61 and 18.9 percent, respectively. To estimate on productivity growth in future, knowledgeable agronomists and breeders of Nepal were consulted. They had shared on high yielding pipeline technologies for different crops for different eco-zones. Based on their experience and knowledge, the anticipated productivity growth in next ten years used in our model was 35 percent in rice, 20 percent in wheat, 25 percent in each of maize and finger millet. Rate of expected research progress represents efficiency criteria for investment in particular crop.

Incidence of poverty: One of the major justifications for investing in research targeted at marginal environments crop is the higher incidence of poverty in these environments. Similarly, the crop such as finger millet grown in marginal area has higher weight in addressing the poverty. The poverty ratio should be explicitly considered for adjustment of congruency in the analysis of research investment.

Data on poverty ratio are available only for politically defined areas such as districts and development regions. By eco-zones, mountain districts contain mostly marginal area with high poverty ratio while hill districts contain less marginal area with low poverty ratio. The terai districts contain mostly favorable area with lowest poverty ratio. Nepal Living Standard Survey (NLSS) of 2003-04 shows that the poverty rate in the mountains (35%) and the hills (33%) is higher than terai region (28%). Poverty ratio assigned to maize and finger millet ecosystem was higher as compared to the ratio assigned to rice and wheat ecosystem. Maize and finger millet are major crops grown in marginal area where livelihood of poor farmers mostly depends upon. The poverty weight for wheat is the lowest since the crop needs favorable environment with better water management, flat land and good fertility. Based on these references, the relative poverty weights of unity for wheat, 1.2 for rice and 1.25 for each of maize and finger millet were used. Official statistics on a separate poverty rate for crop-wise ecosystems (irrigated and rainfed) are not currently available in Nepal. Hence, the poverty rate for crop ecosystems was estimated based on interaction with knowledgeable people like agriculturists and social scientists in Nepal and available poverty data for rural and urban area. The weighted index Ii of the value of the production can be calculated as:

$$Ii = Yi\ Pi\ \Sigma\ (Rj\ \ Wi) \quad -(3)$$

Where,
Yi = expected rate of research progress in commodity i.
Pi= relative price of the commodity in commodity i

Rj = relative incidence of poverty in commodity i..
Wi= production of commodity i

The parameter *Vi* in equation (2) was then $Vi = Ii/ \Sigma Ii$ – (4)

RESULTS AND DISCUSSION

Budget trend in agricultural research and development

Public budget allocation in agricultural sector initially declined from 2001/02 to 2003/04 and then gradually increased till 2009/10. The real budget (inflation adjusted budget) was dwindling in the whole agricultural sector in the initial years of study period. The agriculture sector received decreasing budget from Rs 3795 million in 2001/02 to Rs 2309 million in 2003/04 (Figure 1). Similarly, the research sector budget went down from 558 million to Rs 281 million during the same period.

The public budget started rising since 2006/07 in agriculture sector resulting in substantial increased budget for promotional or development program which went up from Rs. 2861 million in 2006/07 to Rs 4304 million in 2009/10. Unlike the budget trend in agriculture sector, research sector did not receive the investment and remained below Rs 400 million until the end of study period. The share of agricultural research in public investment on overall agriculture sector was declined from 14.5 percent to 7.6 percent during 2000-2010. It was estimated that in developing countries as a group, research investments account for about 6-8 percent of total public spending on agriculture (Pardey et al 1991).

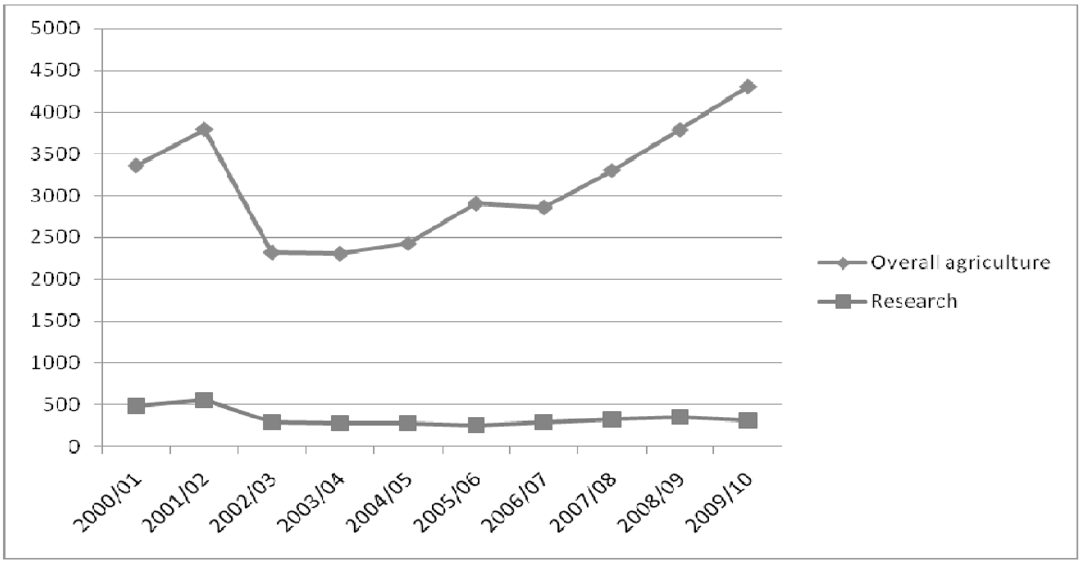

Figure 1. Public investment in overall agriculture and research (million Rs. at constant price)

The AGDP in Nepal had steady growth during last ten years which was increased from 155625 million Rs in 2000/01 to 203585 million Rs in 2009/10 at constant price (2000/01). The agricultural sector budget declined from 2.37 to 1.33 percent and then increased to 2.11 percent of AGDP during the respective years. The research sector budget declined from 0.35 to 0.17 percent and then remained almost stagnant with 0.16 percent of AGDP during the respective years. Although agricultural sector budget comprised research budget, the budget allocation trend simply indicated that government had higher priorities in agricultural extension than the agricultural research. The larger budget in agriculture sector during 2000/01 and 2001/02 was due to World Bank support for Agriculture Research and Extension Project (AREP). The completion of the project prompted to downfall the public budget both in extension and research sector (Stads and Shrestha 2006). Human resource was also declined in research sector as indicated by the declined number of full time equivalent (FTE) researchers in NARC from 331 in 2003 to 278 in 2009 (Rahija et al, 2011). Continuous retirement, discontinuity in recruitment and less retention capacity of the organization were major causes of declined number of researchers. The FTE researchers increased up to 332 in 2010 due to new recruitment after a long gap in NARC, however there was still about 40 percent vacant posts for the researchers.

Investment in eco-zones for agricultural R&D

The past trend of investment in agricultural R&D according to eco-zones shows that hills received about two third budgets every year. Terai received about 29 percent whereas mountain received 4 percent of total budget every year (Table 1). Although larger investment was found in hill eco-zone, it does not imply that all the expenditure was solely for hill agriculture but part of this investment used to spend for other eco-zones. This is because disciplinary research divisions and headquarters office that consume significant portion of the total budget are located in Kathmandu valley which is in hill eco-zone.

Table 1. Research investment trend in eco-zones (NRs thousand at current price)

Fiscal year	Terai	Hill	Mountain	Total
2000/01	138098	328717	19771	486586
2001/02	161777	392888	23111	577776
2002/03	82697	215064	15478	311249
2003/04	86489	199997	14089	300575
2004/05	90488	209308	11093	310889
2005/06	90488	209308	11093	295055
2006/07	88045	196968	10042	355000
2007/08	108218	233093	13689	415000
2008/09	114706	284057	16237	510000
2009/10	134640	354696	20664	560000
Mean %	28	68	4	100

Source: NARC.

There are seventeen disciplinary research divisions, three research units, two national research institutes and one genetic resource center in Kathmandu consuming about 25 percent of the total research budget. Altogether 40 percent of the total budget is spent in Kathmandu including the NARC headquarters' budget.

Research expenditures and full time equivalent (FTE)

Major research work on cereal crops is carried out by Nepal Agricultural Research Council (NARC), however research and development (R&D) activities in these crops are also carried out by the nongovernmental organizations such as Local Initiative for Biodiversity, Research and Development (LIBIRD), Forum for Rural Welfare and Agricultural Reform for Development (FORWARD), and Institute of Agriculture and Animal Science (IAAS).

NARC, a government organization, shares 92 percent in total spending and 93 percent in total staffing in agricultural R&D. LIBIRD, an NGO focused on agricultural research and biodiversity conservation, shares 4.1 percent of total research spending and 3.3 percent of research FTE. Similarly, FORWARD, another NGO shares 2.8 percent of total spending and 2 percent of total FTE researchers. Unlike in governmental agencies, FTE researchers in these NGOs used to be hired on project basis. IAAS, the only one teaching institution owned by the government for higher degree in agriculture, shares about 0.8 percent in total spending and 1.7 percent in FTE researchers (Table 2). IAAS did not have its own budget for research work but received few research projects with financial support from other organizations.

Table 2. Overview of public agricultural R&D spending and research staff levels, 2009/10

Research agencies	Total spending at current price		Research staff	
	Nepalese Rupees million	Shares (%)	Number (FTE)	Shares (%)
NARC	590	92.3	278	93.0
LIBIRD	26	4.1	10	3.3
IAAS	5	0.8	5	1.7
FORWARD	18	2.8	6	2.0
Total	639	100.0	299	100.0

Source: Survey 2010.

Research expenditures mainly incurred in three broad headings viz. staff cost, operational cost and capital cost. Operational cost comprised direct program costs and administrative costs which were associated with the research implementation. The total research expenditure in public and donor funded projects during 2009/10 was Rs 639 million at current price. NARC spent 92 percent of national investment and employed 93 percent of total researchers in the same year. For other agencies, the research expenditure and number of researchers were estimated in consultation with concerned officials since their total expenditure and total staff is quite high as they involve in development and social sector, too. NARC invested 60 percent in staff cost, 30 percent in operational cost that covered administrative cost and 10 percent in capital cost in 2009/10. The research cost per unit FTE in 2009/10 in NARC was NRs 21,22,302 (US $ 29476) that includes all the costs including salary and capital expenditure. In the same year, this cost in other organizations was slightly higher ie NRs. 23,33,333 (US $ 32407) and hence the overall research cost per unit FTE in was Rs 21,37,124 (US $ 29682). In 2003, the per scientist research budget in rice was estimated to be US $ 15,780 for Eastern India and US $ 21,110 for rest of India (Pandey and Pal 2007).

Full time equivalent (FTE) of researchers

Congruency analysis could be carried out through proxy measure of resource allocation with FTE of the researchers in particular crop, domain and environment. There was small scale of research spending and small number of FTE researchers in the agencies like LIBIRD, FORWARD and IAAS. Most of the researchers in these agencies were not involved in cereal crops rather they were involved in other commodities like legumes, vegetables, fishery etc. These agencies had relatively larger spending in other sectors like sustainable livelihood, bio-diversity conservation, ecosystem, and health rather than particular agricultural commodities. Since their investment in cereal crops research was much less ie less than 5 percent of the total and not categorized into specific commodities, the above mentioned agencies were not included in congruency analysis. Among 332 researchers of NARC, 110 researchers in rice, 59 researchers in maize, 94 researchers in wheat and 17 in finger millet were working partly or fully in 2010 (Table 3).

The commodity research does not represent only direct research such as agronomic or breeding but it also encompasses disciplinary researches such as agricultural engineering, food technology, agricultural environment and other sectors which are associated to that commodity. The number of researchers on head count basis was much larger than those on FTE basis which accounts 34.75 researchers in rice, 18.26 researchers in maize, 28.04 researchers in wheat and 1.88 researchers in finger millet in Nepal. Rice researchers and maize researchers use to spend 33 percent of their total time in their respective commodities whereas wheat researchers use to spend 28 percent and finger millet researchers use to spend only 11 percent of their total time in their respective commodities.

Table 3. FTE researchers involved in major cereals, 2010

Crop	No of researchers	Total FTE	Mean FTE	FTE share (%)
Rice	110	34.75	0.33	41.90
Maize	59	18.26	0.33	22.02
Wheat	94	28.04	0.28	33.81
Finger Millet	17	1.88	0.11	2.27

Source: Survey 2010.

Total number of FTE researchers involved in rice in Table 3 was substantially larger from that reported in previous study carried out by Gauchan and Pandey (2011). The previous study reported 25 FTE in rice research which was based on survey in 2009. One of the major reasons for such an increased number of scientists was recruitment of 70 new scientists in 2010 in NARC. The current study has considered seed production as one of the research activity which might not be the case in previous study.

Results of congruency analysis

Congruency analysis was carried out on the basis of actual production share as well as adjusted production share of the commodities in total output value of cereal production. Production share was adjusted with research progress and equity as described in research methods. Poverty, employment, health and environment were considered for adjustment in research priority assessment and impact study of International Potato Center (Fuglie 2007). Rice has good research progress as scientists have identified/developed suitable varieties that resist drought and submergence. Verification trial and up-scaling activities of these varieties have been conducted in farmers' field of the mid hills (Lamjung and Doti), eastern terai and western terai. Regarding equity, maize and finger millet have larger weight as they contribute much in poverty reduction as compared to rice and wheat. Maize and finger millet are widely grown in marginal area where livelihood of large population of poor farmers mostly depends upon.

Congruency analysis in Table 4 shows that FTE share in rice was 15 point below its production share which clearly indicates substantial under investment in rice research. Wheat was substantially over invested as its FTE share was 15 point above its production share. Maize crop was slightly over invested as its FTE share was 3 points above its production share. Finger millet was under invested as its FTE share was 2 points below its production share. Although there was mismatch between FTE share and production share to various extents depending upon individual crop, the overall congruency is 95 percent. When the production share was adjusted with anticipated research progress, it became 3 points larger in rice and one point smaller in wheat but not substantial difference in maize and finger millet.

Table 4. FTE allocation and production share

Particulars	Rice	Wheat	Maize	Finger millet	Congruency
FTE share	41.90	33.81	22.02	2.27	
Actual Production share	56.57	18.47	19.35	4.68	0.95
Adjusted production share (research progress)	59.08	17.15	19.61	4.16	0.94
Adjusted production share (equity)	57.65	15.68	21.53	4.97	0.94
Fully adjusted production share (research progress and equity)	60.20	14.56	20.82	4.42	0.93

The congruency percent for overall cereal crops was still high (94%) indicating that there was considerably balanced investment across the cereal crops. When the production share was adjusted with equity, the congruency percent was as high (94%) as adjusted with research progress. When the production share was adjusted with equity and research progress in combination, the congruency percent declined to 93 percent. The discrepancy or mismatch between FTE share and production share in rice and wheat in adjusted as well as unadjusted case suggests that more resource is required in rice at the cost of resource allocated in wheat. Researchers in rice need to be recruited in large number so that there will be balance between resource allocation and production share. Many studies in past had carried out congruency analysis across the environments and eco-zones. Byerlee and Morris (1993) found that share of research resources invested in wheat in marginal environment had been adequate or even a bit high relative to the share the value of wheat produced in these environments at the international level and for India.

Pandey and Pal (2007) found rice research in rainfed environment was moderately under-invested in India. Unadjusted production share indicated a high congruence (99.5%) in resource allocation while adjusted production share resulted in low congruence (90.4%) across the production environments. Gauchan and Pandey (2011) found a substantial underinvestment in rice research in general but more so in rainfed areas and in terai agroecological zone in Nepal.

CONCLUSIONS

The investment in agricultural research was low and not increasing in real price although there was increasing trend in the national budget. The past trend of investment in agricultural research according to eco-zones shows that hills received about 55 percent, terai received about 39 percent and mountain received 6 percent of total budget, excluding headquarters' and directorates' budget every year. Congruency analysis among the cereal crops shows that there was substantial under investment in rice and over investment in wheat. Maize and finger millet were slightly under invested. The congruency percent for overall cereal crops based on actual production share was high indicating that there was almost balanced investment in cereal crops. When the production share was adjusted with research progress and equity, the under investment in rice was amplified. The congruency percentage based on actual production share was 95 which declined to 93 when it was adjusted with research progress and equity in combination. It indicates that despite the mismatch between investment and output value in individual crops, the overall congruency was still high. Increased investment with financial as well as human resource in rice research is required to minimize the discrepancy in resource allocation and production share.

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Value Chain Analysis of Turmeric (*Curcuma longa*) in Eastern Nepal

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ABSTRACT

A study on value chain analysis of turmeric (*Curcuma longa*) was conducted in December, 2010. Forty turmeric producing farmers were selected randomly from the list of District Agriculture Development Office, Sunsari. Similarly, 25 traders, wholesalers and retailers from Sunsari and Biratnagar were selected purposively. The study revealed that more than 38 percent of the cost goes to the labor as all the operation was labor intensive. There were no formal institutions involved in the promotion of turmeric in the study area and hence local seeds were used since time immemorial. Majority of the farmers practiced mother rhizome retrieval and this practice contributed to reduce the cost of production. The average cost of production was NRs 63 per kg for dry turmeric (*Sutho*). At the producer level, grading, cleaning and *Sutho* making were the major value addition activities, where the marketing margin was NRs 129 per kg *Sutho*. At the processor level, milling and packaging were the major value addition activities, where marketing margin was NRs 65 per kg turmeric powder. Around 2 percent of the total production was used for seed and 2 percent was used for powder making for their own use and rest of the production was sold to collectors. Collectors directly sell 50 percent of the product to the retailers, 16 percent to mill and 5 percent to spice industry (*Masala Udyog*). The production side actors are typically interested in access to capital (credit/loans) and technical inputs, market assurances (price and quantity) and higher prices from the market actors. The market actors expect for sharing risks and inputs, quality and quantity assurances and market lead price fixing from the production side actors. These divergent areas of interest often result in conflict of interest and thus loss of trust. SWOT analysis was carried out, constraints were identified, and interventions were proposed.

Key words: Cost of production, dry turmeric, functional upgrading, margin, SWOT analysis, value addition

INTRODUCTION

Turmeric is an important spice among the rice-eating peoples of Nepal, India, South East Asia and Indonesia and is indispensable in the preparation of curry powder. According to MoAC 2010 and annual Report of National Spice Crop Development Program (NSCDP 2009) about 39,203 mt of turmeric has been produced in Nepal during the year. The production was 24,470 mt, 25,328 mt and 23,570 mt during 2007/08, 2006/07 and 2005/06, respectively. The production is found in increasing trend along with the increase in area of cultivation. However, productivity is almost stagnant or somewhat decreased. There is an import of turmeric from India to Nepal. In 2006/07, there was 82 metric tons of turmeric imported from India. However, turmeric is being exported exclusively in dust form in third country like Japan (ABPMDD 2010). So, there is great potentiality of import substitution and export promotion of turmeric in Nepal. On this ground, this study was carried out to analyze value chain function of turmeric as a broad objective. Whereas the specific objectives were to; identify the existing status of value chain stakeholder; identify the value chain performance in the chain function; identify the strengths, weaknesses, opportunities and threats, and recommend the possible options for further improvement of the turmeric sub-sector.

METHODOLOGY

The value chain is a concept which can be simply described as the entire series of activities required to bring a product from the initial input-supply stage, through various phases of production, to its final market destination. The production stages entail a combination of physical transformation of the product and the participation of various producers and services, and the chain includes the product's disposal after use. As opposed to the traditional exclusive focus on production, the concept stresses the importance of value addition at each stage, thereby treating production as just one of several value-adding components of the chain**.** Considering the importance of value chain, this study was carried out as one of the parts of the value chain analysis of turmeric with the following methodology.

Selection of the study area

Sunsari district was selected purposively for the value chain study of turmeric in December 2010; this was the leading district for turmeric production in Nepal. Hence, keeping in view the potentiality of turmeric production, accessibility of researcher, and budget and time constraints, this district was selected.

Review of the existing information

All the available documentation was collected and reviewed. The review work was carried out during the initiation stage and based on the review outcome, the field work was planned.

Sampling of chain actors

The farmers, traders/wholesaler, service providers were the focus of this research. Incase of farmers, 40 turmeric producing farmers were selected randomly from the list of District Agriculture Development Office (DADO), Sunsari. 25 traders/wholesaler/retailers from Sunsari and Biratnagar were selected purposively. Similary, other chain actors like transporters, representative from cooperatives, commodity association and network, and the service providers like, DADO, District Development Committee (DDC), District Chamber of Commerece and Industried (DCCI), Commericial Agriculture Alliance (CAA) etc. were also selected purposively. Identification and selection of such stakeholders were done in consultation with the CAA officials and DADO, Sunsari.

Interview/FGD of key chain actors

A semi-structure questionnaire was developed and used for the purpose. In addition, checklists were also developed and used for producers, traders, and other stakeholders. An in-depth interview was held with value chain actors. Focus Group Discussions (FGD) was held with concern stakeholders specially the producers and traders.

Tools and techniques of data analysis

The primary and secondary information collected from the field survey and other methods were analyzed by using Statistical Package of Social Science (SPSS) and Micro-Soft Excel for calculating benefit cost ratio, producers share, chain performance, value addition etc. at different stages of value chains.

Strength, weakness, opportunities, threats (SWOT) analysis

It was used for identify SWOT of turmeric commodity.

S - What are the commodity's internal strengths?
W - What are the commodity's internal weaknesses?
O - What external opportunities might move the commodity forward?
T - What external threats might hold the commodity back?

RESULTS AND DISCUSSION

Major actors involved in the value chain and their relation

Production stage: Two types of farmers are engaged in turmeric production: I) Small farmers with subsistence turmeric production, II) Semi-commercial farmers characterized by small production volume but still targeting the market. The produce from the first category of farmers generally does not enter the market or enters in a very limited quantity especially in the local hat-bazaar. Small commercial farmers sell most of their produce to collectors. The producers generally deal with collectors at hat-bazaar area. Sometimes, the traders make a small finance for crop cultivation and or to meet social obligations of the farmers. In this situation, whenever farmers have something to sell, they are required to sell to the same trader. In most cases market information was inaccessible to general farmers and they have to depend on the information provided by village level traders or collectors at hat-bazaar. Generally, the farmers have small and low cash resources. The financial need is sometimes met by the collectors who provide certain amount of loan to farmers in condition that the produce has to be sold to the loan providers.

Collection/assembly stage: Farmers in the area and collectors in the hat-bazaar are involved in marketing business directly. The small farmers carry the produce on their own and sell to collectors. The collectors, based on the information from other various marketing centers, fix the price by themselves and this price has to be agreed upon by the farmers. Then keeping some profit margin on the produce they sell it to retailers in Dharan, Itahari, Biratnagar, Rajbiraj, Lahan, Janakpur, and Kathmandu and processing units like *Masala Udhyog* and mills in Biratnagar.

Processing and logistics stage: There are three types of *Masala* producers in the study area. The first type is mill operators who grind the dry turmeric pieces and sell them in packaged and loose powder form to the retailers. The second type of *Masala* producers are small cottage industries who also grind turmeric and other spices and sell packaged materials to the distributors and retailers. The third type are *Masala* factories who produce different types of *Masala* and also used turmeric powder to pack in different weights as well as mix in the other types of *Masala*. However, they supply to the retailers through distributors. The first and second type of *Masala* producers get raw material ie dry turmeric pieces from the collectors whereas the third type (Masala factory) imports raw materials mainly from India but its statistics has not been available. All the produce collected by the collectors in particular places like Barahchhetra, Chatara/Haatkhola and Bishnupaduka on a particular day is transported to Jhumka road center in three wheelers. From Jhumka, it is transported to other market places on the roof of buses. Normally, the fare for three wheelers and buses is fixed which is NRs 50 for Barahchetra/Chatara/Haatkhola to Jhumka; NRs 50 for Jhumka to Biratnagar or Lahan or Rajbiraj, and NRs 80 for Jhumka to Janakpur per 75 kg of bag.

Retailing stage: These actors are found very effective in turmeric marketing. The retailers are of three types. One, they procure it from wholesalers and sell it to consumers in local hat-bazaars. Other type gets the powder from *Masala Udhyog* or grinding miller and sell it in powder forms. Third type of retailing is done by food sellers in their retail (*Kirana*) shop. These *Kirana* shoppers are retailers of foodstuff and other goods of basic needs. Generally, they have permanent customers who usually purchase food-stuff and other requirements of daily need. The retailers do not have direct relation with farmers. They procure it from collectors and do not have any idea of price what is paid to farmers. The price between them is fixed by collectors.

Relationship between production and market actors

- There often is absence of or limited/weak dialogue between the market and production actors. The "contracts" are often not binding, as the legal basis are not always ensured, they usually act as a document of "mutual trust".
- There is an informal nature of relationship among the actors, with general lack of professionalism in terms of technical knowledge, in terms of production and processing. Additionally, limited investment in technical capacities, enforcement and abidance to contracts hamper ensuring quantity and quality of production.
- Farmers have been using their own seeds and sometimes buy from the villagers as well as from local markets at Bishnupaduka and Chatara.
- There were no formal institutions involved for the promotion of turmeric in the study area so the farmers have been cultivating local seeds since time immemorial. However, Dharan wholesale market is providing space for its marketing at regional level.
- There exist very divergent interest areas in terms of actors in production side and market side. The production side actors are typically interested in access to capital (credit/loans) and technical inputs, market assurances (price and quantity), highest prices from the market actors. While, the market actors expect shared risks and inputs, quality and quantity assurances and market lead price fixing from the production side actors. These divergent interest areas often result in conflict of interest and thus loss of trust. The production side actors are looking to up-scale their activities to reduce the number of intermediaries between the markets, while the market actors are also looking to have backward integration towards the production side. The reasons for this are to reduce the logistic costs and capture upstream or downstream profit margins.

Chain performance

Cost of production: The average cost of production was found to be NRs 62.67 per kg of dry turmeric (*Sutho*). This cost includes the opportunity cost of household labors. The farm gate price highly fluctuates over the years, which is influenced by the supply situation in the markets. The B/C ratio was 3.55 (without land renting cost) and 3.21 (with land renting cost) (Table 1). The study showed that more than 38 percent of the costs goes to the labor (opportunity cost) for planting rhizome, inter-culture operation, harvesting, drying and transporting to collection centre. Similarly, 21.28 percent goes to organic manure, 20.74 percent goes to the firewood for drying rhizome, 11.17 percent for seed purchasing and 8.51 for land renting (Figure 1).

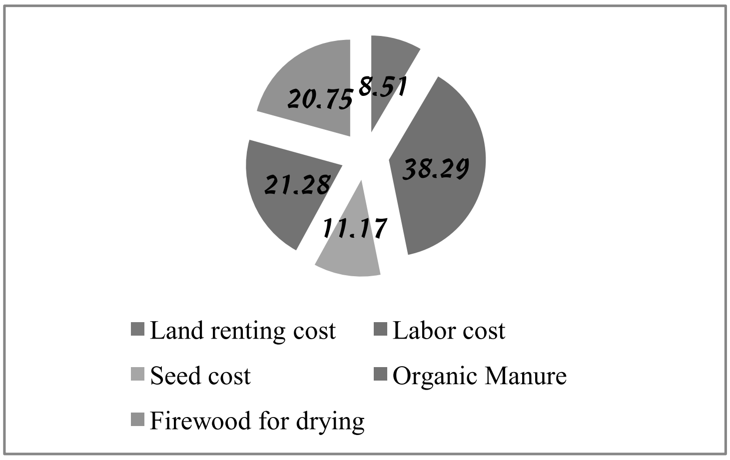

Figure 1. Cost share in turmeric production.

Chemical fertilizer was not used by farmers, all the farmers used FYM/Compost. Manual labor and seed were the two major inputs and in most cases, are owned by the producers themselves. The farmers were also found using various practices, which ultimately increase the efficiency of their production system and keep the production cost low. Majority of the farmers practice mother rhizome retrieval, the practice reduces the cost of production. All the operations were labor-intensive and generally carried out by the household members and their relatives or villagers as a *Perma* (labor exchange system). In very few cases farmers hire laborers on daily wage basis. The average size of the plot per farmer was around 5 kathha (0.167 ha). The analysis showed that the average farmers could earn gross margin NRs 21,050 (Twenty one thousand fifty) from cultivating turmeric in one kathha of land.

Table 1. Details of production cost and benefit of dry turmeric (*Sutho*) per kattha

Cost items	Unit	No	Rate (NRs)	Cost (NRs)
Labor Cost (planting, harvesting and drying)				3600
Land preparation including ploughing and leveling	Plough with ox and man	3	200	600
Planting rhizome	Man days	2	150	300
Weeding and retrieval of mother rhizomes	Man days	4	150	600
Harvesting and post harvest	Man days	7	150	1050
Transportation to market	kg	150	2	300
Labor	Man days	5	150	750
Input Cost				3050
Seed	kg	70	15	1050
Organic fertilizer	kg	1000	2	2000
Processing (fire wood)	kg	1300	1.5	1950
Total (A + B + C) without land renting				8600
Cost for land renting				800
Total cost including land rent				9500
Income from mother rhizome	kg	50	20	1000
Total production main crop per kathha	kg	150	197	29550
Total income from main crop				30550
Cost of production per kg				62.67
BC ratio (without land renting)				3.55
BC ratio (with land renting)				3.21

** Average price of dry turmeric piece @ NRs 197 per kg. Buying rate of 1 US$=71. 29 NRs5 (Nepalese Rupees) as of 30 September 2010.*

***Source:** FGD and interaction with turmeric growers and traders, 2010 (above calculation based on average of 2009 and 2010.*

Value addition, value chain map and margins of turmeric: At the production and assemble level, grading, cleaning, turmeric *Sutho* making were the major value addition activities in the study area. The cost incurred for cleaning and grading per kg of turmeric was Rs 0.55, whereas, the total production cost of dried turmeric (*Sutho*) was Rs 63 per kg. Farmers were getting Rs 128 per kg of turmeric *Sutho,* if they sold it directly to the consumer. Milling and packaging were the major value addition activities at processor level. It cost around NRs 17 per kg turmeric (Table 2).

In the value chain map, the channels have been identified on the basis of core business units, ie the supply, production and distribution involving physical product flow from supplier to the end-user. In general, the more the units are integrated, the more competitive advantage they gain and more capital-intensive they are. Around 2 percent of total production is used for seed and 2 percent is used for powder making for their own use. Rest of the product goes through collectors. After collection, collectors directly sell 50 percent of the product to the retailers, 16 percent goes to mill and only 5 percent goes to spice industry (*Masala Udyog*) from collectors. The most important factor governing the ratio of the share or margin distribution was the flow of market information from the demand side to the supply side. It was found that the market information flow system was very weak; however the analysis of value addition and margins indicated that the price received was very favorable for the value chain actors in the study area during study period than previous year due to increasing trend of price of *Sutho*.

Table 2. Value addition and margin of turmeric in study area

Producer Level/Assemble Level/Collector/Whole seller		Processor Level	
Description	Cost NRs/kg	Description	Cost NRs/kg
Total average production cost of dried Turmeric (*Sutho*)	62.67	Acquisition cost	197.00
Load and unload	0.03	Milling and packaging cost	17.00
Storage and other cost	0.02	Transportation cost	0.80
Transportation cost to Markets	0.65	Losses (10% of *Sutho*)	19.70
Tax	0.45	Tax	0.25
Cleaning, grading	0.55	Total cost[C]	234.75
Losses 2%	3.94	Wholesale price of Turmeric powder[D] (Avg of 2009/2010)	300.00
Total cost at district[A]	68.31	Margin (D-C)	65.25
Price of dry turmeric at district[B] (avg of 2009/2010)	197.00		
Margin (B-A)	128.69		

Production, trade flows and volumes of turmeric

In the year 2009/2010, the production of turmeric in Eastern, Central, Western, Mid-western and Farwestern Development regions was recorded as 6324 mt, 9293 mt, 8388 mt, 8722 mt and 5219 mt, respectively (MoAC 2010). Taplejung, Sankhuwasabha, Udaypur, Sunsari, Morang, Saptari, Okhaldhunga, Ilam, Dhankuta etc are major turmeric growing districts of Eastern Development Region. Turmeric is one of the most important income-generating crops of Eastern region. It can be grown practically in all districts for cash income. Among the different districts of Eastern region, Sunsari is known as the potential district for Turmeric production. In 2009/2010, the area and production of Turmeric in Sunsari district was 110 ha and 830 mt, respectively (MoAC 2010). In the study area, market is controlled by few actors involved in trading in one hand and on the other hand, producers lack appropriate skill, knowledge and information on turmeric marketing and are compelled to sell their produce at relatively low price to those few traders. Similarly, farmers have very little stake over the value chain because the market system is mainly operated by the collectors who have access to the market traders. The collectors in Barahchhetra, Chatara/Haatkhola and Bishnupaduka are the primary collectors in the study area. They can also be known as traders, wholesaler and distributors as they perform all the works. Farmers themselves bring the turmeric to collection point by carrying on their back. Few porters are also involved in carrying the turmeric charging NRs 2 per kilogram. The number of producers was quite low; the collectors in the area manage the marketing of the product by themselves. However, there is a need to ensure high level of farmers' participation and integration in the value chain to get more benefits. The dry turmeric collected at above mentioned locations were distributed to the retailers, mill owners and small cottage industries of Itahari, Biratnagar, Rajbiraj, Lahan, Janakpur and Kathmandu. An export trend reveals that the major share goes to India and overseas countries. Available data received from annual reports of the Agri-Business Promotion and Market Development Directorate (ABPMDD 2010) indicated that total of 87 mt of dry turmeric was exported to India and overseas countries during 2008/09. Japan is another country where turmeric is being exported in dust form.

Price trend analysis
Annual price trend of study districts was found in increasing trend. As expressed by the farmers and traders, from year 2008 to date, price was relatively good. In year 2005, average price of dry turmeric per kg was Rs 34, whereas the price was Rs 243 in 2010 (Figure 2). Price setting at the collection markets in the study area was mainly based on market information and volume of transaction. The actors of the major collection markets rely for market information on Hariwan and Lalbandi market traders. Generally, the supply and demand position in Janakpur and Biratnagar greatly influences the price. However, farmers do not know how price is being fixed in the market as it is solely based on the traders.

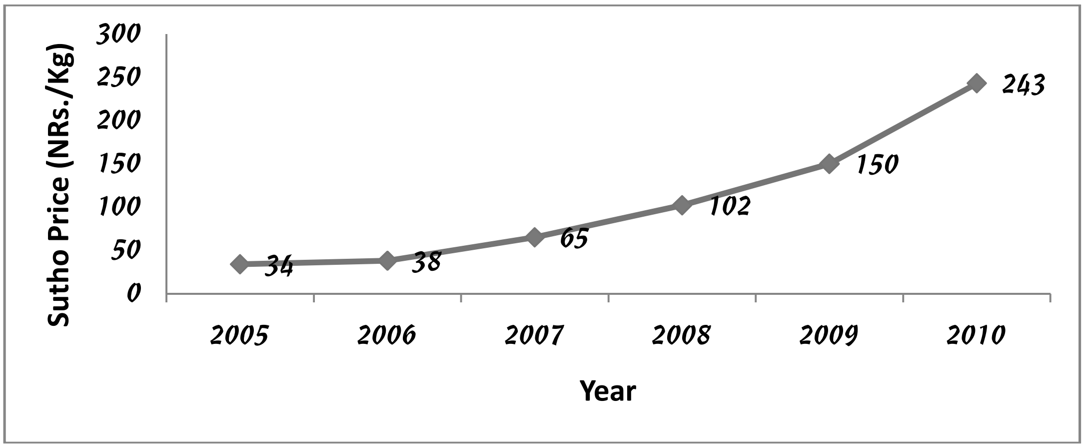

Figure 2. Price trends of dry turmeric in study district (Source: FGD 2010).

Monthly price situation of dry turmeric in the study area revealed that, the price was relatively lower from January to March and increased from September to December in both years. During January to March, farmers dig out the produce, dry it using boiling and drying method, grade it out and bring to market for sale. However, price relatively goes on increasing from late September-October. Moreover, during October to November, the preserved rhizomes are taken out, dried using local techniques, carried to the market to meet the cash needs of farmers for festival (Dashain-Tihar) celebrations. However, harvesting of mother rhizomes and selling in the market was common in July to August. The price was relatively lower in this time. Therefore, some of the farmers stored it and sold it later by benefiting from time utility. The details of price at different periods are given in Figure 3.

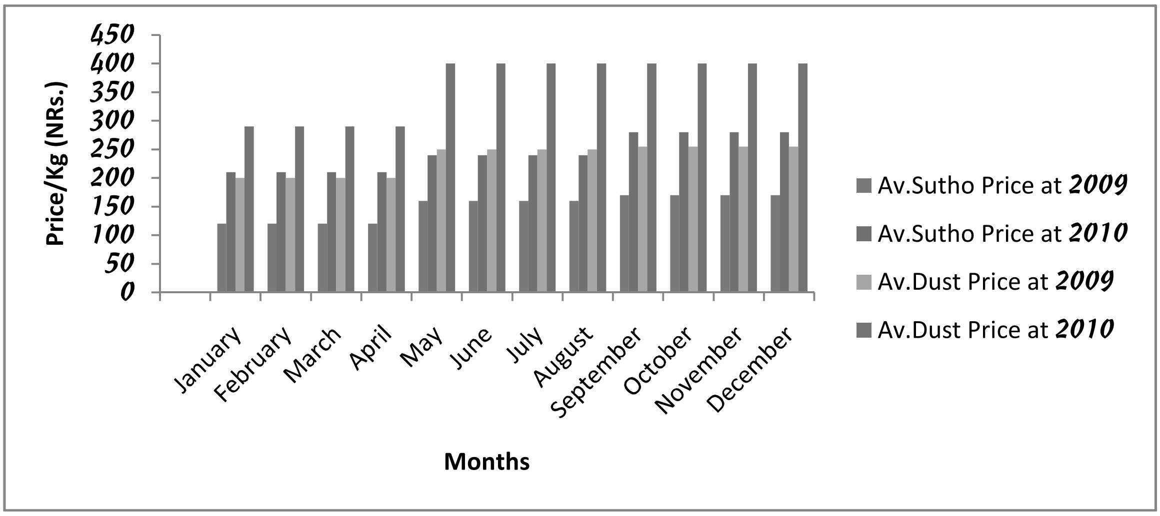

Figure 3. Monthly price of dry and dust turmeric in study district (Source: FGD 2010).

SWOT analysis

The following SWOT analysis of the turmeric sub sector shows a number of strengths and opportunities for value adding interventions. During the designing of interventions adequate provisions need to be created for addressing the weaknesses and threats for the strengthening and growth of the turmeric (Table 3). Major constraints and proposed interventions are given in Table 4.

Table 3. SWOT analysis of turmeric value chain

Levels	Strength	Weakness	Opportunity	Threat
Input	1. Less irrigation required 2. Required Less capital investment	1. No research work started yet 2. Less priority of government 3. Lack of drying yard 4. No chemical fertilizer use 5. No improved variety available 6. Only traditional farming practices 7. No technology transfer training received by farmers	1. Research needs on varietal improvement and other practices 2. Increase in no. of service providers	1. Degeneration of local land races
Production	1. Less infestation of insect and diseases 2. Can be grown in marginal land 3. Pro poor family can grow 4. Good for soil fertility maintenance 5. Local Nepalese variety contains more curcuma	1. No technical services available 2. Low productivity 3. Only sole cropping adopted 4. No cropping plan 5. Commercial farming not adopted	1. Employment generation 2. Increasing outreach potential 3. Environmental friendly 4. Good cover crop 5. Reduce soil erosion	1. Rhizome rot and Alternaria leaf spot disease initiated
Output	1. Easy and long storage capacity 2. Daily kitchen requirement 3. Medicinal values 4. Contains high preservative value 5. Industrial use 6. Less transportation losses	1. No group marketing 2. No market information system 3. Price fixing by traders only 4. Insufficient linkage among service provider 5. Illegal tax collection 6. Seasonal marketing 7. Low daily consumption 8. Actual market demand not known	1. Unmade demand 2. Import substitution 3. High export potential 4. Value addition potential 5. Increasing demand by mills and factory	1. Trade depend on India 2. Monopolistic marketing system 3. Deforestation – more firewood required for drying

CONCLUSION AND RECOMMENDATION

The overall value chain study of turmeric in Eastern region guided that attention should be paid in seed production, introduction of appropriate variety, quality of seed, disease and pest management, drying and processing methods, post-harvest management, value addition and access to finance. There exist very divergent interest areas in terms of actors in production side and market side. The production side actors are typically interested in access to capital (credit/loans) and technical inputs, market assurances (price and quantity), highest prices from the market actors. While, the market actors expect shared risks and inputs, quality and quantity assurances and market lead price fixing from the production side actors. These divergent interest areas often result in conflict of interest and thus loss of trust. The production side actors are looking to up-scale their activities to reduce the number of intermediaries between the markets, while the market actors are also looking to have backward integration towards the production side. The reasons for this are to reduce the logistic costs and capture upstream or downstream profit margins.

Some of the major interventions recommended for turmeric value chain in the inputs level are: selection and production of improved seed from local cultivars having high curcuma content; raising awareness among farmers for quality seed production; replacement of old seed by improved seed; establishment and strengthening of input service providers in production area to cater the need for timely supply of required inputs. Similarly, at the production level, some recommendations are: research must be started on varietal screening and development, development of cost effective production packages, input responsive and cost effective practices; capacity enhancement of farmers by providing them trainings on cultivation practices, arranging farm visits conducting field demonstrations; DADO should proactively motivate the farmers towards adoption of improved technology in various stages of crop production; measures have to be taken to reduce production costs. Reductions in production costs can be achieved by adopting practices like seed rhizome re-utilization, mother rhizome retrieval during the lean period and intercropping. The cost of production can also be reduced by improving drying method like using solar dryer. Moreover, some recommendations at output level are: harvesting and selling during the months of September to December when price is high; timely delivery. Regularity in supply (supply interval), uniform packing size, quality of packing are some of the important aspects in marketing. Shift from dried turmeric to powder and timely flow of market information to all actors in the value chain are recommended.

Table 4. Major constraints and proposed interventions for turmeric value chain

Category	Constraints	Interventions
Infrastructure	Lack of improved drying facilities at farm level	Construction of community drying yards at accessible allocation. Demonstration of appropriate drier systems Improvement in existing processing technique of drying
	Lack of link road	A link road should be constructed
	Lack of storage facilities at market	Market yards and storage structures should be made at Chatara/Haatkhola bazaar
	Lack of weighing facilities at production level	Collection centre should be established at farm level with proper weighing equipments
Technical	No research work started	Research work must be started on varietal improvement on local races Promising varieties from India should tested and verified by NARC Improved cultivation practices should be tested and reached to farm level NARC should initiate research work on value addition possibilities
	No training provided to farmers	Training needs to be identified and organized accordingly
	No cropping plan	Cropping calendar should be identified and intercropping should practiced instead of sole cropping
	Less productivity	High yielding, high curcuma content, fertilizer responsive and disease-pest resistant variety supported with improved cultivation practices should be introduced in the farm level
Marketing	No group marketing	Farmers group should be formed to marketing
	No market information system and price fixing mechanism	Market information system should be established and turmeric must be added in priority crop
	Illegal taxing	Government should abolish the illegal taxes
	Actual market demand not known	Market study should be done to understand the actual domestic as well as international market demand
Input supply	No improved seed available at farm level	Input service providers should reach to the farm level Inputs service providers should be trained on improved varieties and other inputs
	Irrigation requirement not known	Appropriate quantity and time of irrigation should be identified
Lobbying	Illegal and multiple taxes	Farmers and traders jointly approach to abolish the illegal as well as multiple taxes Awareness on government regulations, farmers rights
	Less government priority	Government should keep Turmeric in their priority so as each DADO could plan in their annual target
Finance	No financial system at traders and Farm Level	Saving and credit system should be established at farm level

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Evaluation of Pole-type French Bean Genotypes in the Mid Hills of Western Nepal

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ABSTRACT

Eight pole-type French bean (*Phaseolus vulgaris* L.) stringless, snap bean genotypes (Four Season, LB-31, LB-39, Madhav, Trishuli, Chinese Long, Makwanpur and Samjhana) were evaluated during summer seasons of 2010 and 2011 at the Agriculture Research Station, Malepatan, Pokhara (848 msl) to assess the variability in the genotypes and their potential for utilization in improvement programs. The experiment was conducted in randomized complete block design (RCBD) with three replications. Results showed that the variability was higher in adaptation, vegetative growth, floral and pod characteristics and fresh pod yield. The highest number of branches and nodes per plant were produced by Chinese Long (5.30) and LB-31 (32.50), while the lowest number of branches (3.52) and nodes (24.33) were produced by LB-39, respectively. Makwanpur was the earliest in flowering (37.50 days) and the Trishuli was late (47.17 days). The tallest plants were found in Chinese Long (277.20 cm) while the shortest in LB-31 (217.17 cm). Madhav produced the highest number of flower buds per inflorescence (5.77) and LB-39 produced the lowest number (4.67). Pod length was the highest in Chinese Long (20.46 cm) and the lowest in Trishuli (16.24 cm), while pod width was the highest in LB-39 (10.14 mm) and the lowest in Makwanpur (8.35 mm). Four Season produced the highest fresh pod yield (25.75 t/ha) followed by Makwanpur (24.89 t/ha) and the lowest by LB-39 (15.93 t/ha). The variability indicated that the collected genotypes were distinctly different. The existing diverse variability could be utilized for the improvement of French bean genotypes in the future.

Keywords: French bean, fresh pod yield, genetic variability, pole bean, snap bean, string less bean

INTRODUCTION

French bean (*Phaseolus vulgaris* L.), a native crop of central and South America (Swaider et al 1992) has one of the longest history of cultivated plants. It is widely cultivated in the temperate, subtropical, tropical regions and is one of the important legume worldwide for human consumption (Singh 1999). Bean is an important vegetable of Nepal and is cultivated in a wide range of agro-climatic conditions from plains at 300 m above sea level (masl) to the high hills at 2,500 masl in different seasons (Neupane et al 2008). Beans, the "meat of the poor", contribute essential protein to the undernourished people living in the hills. In Nepal, green bean pods are used as fresh vegetable and the dried seeds for pulse while the foliage is consumed as fodder and is used to restore soil fertility.

Different ethnic groups have their own ways of preparing beans and there are special occasions and festivals when the products are relished. Red kidney beans are cultivated during the winter season on the plains (below 500 masl) and are an integral part of the cuisine as a socio-cultural identity in the plains of Nepal. Different Indian rajma (red kidney bean) genotypes have been introduced in production areas and the predominant cropping pattern is as a mono crop. In the mid hills, fresh green pods are important and widely consumed as a vegetable. Both pole- and bush-type French beans are cultivated for green pods in the hills (500–1600 masl) during summer to autumn. Green stringless French bean (snap bean) is a very popular vegetable crop among the hill people. These beans are grown as a mono crop in the commercialized peri-urban areas using staking for pole beans. Pole beans are also inter-cropped with maize as a rain-fed crop in the hills. Dried shelling beans are usually produced from summer to autumn in the high hills and mountains (1,600–2,500 masl). These beans are long duration vegetables, which are grown either with maize or in apple orchards. In the high hills, they are the major source of protein for households and are also a cash-generating crop. Dried beans produced in the high hills are considered to be high quality beans and find their way to distant markets and cities. Farmers regard beans as a cash-generating crop in the hills and grow a number of landraces with varying morphologies (Neupane and Vaidya 2002). The current research was initiated with the objectives of collection, evaluation and characterization of available exotic and indigenous germplasms so that they can be utilized for varietal improvement and commercial cultivation.

MATERIALS AND METHODS

French bean germplasms were collected from government farms, markets and farm households during February-March 2010. A total of 11 landraces were collected, planted and observed in the previous year for their type and character. Eight were found snap type beans, stringless and green pods are used as vegetable. The genotypes selected for evaluation were Samjhana, Madhav, Chinese Long (Run Long), Four Season, Trishuli, LB-39, LB-31 and Makwanpur. The genotypes were evaluated at the Agriculture Research Station (Horticulture), Malepatan, Pokhara and the agro-morphological characteristics and fresh pod yield were recorded. The station is situated at a latitude of 28°13'6.18" N and a longitude of 83°58'27.72" E at an elevation of 848 masl and is characterized by a sub-tropical climate. For characterization, the experiment was conducted from April to July 2010 and 2011. The experiment was arranged in a randomized complete block design with three replications. Spacing was maintained at 75 × 45 cm and experimental plot sizes were 3.6 × 1.1 m. The crop was planted on 27 April 2010 and 2011. Manure and fertilizer were applied as compost (20 t/ha) and 40:60:50 kg NPK/ha, respectively. Scoring of agro-morphological characters was done following descriptors for *Phaseolus vulgaris* (IBPGR 1982). The data were analyzed using Genstat software (version 12.1 VSN International, Hemel Hempstead, UK). The count data was analyzed by square-root transformation method.

Plant survival was recorded as a percentage at the two different stages of 15 days and harvest. Days to flowering was recorded when 50 percent of plants had set flowers. Node number was recorded after flower set from base to first axillary inflorescence and the mean was averaged from 10 randomly selected plants. The number of flower buds per inflorescence was recorded from the lateral (third inflorescence from the apex) and the mean was averaged from 10 randomly selected plants. The number of branches was recorded from the base to the first inflorescence and averaged based on measurements from five randomly selected plants. Plant height was measured at the green pod maturity stage from the cotyledon scar to the highest tip of the plant. Height was recorded in centimeters and the measurements from five randomly selected plants were averaged. Pod length and pod width were measured on the largest, fully expanded, immature, green pod and measurements were averaged from 10 randomly selected plants (followed descriptors). Fresh pods were harvested five times at 10 days interval when the pods were fully expanded, immature and green stage.

RESULTS AND DISCUSSION

Plant survival

Plant survivability was distinctly different (Table 1). The plant survival percentage at 15 days was the highest at 100 percent (transformed data 10.00) in Four Season, Madhav, Chinese Long, LB-31 and Samjhana. Survivability was the highest at 97.92 percent (transformed data 9.73) in Four Season and Chinese Long at harvest. The lowest percentage of plant survival was found in LB-39 with an average of 89.58 percent (transformed data 9.46) at 15 days. Similarly, the lowest percentage of plant survival at harvest was found in LB-39 with an average of 64.58 percent (transformed data 8.48). Plant survival of the crop varieties is one of the important parameters for seasonal and off-season production of vegetables, which determines the resistance and tolerance of the variety to a particular environment. Similar findings have been reported by other researchers. Pandey (2004) reported that the survivability of a particular variety of cauliflower is affected by adverse climatic conditions and it is obvious that the genetic characters show resistance and susceptibility to a particular environment. Al-Soqeer (2010) reported that plant survival of jojoba clones was affected by the genotype and growing season ranging from 91.7 to 98 percent. NeSmith (2003) also reported that the plant survival and vigor of southern highbush blueberry were highly affected by the genotype and growing environment.

Table 1. Plant survivability and plant characteristics of pole-type French bean genotypes at different stages during summer seasons 2010-2011

SN	Genotypes	Plant survival at 15 days	Plant survival at harvest	Number of branches	Number of nodes	Plant height, cm
1	Four Season	10.00	9.73	3.63	27.33	249.67
2	LB-39	9.46	8.48	3.52	24.33	239.22
3	Madhav	10.00	9.52	4.40	26.50	230.73
4	Trishuli	9.84	9.52	3.82	25.50	240.32
5	Chinese Long	10.00	9.73	5.30	34.17	277.20
6	Makwanpur	9.71	9.29	4.62	27.17	247.05
7	Samjhana	10.00	9.57	5.25	29.00	257.45
8	LB-31	10.00	9.01	5.08	32.50	217.17
	Mean	9.88	9.36	4.45	28.31	244.90
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	LSD (0.05)	0.18	0.26	0.31	2.38	13.05
	CV, %	1.0	1.6	4.0	4.8	3.0

Branches

The number of branches per plant in different genotypes differed significantly (Table 1). The highest number of branches was produced by Chinese Long with an average of 5.30 branches per plant, whereas the lowest number of branches was produced by LB-39 with an average of 3.52 branches per plant. Plant growth including the number of branches is the result of a variety's genetic potential interacting with the environment and farming practices. Environmental conditions (mainly air temperature and rainfall) greatly affect the growth and development of bean plants. Brewster (1983) reported that temperature influences the responses of many plant species to photoperiod, growth, tuber development and flowering. The temperature is just as important as day length in influencing growth and flowering (Herath and Ormrod 1979). Alghamdi (2007) also reported in a study of faba beans that the genotypes differed significantly for all traits. He found that the number of branches per plant was significantly different. Similar findings have been reported by Al-Soqeer (2010) where the number of branches of jojoba clones was affected by genotype and growing season ranging from 11.9 to 58.7 branches.

Node number

The number of nodes per plant produced by different genotypes was significantly different (Table 1). The highest number of nodes was produced by Chinese Long with an average of 34.17 nodes per plant, whereas the lowest number of nods was produced by LB-39 with an average of 24.33 nodes per plant. Growth, development and yield of vegetable crops are the result of each variety's genetic potential interacting with the environment and farming practices. The results showed that the node number was affected by the genotype and the growing environment. Long days and high temperatures changed the growth habit of the normally determinate lablab bean (*Lablab purpureus*) plant to indeterminate (Kim and Okubo 1995). They also reported that the plants of indeterminate growth habit showed an increased number of nodes and a greater length of internodes with high temperature and day length (13 h at 25^{o}C or 10–11 h at 30^{o}C). Similar results have been reported by Luitel et al (2009) who noted that the ground coverage and stem number of potatoes were directly influenced by the genotype. Islam et al (2010) reported that the genotypes of hyacinth bean showed considerable variation for most morpho-physical traits. They found that the number of nodes per raceme ranged from 2.33 to 14.1 in different genotypes.

Plant height

The plant height differed significantly among the genotypes (Table 1). The tallest and the shortest plants were found in Chinese Long and LB-31 with an average of plant height 277.20 cm and 217.17 cm, respectively. Neupane et al (2008) reported that the plant height in beans was influenced by the genotype. They recorded that the plant height ranged from 28 to 144 cm in different bean genotypes that were planted at the same date. Similar results were also reported by other researchers. Alghamdi (2007) reported that faba bean genotypes significantly differed in flowering date and plant height. Al-Soqeer (2010) reported that the plant height in jojoba clones was affected by the genotype and the growing season ranging from 30.5 to 52.8 cm.

Days to flowering

Days to 50 percent flowering of a particular variety describes the earliness, medium or late to mature. The flowering days in different genotypes differed significantly (Table 2). The earliest flowering genotype was Makwanpur with an average of 37.50 days after sowing. The latest flowering genotype was Trishuli with an average of 47.17 days after sowing. The flowering and fruiting days were influenced by genotypes, day length and temperature. In most of the vegetable crops, early flowering and maturing genotypes are considered preferable. White and Laing (1989) reported that adaptation of the common bean is strongly affected by photoperiod and there is considerable genetic variation for photoperiod response in the bean species. Neupane et al (2008) reported that the flowering days in beans were influenced by the genotype. They reported that flowering varied from 40 to 84 days depending on the bean genotype. Similar results have been reported by other researchers. Adams et al (1985) and Wallace et al (1991) reported that with the common bean, the days to flowering and the length of flowering period vary depending on cultivar and environmental conditions. Imrie and Lawn (1990) reported that the time of flowering of mung bean crop varies appreciably depending on the genotype, day length and temperature prevailing during the period after sowing. Al-Soqeer (2010) reported that the days to flowering in jojoba clones were affected by genotype, with early and late flowering occurring. Islam et al (2010) also reported that the genotypes of hyacinth bean had days to first flower ranging from 47.6 to 136.3 days, indicating the presence of an early variety.

Flower buds

The number of flower buds per inflorescence was significantly different among the genotypes (Table 2). The highest number of flower buds was produced by Madhav with an average of 5.77 flower buds per inflorescence. The lowest number of flower buds was produced by LB-39 with an average of 4.78 flower buds per inflorescence. The number of flower buds per inflorescence produced was influenced by the genotype. Neupane et al (2008) reported that the number of flower buds per inflorescence in bean was influenced by the genotype. All the genotypes were planted on the same date and varied in the number of flower buds from 1.8 to 18 depending on the bean genotype. Peksen (2007) also reported that large differences were found among common bean genotypes for the number of flowers per plant.

Table 2. Floral and pod characteristics and fresh pod yield of pole-type French bean genotypes during summer seasons 2010-2011

SN	Genotypes	Days to 50% flowering	No of flower buds/inflorescence	Pod length, cm	Pod width, mm	Fresh pod yield, t/ha
1	Four Season	43.83	5.17	18.21	9.14	25.75
2	LB-39	44.17	4.78	18.47	10.14	15.93
3	Madhav	45.00	5.77	18.94	9.84	21.10
4	Trishuli	47.17	5.12	16.24	9.61	20.56
5	Chinese Long	42.33	5.07	20.46	9.01	16.42
6	Makwanpur	37.50	5.55	18.13	8.35	24.89
7	Samjhana	41.83	5.35	20.18	9.58	21.40
8	LB-31	42.67	5.22	17.18	9.00	20.17
	Mean	43.06	5.25	18.48	9.33	20.78
	P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	LSD (0.05)	1.60	0.22	0.58	0.46	2.02
	CV, %	2.1	2.4	1.8	2.8	5.6

Pod length

The pod length among the genotypes was significantly different (Tables 2). The combined analysis over the years showed that the longest pods were produced by Chinese Long with an average of 20.46 cm and the shortest pods by Trishuli with an average of 16.24 cm. The results revealed that the pod length was influenced by the genotype. Neupane et al (2008) reported that the pod length in beans was influenced by the genotype. They found that all the genotypes planted on the same date produced varying pod lengths ranging from 6.7 to 17.4 cm. Similar results were also reported by other researchers. Islam et al (2010) reported that the genotypes of hyacinth bean showed considerable variation in pod length varying from 3.96 cm to 18.20 cm. Pengelly and Maass (2001) also reported that the pod length in lablab bean ranged from 2.5 to 14 cm among 249 genotypes.

Pod width

The pod width among the genotypes differed significantly (Table 2). The combined analysis over the years showed that the widest pods were produced by LB-39 with an average of 10.14 mm and the narrowest pods by Makwanpur with an average of 8.35 mm. Neupane et al (2008) reported that the pod width in beans was influenced by the genotype. They recorded pod widths ranging from 10 to 30 mm in different genotypes which were planted on the same date. Islam et al (2010) also reported that the genotypes of hyacinth bean showed considerable variation in pod width that ranged from 1.5 cm to 4.46 cm. Pengelly and Maass (2001) reported that the pod width in lablab bean showed high variation among 249 genotypes.

Pod yield

The fresh pod yield among the genotypes differed significantly (Table 2). The combined analysis over the years showed that the highest fresh pod yield was produced by Four Season with an average of 25.75 t/ha followed by Makwanpur with an average of 24.89 t/ha. The lowest fresh pod yield was produced by LB-39 with an average of 15.93 t/ha followed by Chinese Long with an average of 16.42 t/ha. The results revealed that the fresh pod yield was influenced by the genotype. Similar results were also reported by other researchers. Neupane et al (2008) reported that the pod and dry seed yield in beans was influenced by the genotype. They found that all the genotypes planted on the same date produced varying pod number/plant and seed yield (g/m^2) ranging from 5 to 32 and 5.9 to 306.5, respectively. Smittle (1986) reported that the genotypes of lima bean showed considerable variation in pod yield. Ndegwa et al (2001) reported that the fresh pod yield of snap bean was influenced by the genotype ranging from 5443 to 10636 kg/ha.

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Assessment of Genetic Diversity in Acid Lime (*Citrus aurantifolia*) Landraces Grown at Different Agro-ecological Zones of Nepal, using SSR Markers

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ABSTRACT

Present study has been undertaken to assess genetic diversity of acid lime landraces at three different agro-ecological regions of eastern Nepal. Twelve simple sequence repeat (SSR) loci were used to determine the genetic diversity of 62 acid lime landraces collected from high hills, mid hills and terai agro-ecological zones of eastern Nepal. The average number of allele was detected 2.91 in terai accessions followed by 2.82 and 2.73 in high hill and mid hill accessions respectively. Proportion of polymorphic allele was found 100 percent in terai and high hill. The average polymorphic information content (PIC) value was observed higher in terai (0.53) as compared to mid hills (0.50) and high hills (0.49). UPGMA clustering based on Jaccard's similarity matrix grouped 62 accessions into five main clusters. The average genetic similarity index among the acid lime accessions was observed to be 0.80, the lowest similarity index being 0.77 observed in terai and highest of 0.82 in the mid hills. The result of this study shows that, moderate genetic diversity of acid lime accessions was found in the terai as compared to mid and high hills of Nepal. SSR markers are highly polymorphic and more informative for the genetic diversity assessment and germplasm characterization. The results of this study will be highly beneficial for acid lime breeding program and development of elite variety for commercialization.

Key words: Acid lime landraces, agro-ecological zone, alleles, genetic diversity, polymorphic, SSR

INTRODUCTION

Acid Lime (*Citrus aurantifolia* Swingle) is one of the important commercial fruit crops of Nepal, which is being cultivated in 60 out of 75 districts of Nepal (NCRP 2006). The cultivation of acid lime fruits is scattered in different altitudinal range in Nepal from terai to high hills and from east to west. It is considered as a high value commodity and has been given number one priority by Master Plan for Horticulture Development (MPHD 1990). The total area under citrus cultivation is about 26,681 ha, out of which acid lime (*Citrus aurantifolia*) covers 4,183 ha (MoAC 2008). Within the citrus fruit species, acid lime comes under third position after Mandarin and Sweet orange in terms of area and production. The total production of acid lime in Nepal was recorded as 20,492 tons per year, with productivity 8.4 tons/ha (MoAC 2008). In Nepal, Eastern Development Region occupies the highest position in terms of area (1545 ha) and production (7,987 mt) of acid lime. The fruit crop is used as juice, desert, pickle and for medicinal purposes.

Nepal is the home land of citrus species because of the wide availability and cultivation of wild citrus species (Verma 1994), however limited work has been carried out on the evaluation and characterization of citrus genetic materials. Assessment of genetic diversity within the acid lime landraces is the basis for breeding, variety development and conservation of genetic resources. Traditionally, evaluation of genetic diversity and characterization of varieties have been based on morpho-physiological traits such as color, size and maturity period, which are adapted by most of the *Citrus* breeders. In recent years, development of DNA marker technology has provided efficient tools to evaluate and measure the genetic diversity and cultivar identification (Ni et al 2002).

Molecular markers such as isozymes, RFLP and RAPDs have been applied to study genetic diversity, taxonomy, cultivar identification (Fang et al 1997, Filho et al 1998, Novelli et al (2000), and the construction of genetic linkage maps (Kijas et al 1997, Sanker and Moore 2001) in various *Citrus* species. During the last decade microsatellite or simple sequence repeats (SSRs) based markers have been commonly used in genetic mapping (Kijas et al 1995), genetic diversity study (Herrero et al 1996), and phylogenetic construction (Federici et al 1998). They are regarded as the most reliable marker because they are highly polymorphic and usually co-dominant (Brown et al 1996, Hokanson et al 1998, Liu et al 2003), easy to use, evenly distributed in the genome, transferable between laboratories and also not influenced by environmental conditions (Gupta et al 1996). It is short sequence elements composed of tandem repeat units of one to seven base pairs (bp) in length (Tautz 1989). These repeat sequences have been shown to be polymorphic within the species and highly applicable as molecular markers in population genetics and genome mapping. They have been used in the genetic diversity studies in many plants including citrus (Nunes et al 2002). In citrus and related genera, microsatellites have also been used for phylogenetic studies (Fang and Rose 1997, Pang et al 2003) assessment of genetic variability (Kijas et al 1995, Koehler-Santos et al 2003) and identification of zygotic plants (Ruiz et al 2000, Cristofani et al 2001).

Acid lime is cross-pollinated crop and has wide sexual compatibility with other *Citrus* species and related genera. It also has a high frequency of bud mutations and a high level of genetic erosion (Scora 1988) which results in decreased production and quality of fruits every generation. In this context, the study of genetic diversity is very crucial for the selection of elite genotypes for breeding and variety development programs. The genetic variability of acid lime landraces at different agro ecological zones in Nepal has not been carried out in the past. Therefore, the present study has been undertaken for the assessment of genetic diversity of acid lime landraces in the eastern part of Nepal, using SSR marker technique for the selection of elite genotypes.

MATERIALS AND METHODS

Plant material

Study sites were selected on the basis of major production pockets of the districts, but major emphasis was given to the altitudinal variation. Secondary data and necessary information were collected from the District Agriculture Development Office of each site. About 20-21 lime trees based on the morphological and farmers' preferences were selected for study from each of the three agro-ecosites viz; terai (< 600 m asl), mid-hill (600-1200 m asl) and high-hills (> 1200 m asl). A total of 62 young leaf (6-8 weeks) samples were collected randomly from selected trees at different altitudes (Table 1). All the samples were collected from eastern part of Nepal and washed in clean water, then kept in silica gel and brought to Nepal Academy of Science and Technology (NAST), Molecular Biotechnology Laboratory, Khumaltar for DNA extraction and subsequent molecular study.

DNA isolation

Leaf samples (100 mg) were ground to a fine powder in liquid nitrogen. DNA was extracted by using DNeasy 96 plant DNA extraction kit (QIAGEN, www//qiagen.com). The extracted DNA (200 µl) was stored at -20°C until use. The quality and quantity of DNA were determined by Bio-photometer (Eppendorf Company, Germany).

Table 1. Altitudinal range, accession numbers and locality details of sample collection sites of acid lime landraces

Above 1200 m asl			600-1200 m asl			Less than 600 m asl		
Acc no	Altitude, m	VDC-Ward no	Acc no	Altitude, m	VDC-Ward no	Acc no	Altitude, m	VDC-Ward no
LT-1	1605	Okhre-8	LD-49	1185	Bodhe-1	LM-43	135	Sunpur-2
LT-17	1750	Fachmara-7	LKv-60	1285	Balara-1	LM-44	135	Sunpur-2
LT-18	1710	Fachmara-9	LKm-61	1285	Balara-1	LD-45	135	Sunpur-2
LT-15	1655	Fachmara-9	LKr-62	1285	Balara-1	LD-58	135	Sunpur-2
LD-50	1638	Rajarani-9	LD-48	1181	Bodhe-1	LS-34	128	Narsing-2
LT-8	1505	Okhre-8	LT-25	1180	Balara-1	LS-35	128	Narsing-4
LT-22	1505	Sudap-1	LT-26	1175	Balara-1	LS-36	128	Narsing-4
LT-9	1500	Okhre-5	LT-27	1175	Balara-1	LS-37	128	Narsing-4
LD-21	1485	Fachamara-1	LT-28	1175	Balara-1	LS-38	128	Narsing-4
LD-20	1410	Fachamara-8	LT-29	1175	Balara-1	LS-39	128	Narsing-4
LT-16	1405	Fachamara-7	LT-30	1175	Balara-1	LS-40	128	Narsing-4
LT-19	1350	Fachamara-7	LD-59	1175	Balara-1	LS-41	128	Narsing-4
LT-13	1315	Fachamara-7	LT-4	1155	Okhre-1	LM-42	128	Narsing-4
LT-12	1310	Fachamara-7	LT-5	1155	Okhre-3	LS-56	128	Narsing-4
LT-14	1308	Fachamara-7	LT-6	1150	Okhre-3	LM-57	128	Narsing-4
LD-23	1308	Sudap-7	LD-31	1150	Dhnk -3	LM-51	125	Pathari-2
LT-3	1305	Okhre-8	LT-7	1145	Okhre-2	LM-52	125	Pathari-2
LT-24	1290	Balehara-8	LT-10	1135	Okhre-3	LM-53	125	Pathari-2
LT-2	1285	Okhre-1	LT-11	1130	Okhre-3	LM-54	125	Pathari-2
LD-46	1278	Bodhe-2	LD-32	1130	Balhra-3	LM-55	125	Pathari-2
LD-47	1278	Bodhe-2	LD-33	1130	Balhra-1	-	-	-

LT, Lime Terhathum. LD, Lime Dhankuta. LM, Lime Morang. LS, Lime Sunsari. LKm, Lime madrasi. LKr, Lime Rampur. LKv, Lime Vanarasi, VDC, Village Development Committee. M, meter. asl, Above sea level.

PCR Amplification and SSR analysis

A total of twelve published SSR primer pairs for citrus species were used in the present study (Barkley et al 2006, Gollein et al 2005 and Jannati et al 2009). These primers were selected based on Polymorphic Information Content (PIC) values (Table 2). PCR amplification was performed in BIOER Thermal cycler (Bioer Technology Co. Ltd, China, Version 2001.1.0). The PCR reactions were performed in total volume 25 µl, containing 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 2.5 µl of 10 x *Taq* buffer [100 mM Tris-HCl, pH 8.8 at 25°C, 500 mM KCl 0.8% (v/v), Nonidet P40]; 0.6 U *Taq* DNA polymerase (Fermentas, Life science; 5u/µl), 0.8 pmol of each primer (Eurofins Genomic Test Pvt Ltd, Banglor, India) and 0.8 ng of template DNA. The PCR program consisted of initial denaturation at 94°C for 5 min; 32 cycles at 94°C for 1 min; followed by annealing at 60°C for 30 sec; extension at 72°C for 1 min and final elongation at 72°C for 4 min. The PCR products were separated on 3% agarose gel (Embi Tec. San Diego, CA) and stained with Ethidium bromide (10 mg/mL Promegha) for 35 min and de-stained in distilled water for 15 min. The DNA fragments were photographed and documented using Gel doc system (Syngene UK). Electrophoresis was conducted in 1 x TAE buffer (Tris, acetic acid and EDTA buffer) at 100 volt for 45 minutes (9.0 V/cm).

Data analysis

Bands amplified by each of the primer pairs for all 62 accessions of acid lime were scored as present (1) or absent (0) and beanery data matrix was created for diversity analysis. Polymorphic information content (PIC) at each locus was calculated according to the formula (Nei 1972).

$$PIC = 1 - \sum_{j=i}^{n} (Pij)^2$$

Where, Pi is frequency of the *j*th pattern for marker *i* and the summation extends over *n* patterns.

The similarity matrix was calculated by using Jaccards coefficient (Jaccard 1908). The dendogram based on the UPGMA (Unweighted Pair Group Method with Arithmetic Average) algothrim was generated using SAHM clustering module of clustering NTSYS-pc (Numerical taxonomy and multivariate analysis system) software (version 2.21c) package (Rohlf 1993).

Table 2. SSR primers details used in present study

Primer code	Primer sequence 5'-3' (F) and 5' – 3' (R)	Repeat motif	Allele no	Allele size	PIC value	Reference
TAA45	GCACCTTTTATACCTGACTCGG TTCAGCATTTGAGTTGGTTACG	TAA	10	132-171	0.95	Golein et al (2005)
TAA52	GATCTTGACTGAACTTAAAG ATGTATTGTGTTGATAACG	TAA	8	132-174	0.94	,,
TAA41	AGGTCTACATTGGCATTGTC ACATGCAGTGCTATAATGAATG	TAA	9	122-185	0.91	,,
TAA15	GAAAGGGTTACTTGACCAGGC CTTCCCAGCTGCACAAGC	TAA	9	141-204	0.89	,,
TAA3	AGAGAAGAAACATTTGCGGAGC GAGATGGGACTTGGTTCACACG	TAA	10	133-172	0.87	,,
CAT01	GCTTTCGATCCCTCCACATA GATCCCTACAATCCTTGGTCC	CAT / CTT	12	138-172	0.89	Jannati et al (2009)
CAC15	TAAATCTCCACTCTGCAAAAGC GATAGGAAGCGTCGTAGACCC	CAC	4	148-163	0.36	,,
TAA27	GGATGAAAAATGCTCAAAATG TAGTACCCACAGGGAAGAGAGC	TAA	10	158-230	0.85	,,
CT19	CGCCAAGCTTACCACTCACTAC GCCACGATTTGTAGGGGATAG	CT	14	117-171	0.84	,,
TC26	CTTCCTCTTGCGGAGTGTTC GAGGGAAAGCCCTAATCTCA	TC	7	93-119	0.83	,,
AG14	AAAGGGAAAGCCCTAATCTCA CTTCCTCTTGCGGAGTGTTC	GA	20	119-163	0.85	Barkley et al (2006)
GT03	GCCTTCTTGATTTACCGGAC TGCTCCGAACTTCATCATTG	GT	19	149-197	0.83	,,

RESULTS

SSR polymorphism in acid lime accessions

In total twelve SSR primer pairs were used to profile 62 accessions, among which eleven pairs produced amplification products. The total number of alleles amplified by the eleven primer pair was 33, with an average of 3 alleles per locus. The highest number of alleles detected was 4, at TAA45, TAA41 and CAT01 loci and lowest of 2 was detected at TC26, AG14 and GT03 loci. The average PIC value was 0.50, ranging from 0.18 to 0.75. The highest PIC value was 0.75 at CAT01 locus and lowest was 0.18 at GT03 locus. Approximate size of the allele amplified by the eleven primer pairs ranged from minimum of 50 to maximum of 225 bp (Table 3).

Genetic variation was observed within the acid lime landraces according to the agro-ecological zones. A little variation was observed in the total number of alleles which were 32, 31 and 30 for terai, high hill and mid hill, respectively (Table 3). All the tested SSR loci were found polymorphic for the lime accessions in terai and high-hills, except GT03 locus which was found monomorphic for the accessions in mid hill. Small variation was observed in average number of allele per locus. Variation in the average polymorphic information content (PIC) value was observed as 0.49 in high hills, 0.50 in mid hills and 0.53 in terai. Among the three agro-ecological zones, PIC values were 0.25 to 0.75 in terai, 0.23 to 0.75 in high hills and 0.40 to 0.75 in mid hill accessions. Highest PIC value 0.76 was observed in CAT01 locus in terai accessions (Table 3). CAT01 locus amplified remarkable bands in all accessions. Amplification of double and triple bands was also visible in the gel lane in many accessions by different primers (Figure 2).

Table 3. Genetic diversity parameters (number of alleles per locus and PIC values) of acid lime landraces at different altitudinal range

SN	Locus	All accessions (n = 62)			>1200 m asl (n = 21)		600-1200 m asl (n = 21)		< 600 m asl (n = 20)	
		Alleles /locus	Allele Size (bp)	PIC	Allele /locus	PIC	Allele /locus	PIC	Allele /locus	PIC
1	TAA45	4	80-150	0.65	3	0.55	4	0.62	4	0.71
2	TAA41	4	50-170	0.63	3	0.56	4	0.65	4	0.63
3	TAA15	3	160-200	0.51	3	0.52	2	0.49	3	0.52
4	TAA3	3	130-170	0.62	3	0.54	3	0.62	3	0.67
5	CAT01	4	60-180	0.75	4	0.74	4	0.75	4	0.76
6	CAC15	3	180-225	0.43	3	0.34	3	0.42	3	0.48
7	TAA27	3	70-125	0.62	3	0.57	3	0.61	3	0.58
8	CT19	3	60-155	0.34	3	0.52	2	0.40	2	0.26
9	TC26	2	125-145	0.49	2	0.48	2	0.50	2	0.51
10	AG14	2	130-160	0.36	2	0.38	2	0.42	2	0.49
11	GT03	2	120-175	0.18	2	0.23	1	0.0	2	0.25
	Total	33	-	5.58	31	5.43	30	5.48	32	5.85
	Average	3	-	0.51	2.82	0.49	2.73	0.50	2.91	0.53

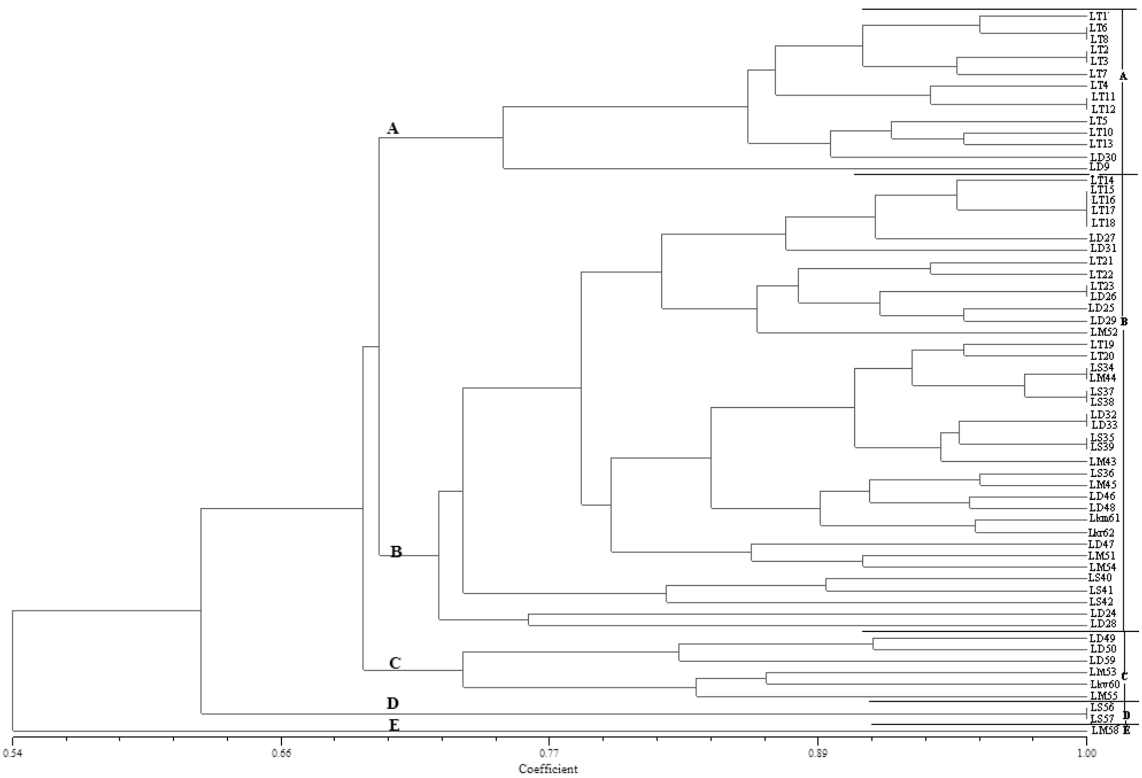
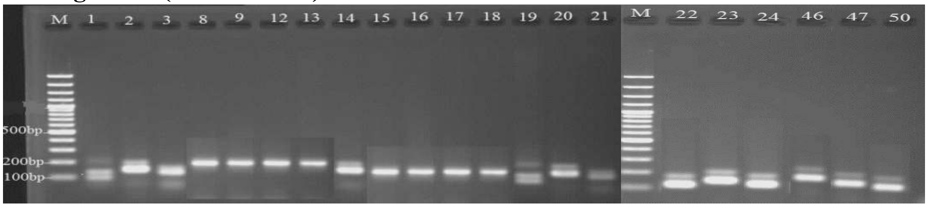


Figure 1. UPGMA dendrogram derived from similarity matrix of Jaccard's coefficient, demonstrating the genetic relationships among 62 acid lime landraces, based on 11 microsatellite markers.

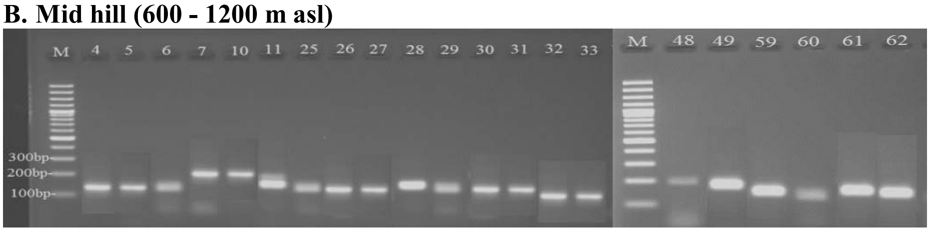
Genetic diversity and relationship among the acid lime accessions

Based on the dendrogram and Jaccard's coefficient similarity level, narrow genetic relationship was observed within 62 acid lime accessions. Total accessions were separated into five major cluster groups. Majority of the accessions (39) was grouped under the cluster B whereas single accessions (LM58) was in group E. Cluster group A consists of 14 accessions and group C consists of 6 accessions where as cluster D consists of only two (LM56 and LM57) accessions (Figure 1). There was small genetic variation between the cluster groups A, B and C (75%, 72% and 73%), whereas group E showed wide variation (54%). Average genetic similarity among the 62 lime accessions was found as77 percent, ranging from 54 to 100 percent.The cluster groups A, B, and C had narrow genetic relationship than of D and E. The result shows that, accessions LT2 and LT3, LT6 and LT8, and LT11 and LT12 are in equal genetic level in group A, where as LT15, LT16, LT17 and LT18, LT23 and LD26, LS34 and LM44, LS37 and LS38, LD32 and LD33, and LS35 and LS39 accessions are in equal genetic level in group B. The accessions LS56 and LS57 are also of same genetic level in group D where as LM58 with wide dissimilarity was in group E (Figure 1). These accessions were also studied for their genetic relationships within the agro-ecological zones. Among the three ecological zones the accessions from high and mid hills were found closely related with a high average genetic similarity (73% and 81%) as compared to terai (69%).

A. High hills (> 1200 m asl)



B. Mid hill (600 - 1200 m asl)



C. Terai (< 600 m asl)

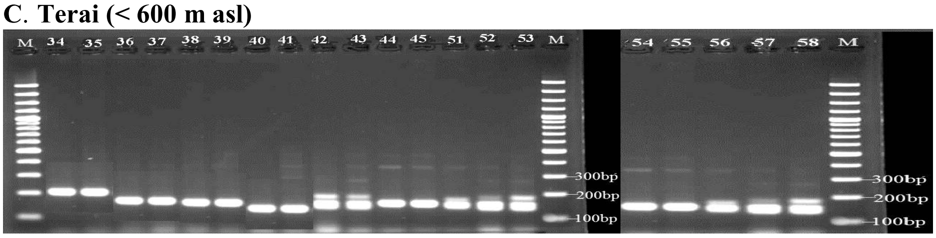


Figure 2. SSR Profile generated for 62 acid lime landraces CAT01 primer pair. Lanes marked M are 100bp plus molecular marker. A represent high hill, B represent mid hill and C represent terai accessions.

Two-dimensional plots of the principal component analysis (PCA) classify the 62 acid lime accessions based on allelic variation (Figure 3). PCA also shows the remarkable variation among the accessions. The first principal component axis accounted 15.4 percent and the second accounted 12.2 percent of the total variation. Three PCA accounted 35.3 percent of the total variance (Table 4). The PCA exhibited genetic variation among the landraces in the study.

Table 4. Eigen vectors, total variance and cumulative variance of acid lime landraces

Characters	PC1	PC2	PC3
Eigen values	5.0900	4.0182	2.5446
Percentage of total variance	0.154	0.122	0.077
Percentage of cumulative variance	0.154	0.276	0.353

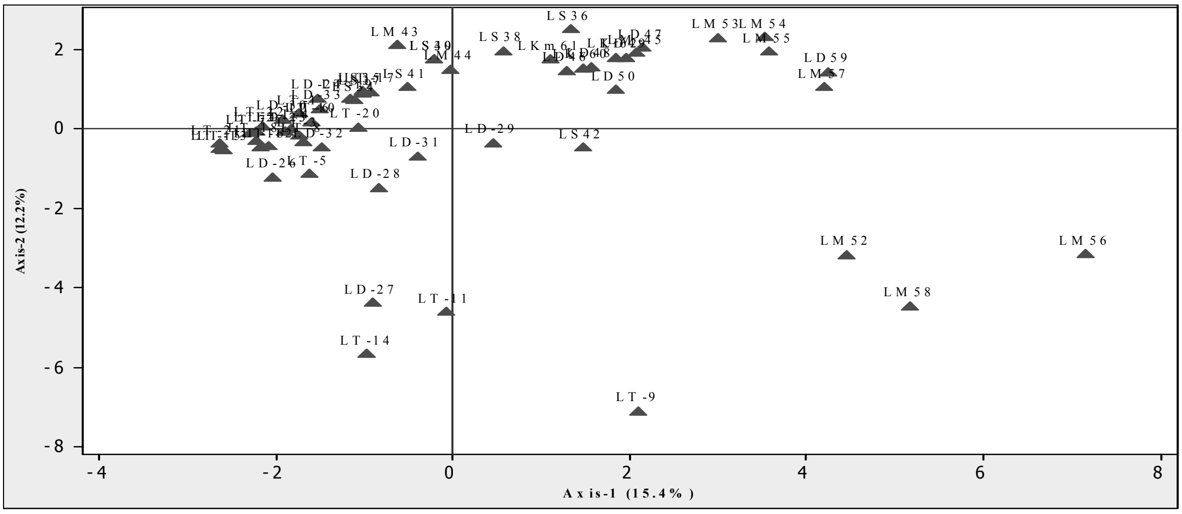


Figure 3. Scatter plot of first two components for 62 acid lime accessions showing 27.6% of total alleles variation measured at 11 polymorphic loci.

DISCUSSION

Normally three types of lime are grown in Nepal, namely acid lime, eureka Lime and natural hybrid types (Paudyal and Shrestha 2004). Among them acid lime is a key lime which is commonly known as Pahade Kagati or Sun Kagati in Nepali. It is an indigenous type found in mid hills from east to west of Nepal. Previously, all three types were cultivated in kitchen garden scale but now a days few farmers have started to grow acid lime in commercial scale.

Acid lime is a cross pollinated crop and large amount of genetic variation exists between the accessions. These variations can be potential sources for the selection of elite genotypes in breeding and variety development programs. Genetic diversity of acid lime landraces in the molecular level has not been studied in the past in Nepal. Therefore, twelve SSRs primer pairs were used for the study of genetic diversity of 62 acid lime landraces collected from the different agro ecological zones of eastern part of Nepal. Among the twelve primers eleven primer pairs were amplified computable bands. In the present study small allelic variation was observed according to agro ecological zones. Average number of alleles per locus provides complementary information of polymorphism and best to apply in co-dominant markers (IPGRI 2003). In Satsuma orange, Ghanbari et al (2009) reported that maximum 5 allele amplified by CAT01 and TAA14 primer pairs while minimum 2 amplified by TC26 primer. Li et al (2006) and Golein et al (2006) reported that two to nine alleles (average six) were observed in Mandarin and Lemon, amplified by the SSR markers. On the other hand Santos et al (2003) reported 32 fragments amplified by nine SSR markers when they characterized the Mandarin germplasm in Brazil; the number of bands varied from one to six per primer and the size ranged from 100 to 400 bps. Qing-Qin et al (2007) obtained 4.4 alleles per locus in citrus by SSR markers.

In this study the average gene diversity (PIC value) was observed higher in terai as compared to high and mid hills accessions. It means genetic variability of terai accessions was higher as compared with high and mid hills accession of acid lime landraces. In the present study, double and triple bands also amplified by the SSR primer pairs in few accession. These accessions may be the product of crossing with other citrus species (hybrid) in the previous generation, because most of the DNA samples were collected from the seedling trees. It is also reported that acid limes, which are apparent hybrids of citrons and papedas (Scora 1975, Nicolosi et al 2000) or a tri-hybrid cross of citron, pummelo and *Microcitrus* (Barrett and Rhodes 1976) and had the highest variability of all the taxonomic groups.

Jannati et al (2009) reported the SSRs marker detected considerable level of genetic variability within the acid lime landraces.

Based on the dendogram, acid lime landraces are separated in five major cluster groups and display the relationship between the accessions. The cluster group D and E were observed with wider level of genetic distance in the group, because these accessions are morphologically hybrid type and collected from the terai zone. In group C genetic similarity level was 73 percent and consisted 6 accessions, trees are similar with other landraces but have thicker peel and higher juice percent. In the cluster group A genetic similarity level was 75 percent, collected from high and mid hills and fruits have thin peel and are highly acidic. In addition to the cluster analysis, principal component analysis (PCA) was carried out to determine the genetic variation of acid lime landraces. The first three principal components with eigen value was greater than 35.3 percent of the total variance among the accessions. Therefore, PCA using the first two principal coordinate provide a good grouping of the accessions in the co-ordain system confirming the relationship with the dendogram.

CONCLUSION

Among the three agro ecological zones high genetic diversity was observed in terai landraces than mid and high hills. Maximum genetic variation (54%) was observed within 62 accessions from terai to high hill. It may due to the planting materials carried by the farmers from different hill districts during migration and introducing from neighboring country. On the other hand, less genetic variability was observed in the high and mid hills due to acid lime trees being established in natural condition. The trend of genetic variability of acid lime landraces was decreasing with increasing altitudinal range.

Identification of specific marker can be a useful and an efficient tool in the breeding program of citrus species. In this study, SSR marker proved highly polymorphic and more informative within the single species of acid lime landraces. Among the tested SSR primer pairs CAT01, TAA45, TAA41, TAA15 and TAA3 had high PIC values and were found more informative for diversity study in citrus species. The result of this study has provided valuable information to the breeders for efficient development of new variety of acid lime. On the basis of above results we can select economically viable genotypes of acid lime from the terai rather than mid and high hills.

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Evaluation of Some *Trichoderma* spp. for Clubroot Disease Management

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ABSTRACT

Clubroot disease caused by *Plasmodiophora brassicae* Woronin has been causing huge losses in cabbage and cauliflower production in Palung and Bajrabarahi areas of Makawanpur district. Three isolates of *Trichoderma* effective in seedling assay were evaluated during 2008/9 and 2009/10 at Palung and Bajrabarahi. The experiments were conducted in the farmers' infested fields using 'Rami' variety of cauliflower. *Trichoderma* isolates multiplied in rice husks were applied in planting holes at the rate of 25 gm during transplanting. In the first year of experiment, effect of only TS isolate was significantly different from untreated control and reduced clubroot severity and incidence by 47 percent and 32 percent, respectively. In the second year, the effects of T22, TS and T69 for disease severity were significantly different from the untreated control at p = 0.05 level and reduced disease severity by 37.8, 33.0 and 31.1 percent, respectively. In terms of clubroot incidence, only T69 (61.66%) was significantly different from untreated control (84.22%). It revealed that these *Trichoderma* spp. could be used as biological control agents for integrated management of clubroot disease in the mentioned locations.

Key words: Cauliflower, clubroot, incidence, severity, *Trichoderma*

INTRODUCTION

Cauliflower (*Brassica campestris* var. *botrytis*) and cabbage (*Brassica campestris* var. *capitata*) are important commercial winter vegetables of Nepal, which are being cultivated in large scale. For the last several years, clubroot disease caused by *Plasmodiophora brassicae* Woronin has appeared to be an economically important disease in some of the potential brassica vegetable cultivating areas, including Kathmandu valley and Tistung/Bajrabarahi/Palung/Daman areas of Makwanpur district. Tistung/Palung/Daman are located in between 1800 - 2050 meter above sea level (masl). In Makwanpur, area of cauliflower cultivation has reduced from 13830 ha in 2006 to 478 ha in 2008 (VDD 2006 and 2009) due to epidemics of the disease. Now the cauliflower crop is getting replaced by maize crop. Observations made in the past years showed increase in clubroot index resulted in yield loss in terms of both biomass and curd weight. It has been observed that this disease could cause yield loss of 27-81 percent in total biomass and 18-87 percent in curd yield of cauliflower and sometimes 100 percent crop loss. According to the growers of Palung, the production of cauliflower went down to 300 kg from 5-6 mt in one of the years (2003/2004) due to this disease (Timila et al 2008).

In general, the ecofriendly approach is drawing much attention in integrated disease management (IDM) strategy because of health and environment concerns towards using chemical pesticides. There are several reports on the use of biological control agents (BCAs) as a component of integrated disease management strategy for clubroot disease. Some of the BCAs reported for the management of clubroot are *Paecelomyces liliacenus* (Villanueva and Binaliw 1989); *Heteroconium chaetopspira* (Narisawa et al 2000); *Trichoderma* spp. (Cheah and Page 1997) and *Streptomyces* sp. (Cheah et al 2000). During 2005 and 2006, 18 isolates of *Trichoderma* were screened against clubroot disease under screenhouse conditions (seedling assay) at Khumaltar, Lalitpur, Nepal. Of them, three isolates, *T. harzianum* (T22), *Trichoderma* sp. (T69) and *Trichoderma* sp. (TS) were found significantly effective in reducing clubroot severity (Timila 2008).

For the verification of the efficacy of above-mentioned *Trichoderma* spp. under field conditions, experiments were conducted at Palung and Bajrabarahi of Makwanpur district.

MATERIALS AND METHODS

The field experiments were conducted in three and four farmers' fields infested with clubroot as replications at Palung and Bajrabarahi VDCs of Makawanpur district in 2008 and 2009, respectively. The fields having high incidence of clubroot in the previous season were selected.

***Trichoderma* spp. as treatments**

1. T22 (*Trichoderma harzianum*): Commercial isolate, isolated from PlantShield (Bio Works Inc. USA).
2. TS (*Trichoderma* sp.): Native isolate, isolated from clubroot infested field at Bhaktapur.
3. T69 (*Trichoderma* sp.): Native isolate, isolated from soil at Beluwa of Parsa.
4. Untreated control

The pure isolate was multiplied in sterilized rice husk with 10% rice bran, and used when rice husk was fully covered with green spores of the organism. Handful (25 g) of the rice husk was applied in planting holes with compost and mixed during transplanting. Each treatment received 21 plants per replication in randomized complete block design.

Seedlings of Rami variety of cauliflower were produced at Daman farm to assure infection free from clubroot pathogen. One month old seedlings from the date of seeding were transplanted in the last week of June, 2008 and third week of June, 2009. Row to row and plant to plant distances were maintained as 50 cm and 40 cm, respectively. Recommended dose of chemical fertilizers and compost as per farmers' practice were used. All other agronomical practices were followed as per farmers practice. pH of the soil of each field was recorded before transplanting. Clubroot severity and incidence were assessed uprooting each plant 7-8 weeks after transplanting. For disease severity assessment 1-6 scale was used with the following categorization. It was modified from 0-4 scale (Mitani et al 2003).

1 = no disease or club
2 = few clubs on lateral roots only
3 = clubs in lateral or tap root slightly clubbed
4 = well developed clubs in lateral or tap root but with lateral roots
5 = > 50% root converted to club with poor lateral roots, tap root clubs decayed or not
6 = plant death/ entire clubs decayed

Yield parameters such as biomass and marketable curd weight were not taken due to missing of the data in some replications. Data was processed and analyzed with MSTATC.

RESULTS AND DISCUSSION

The soil of the experimental fields was acidic with the pH values ranging from 4.6 to 5.8 (Table 1). Data presented in Table 2 shows that the clubroot severity and incidence were obviously reduced in *Trichoderma*-applied plots. However, only isolate TS was significantly different from the untreated control in 2008 and at par with T22 and T69. In the following year, all three isolates were significantly different from the untreated control and at par among themselves in their effectiveness.

Table 1. Altitude and pH values of the experimental farmers fields at Makwanpur district (2008)

Location	VDC	pH value	Altitude, masl
Karkichhap	Bajrabarahi - 5	5.7	1900
Phant Bazar	Palung – 6	5.1	1760
Phant Baza	Palung – 6	5.3	1760
Phant Bazar	Palung – 7	4.6	1850
Phant Bazar	Palung – 7	5.0	1850
Bajrabarahi	Bajrabarahi - 2	5.1	1680
Bajrabarahi	Bajrabarahi - 2	5.8	1680

VDC, Village Development Committee. masl, Metre above sea level.

Table 2. Effect of different *Trichoderma* spp. on mean clubroot severity (index) and the mean clubroot incidence (%) in cauliflower at Makwanpur district during 2008/9 (2065/66) and 2009/10 (2066/67)

	Treatments	Source	Mean clubroot severity index (1-6) scale		Mean clubroot incidence, %	
			2008	2009	2008	2009
1	T22	Plant Shield	2.55 ab	2.72 b	41.80 ab	63.74 ab
2	TS	Soil	2.15 b	2.67 b	35.93 b	64.93 ab
3	T69	Soil	2.45 ab	2.75 b	40.84 ab	61.66 b
4	Control	---------	4.09 a	3.99 a	68.23 a	84.22 a
	P value		O.0492	0.0010	0.0510	0.0492
	CV, %		12.48	4.96	19.61	19.44

Means followed by the same letter are not significantly different at P = 0.05 by Duncans Multiple Range Test.

During 2009, effect of all three *Trichoderma* spp. was significantly different from the untreated control and at par among themselves at (P = 0.05). In relation to incidence, only TS and T69 were significantly different from the untreated control in both the years. Among *Trichodermas,* their effects were at par in both the years. The reduction percent of clubroot severity and incidence in both the years were nearly similar (Figure 1 and 2). In all *Trichoderma*-applied plots, however, incidences were higher but lesser than in the untreated control. Concerning yield parameters, clubroot severity is more important than incidence, meaning higher the severity, lower the yield. Positive coorelation between clubroot severity index and curd yield/biomass was observed (Timila, unpublished data). Previously, the author had observed in one of the farmer fields during monitoring of clubroot disease that high disease severity could cause yield loss of 27-81 percent in total biomass yield and 18-87 percent in curd yield of cauliflower (Timila RD, unpublished data). Hundred percent crop losses were also common in some of the fields.

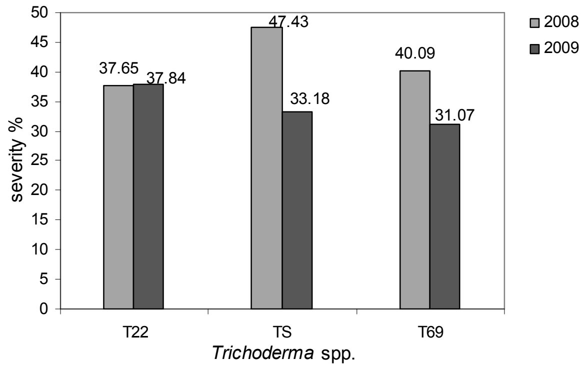

Figure 1. Reduction percent of clubroot severity by different *Trichoderma* spp.

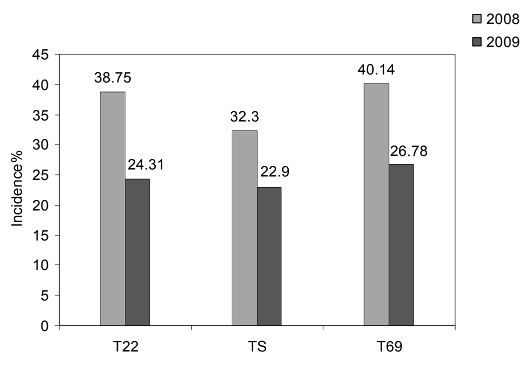

Figure 2. Reduction percent of clubroot incidence by different *Trichoderma* spp.

In both the years (2008 and 2009), TS isolate was found much effective in reducing clubroot severity compared to the other two isolates. Reduction percent of clubroot severity and incidence was lesser in the second year compared to the first year. Disease severity reduction in 2008 was 37-47 percent while in 2009 it was 31 to 37 percent (Figure 1). Similarly, disease incidence reduced from 32 to 40 percent in 2008 and from 22 to 27 percent in 2009 (Figure 2). Less reduction in incidence in the second year could be due to higher resting spore density of the pathogen in the field because in one of the experimental fields, cauliflower crop was severely infected in the previous year. Almost 100 percent plants were observed to be infected.

The eco-friendly tactic is drawing much attention in integrated disease management (IDM) strategy. However the success stories are limited mostly in the controlled conditions. Significant reduction of clubroot severity in Chinese cabbage by 17 isolates of *Trichoderma* has been reported by Cheah and Page (1997). But only three isolates were effective in reducing clubroot severity in cauliflower in the field (Cheah et al 2000). Similar types of results for *Trichoderma* spp. against clubroot were observed by different researchers, but very few were effective in the field conditions. In a previous field study, Timila and Shrestha (2000) also found reduction of clubroot incidence and severity by the use of wheat grains colonized with *T. harzianum* (isolated from Niprot, the commercial product) that was applied in planting holes during transplanting. The commercial product, Sanjevani (*T. viride*) is one of the biofungicides which appeared in the market. Budhathoki et al (2005) reported its promising effect in reducing 94% clubroot disease in cauliflower and cabbage. But farmer's perception revealed its inconsistent effects in their fields.

However, efficacy of BCA varies with the local environmental influence on their establishment and survival (Khetan 2001). Its effect could be influenced by soil moisture level, pH of the soil, and pathogen density, the resting spore density present in the soil etc. Its degree of effectiveness may be varied from field to field and also from location to location. At low resting spore density, another BCA, *Heteroconium chaetospira* the endophytic fungus could be a very good for clubroot disease if the crop is grown under suitable drainage conditions as mentioned by Narisawa et al (2005). It may be applicable for *Trichoderma* also. So, at high spore density *Trichoderma* may not give satisfactory level of control as observed in the present study. However, the mean effect of two years data shows all the three *Trichoderma* spp. were effective in reducing clubroot severity and incidence compared to untreated control (Table 3). In the screening of 16 isolates of *Trichoderma* spp. against clubroot disease, Timila (2008) found T22, T69 and TS significantly better in reducing clubroot severity compared to other isolates.

Table 3. Mean clubroot severity index and incidence percent of two years in different *Trichoderma* spp.

	Treatments	Severity index	Incidence, %
1	T22	2.63	52.77
2	TS	2.41	50.43
3	T69	2.60	51.25
4	Control	4.04	76.21

However, there are effective chemicals identified such as Nebijin for the control of the disease. Other management measures are transplanting seedlings from disease free areas, maintaining good drainage system, raising the pH of the soil up to 7.2, and rotating susceptible crops with non-cruciferous crops. Absence of cruciferous crops for 6-7 years in the field might help in reducing the disease significantly by preventing buildup of inoculum of *P. brassicae* (Toit 1990). Such a long period rotation is not practicable for those farmers with small land holdings and the farmers are compelled to cultivate brassica vegetables intensively for their livelihood. Also there is no other potential fungicide available yet, that would be acceptable on an economical and ecological basis (Ludwig-Mullar 1999). Hence, despite all those measures, there are no sustainable management alternatives available yet. In such conditions, use of *Trichoderma* could be one of the options. Composting can be well enhanced and improved by *Trichoderma* (Khadge 2003). Since farmers in Nepal commonly use composts, composting with *Trichoderma* could be more effective in suppressing clubroot disease. Their effectiveness under diverse environmental conditions could be enhanced by the use of other components or compatible chemical fungicide in mixture or in alternation as revealed by Shtienberg and Elad (1996). This will help in minimizing chemical fungicide use if it has to be applied. In case of clubroot disease, use of *Trichoderma* in combination with compatible chemical is still a field of research.

With the results of those two years experiments, it can be concluded that T22, TS and T69 could be used as BCAs as one of the components of integrated disease management for clubroot disease in Palung/ Bajrabarahi of Makwanpur conditions.

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Mushrooms Used in Ceremonies of Newar Community (Ethnomycology) in Kathmandu Valley: New Record for Nepal

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ABSTRACT

Three species of fungi [*Stereum hirsutum* (Willd.: Fr.) Fr.; *S. ostrea* (Blume & Nees) Fr. and *Schizophyllum commune* Fr. :Fr.] were gathered during the market survey of Lalitpur district, Kathmandu valley, which were used by Newar community in special ceremony. In addition to those, *Stereum rugosum* (Pers.:Fr.) Fr. and *Stereum striatum* (Fr.) Fr. were also found deposited previously in Natural History Museum. Among them, *Stereum striatum* (Fr.) Fr. is recorded to be new record to Nepalese mycoflora.

Key words: Ethnomycology, Kathmandu market, *Schizophyllum*, *Stereum*

INTRODUCTION

Nepalese literature

The ethnomycological knowledge and the utilization of mycodiversity can be seen in following papers viz Adhikari and Adhikari 1996-1997 and 1999, Adhikari et al 1996, Adhikari 1987, 1988, 1995, 1996, 2000, 2004, 2008abc and 2009ab, Adhikari et al 1992, Adhikari and Devkota 2007, Adhikari et al 2005, Adhikari and Durrieu 1996, Adhikari et al 2003, Adhikari and Pokharel 1999, Balfour-Browne 1955 and 1968, Berkeley 1854abcd, Bhandary 1991, Bills and Cotter 1989, Christensen and Larsen 2005, Christensen et al 2008a, Christensen et al 2008b, Giri and Rana 2007 and 2008, Giri et al 2005, Hattori et al 2002, Hjortstam and Ryvarden 1984, Joshi and Joshi 1999, Kharel and Rajbhandary 2005, Kuo 2008ab, Pandey 1971-1976, Pandey and Budathoki 2002, Pandey and Budathoki 2007, Pandey et al 2007, Parajuli et al 1999, Rana and Giri 2008, Ryvarden 1977, Sacherer 1979, Schroeder and Guzman 1981 and Spoon 2008.

Survey

During the course of mycological foray in and around the Kathmandu valley, the junior author (KS) came across the dried wild mushrooms packed in plastic bag and sold open in the market of Mangal Bazaar, Lalitpur. It was found that these mushrooms were sold whole round the year. The study revealed that the market packed plastic bag contained three species [*Stereum hirsutum* (Willd.: Fr.) Fr.; *S. ostrea* (Blume & Nees) Fr and *Schizophyllum commune* Fr. :Fr.]. These species are cited as inedible mushroom by various literatures (Phillips 2006, Courtecuisse and Duhem 1994, Arora 1986, Lincoff 1981, Imazeki and Hongo 1979 and Lange and Hora 1963).

Reported species

Till now eight species of *Stereum* have been reported from the world. Among these, the previous records of *Stereum* species reported from Nepal are viz *Stereum endocrocium* Berk. from Nangki (Berkeley, (1854); *S. ostrea* (Blume & Nees) Fr. on rotten log, from Khandabari (Balfour-Browne 1968), *S. gausapatum* (Fr.) Fr. from Godavary (Pandey 1976) (Fig. 6), *S. hirsutum* (Willd.: Fr.) Fr. on rotten log from Hatier, Chipli, Kasuwa Khola, Milke Danda, Sanghu, Simbrung (Balfour-Browne 1968) and Phulchowki (Singh and Nisha 1976), *S. rugosum* (Pers.: Fr.) Fr. from Godavary (Pandey 1976), *S. sanguinolentum* (Alb. & Schw.: Fr.) Fr. on branches, from Gurjakhani (Balfour-Browne 1968), Godavary (Pandey 1976) and Kuldi (Hjort. and Ryv. 1984) and *Stereum* sp. on rotten tree trunk from Phulchowki (Singh and Nisha 1976), Bajrabarahi, Godavary, Kakani and Nagarjoon (Pandey 1976). *S. endocrocium* Berk. is said to be endemic to Nepal (Adhikari 2009) till now.

Schizophyllum commune Fr.: Fr. has been previously recorded from Ranipauwa, Kaligandaki (Balfour-Browne 1968), Bagarchhap (Bhandary 1991), Pokhara (Adhikari 1996, Adhikari et al 1996), from Langtang (Pandey and Budhathoki 2006), on dead wood and branches of sissoo, Gamaharia–Maheshpur (Parajuli et al 1999), and Dakshinkali, Nagarkot, Sundarijal and Langtang (Pandey et al 2006).

Ethnomycological findings

This is the new report on the ethnomycologcial aspects of these species [*Stereum hirsutum* (Willd.: Fr.) Gray, *Stereum ostrea* (Blume. & Nees.) Fr., *Stereum rugosum* Pers.: Fr., *Stereum striatum* (Fr.) Fr. and *Schizophyllum commune* Fr.: Fr.] from Nepal. It is still believed that besides these three species *Stereum sanguinolentum* (Alb. & Schw.: Fr.) Fr. and *Stereum gausapatum* (Fr.) Fr. are also mixed and sold in the market. In appearance as they become blackish brown after drying and mixed together, they are called Haku (= black) mhhukan (= mushroom). *Schizophyllum commune* Fr.: Fr. (with split gills) has been reported used by Magar and Newar communities (Adhikari 2000), while the other two species of *Stereum* (hymenial surface is smooth and without pore) was utilized by Newar community during the grand celebration and auspicious ceremonies (viz worship of lord Laxmi, weddings, wearing of sacred fibre, bhai tihar, Tika offering ceremonies, nakhatya and others). But none of the rest authors has published on the utilization aspects of these fungi.

These species are cooked as vegetable for eating. While cooking curries, they mix these species with five other vegetables, which is called “Pancha gole” (= mixture of five things). The ingredients are legumes, cheeps of dried radish, potato, bamboo shoots, etc. They cook mixed with spices like onion, garlic, dust of ginger, coriander, foenicum, black pepper and other spices. The basidiocarp of *Stereum* is hard and woody. Before cooking the mushroom is kept in boiled water mixed with common salt for few minutes. Then the water from mushroom is rinsed-off and washed thoroughly with cold water till the greasiness of the mushroom is driven-out. The quantity of mushroom mixed is very low (about 5 to 10%). In our opinion, it is supposed that the toxic or hallucinogenic activity or properties present in the mushroom is either driven out or reduced to very low effect. Moreover, the citric acid in the bamboo shoots also helps to detoxify the amount of toxic substance present. Cooking makes the mushroom soft, cartilaginous and tasty. The cost of these mixed mushrooms were said to be Rs 4000/kg.

Besides these [*Stereum hirsutum* (Willd.:Fr.) Gray, *Stereum ostrea* (Blume. & Nees.) Fr., *Stereum rugosum* Pers.: Fr., *Stereum striatum* (Fr.) Fr. and *Schizophyllum commune* Fr.: Fr.] fungi, the taxon *Stereum striatum* (Fr.) Fr has been recorded for the first time as new record to the Nepalese mycoflora, which was deposited previously in Natural History Museum, Swayambhu (NHM). The taxonomic descriptions of these Nepalese mycoflora were not provided before.

Ethnomycologically none of the species discussed below was reported previously used by Newar communities in Nepal. The specimens are deposited in Natural History Museum (NHM), Swayambhu, Kathmandu, Nepal.

Enumeration of species

Characters of *Stereum*

Basidiocarp, effuso-reflexed to resupinate, zonated, hard, woody. Upper surface hairy to glabrous in age. Hymenium smooth, white to gray to gray brown, pale brown to reddish brown. Basidia one-celled, four-spored. Spores elliptic to cylindrical, hyaline, thin-walled. Without setae.

Key to the *Stereum* species of present collections

A. Basidiocarp surface white to gray to gray brown. Not exudating red juice when cut ------------ B
B. Basidiocarp surface white to grayish or gray brown ---C

C. Basidiocarp surface white, covered with silky hairs, fan like to conical, with striate surface. Hymenial surface yellowish grey -- *Stereum striatum*

CC. Basidiocarp surface white to gray brown, covered with hairs, which fall off, effuso-refllexed, not conical, zoned. Hymenial surface wood brown after drying - *Stereum hirsutum*

BB. Basidiocarp hairy, dark brown. Hymenial surface yellow to gray brown to brown in age-------- --- *Stereum ostrea*

AA. Basidiocarp surface of various colors. Exudating red juice when cut --------*Stereum rugosum*

***Stereum hirsutum* (Willd.: Fr.) Gray** in Eyssortier & Roux, 2011. *Le guide des champignons France et Europe.* 1018; Phillips, 2006. *Mushrooms.* 316. (Fig. 2).
Stereum hirsutum (Willd. Fr.) Fr. in Courtecuisse & Duhem, 1994; *Guide des champignons de France et D'Europe.*138.
Stereum hirsutum (Willd.: Fr.) S. F. Gray in Lincoff, 1981. *The Audubon Society Field Guide to North American Mushroom.*518; Chaumeton *et al.,*1985. *Les Champignons de France.* 47; Imazeki, Otani & Hongo, 1988. *Fungi of Japan*. 517; Courtecuisse, 2001. *Photo guide des champignons de France et D'Europe.* 332; Gerhardi, 2001. in *Guide Vigot des Champignons.* 680.
Stereum hirsutum Fr in Imazeki & Hongo, 1979, *Coloured illustrations of fungi of Japan*. 134. Arora, 1986. *Mushrooms demystified*. 605; Lange & Hora, 1963. *Collin's guide to mushrooms & Toadstools.* 56.

Taxonomic rank: Formerly **t**his species belonged to the family Steriaceae in the order Corticiales of Basidiomycotina. Recently, it has been included in the order Russulales and the family Stereaceae.

Description: Basidiocarp 5-3 cm across, often fused together; fan-shaped, semicircular or irregular; densely velvety, hairy, or with appressed hairs, with concentric zones of texture and color; colors variable, ranging from light yellow to tan, brown, reddish brown, or buff, laterally attached, without a stem. Hymenial surface smooth, yellowish to yellow-brown darker grayish brown with age on drying. Spores 5-8 × 2-3.5 µm, smooth, cylindric or narrowly elliptical, amyloid. Spore print white, difficult to obtain.

Specimen examined: Gathered from market, Mangal Bazaar, Lalitpur, 2067/9/15, no. 2011102, MK Adhikari and KS Adhikari, sold at the rate of Rs 4000/kg).

Common name: Chin mmuhkan, Haku Mhhukan (Newari), Hairy curtain crust, Hairy parchment (English), Steree hirsute (French).

Edibility: Most of the literatures cite this species as inedible species.

Comments: In *Stereum hirsutum* although the color of basidiocarp varies from white to brown, it is recognized by its smooth wood brown hymenophore and hairy basidiocarp. The basidiocarp of *S. sanguinolentum* and *S. gausapatum* in fresh, when cut bleeds. *S. hirsutum* does not bleed. *S. hirsutum* is smaller and more frequently fused than *S. ostrea* and not as orange as *S. complicatum.* It does not bleed red latex when injured, like several other *Stereum* species do. *S. hirsutum* has antimicrobial activity.

Ecology: On dead wood of hardwoods, gregarious, fusing together laterally; causing a white rot of the heartwood.

Distribution: It is subtropical to temperate species. Common. Reported from North Africa, North America, Europe, Japan and Nepal.

***Stereum ostrea* (Blume. & Nees.) Fr.** in Blume & Nees, 1826; Fries, 1838; Saccardo, 1888; Phillips, *Mushrooms.* 316, 2006. (Fig. 1).

Stereum ostrea (Blume. & Nees.: Fr.) Fr. in Lincoff, *The Audubon Society Field Guide to North American Mushroom.* 497, 1981.

Arora (1986, 606 pp.) has treated *Stereum ostrea* as synonym of *Stereum fasciatum* (= *S. lobatum*). Lincoff (1981) has treated *S. fasciatum, S. lobatum* and *S. versicolor* as synonyms of *S. ostrea.*

Taxonomic rank: Formerly, this species belonged to the family Steriaceae in the order Corticiales of Basidiomycotina. Recently, it has been included in the order Russulales and the family Stereaceae.

Description: Basidiocarp 1-7 cm across, fan shaped, semicircular or irregularly kidney-shaped, leathery, densely velvety or hairy at first, but often smoother by maturity; with zones of brown, red, orange, yellowish, brown, and buff shades, zoned radially in colours from dark brown in texture, slightly hairy, near the base, out through oranges and chrome yellow, with a very light margin, sometimes green algae also colonizes the top, without a stipe. Hymrnium smooth, with wrinkles or bumps, colour from white to grayish or pale reddish brown, bearing basidiospores. Spores 5.5-7.5 × 2-3 µm, smooth, cylindric, amyloid. Spore print difficult to obtain.

Specimen examined: Gathered from market, Mangal Bazaar, Lalitpur, 2067/9/15, no. 2011101, NHM, MK Adhikari and KS Adhikari, sold at the rate of Rs 4000/kg.), Mangal Bazaar, Lalitpur, 2045/10/7 (no entry number, HRB), no 2011106, NHM, Adhikari.

Common name: False Turkey Tail, Golden Curtain Crust (English), Haku Mhhukan (Newari).

Edibility: Most of the literatures cite it as inedible species.

Comments: *Stereum ostrea* is more red. Common thin wood-rotting shelf fungus. It is distinguished from *Trametes versicolor* by having fine whitish pores underneath.

Stereum ostrea is inedible due to its tough, leathery texture. It is found to play important role to folk remedy (Adhikari et al 2005). Recently, some new compounds such as a sesquiterpene, three aromatic compounds and a known compound methyl 2,4-dihydroxy-6methylbenzoate were isolated from a culture broth of the fungus *Stereum* sp. The novel sesquiterpene was determined to be stereumone and the three new aromatic compounds were elucidated together with the known compound. The combination of these compounds showed evident nematicidal activity against nematode *Panagrellus redivivus*.

Ecology: On the dead wood of hardwoods; gregarious causing a white rot of the heartwood in spring, summer and winter.

Distribution: It is subtropical to temperate species. Common. Reported world-wide on dead wood, often in overlapping tiers (North Africa, North America, Europe, Australia, Japan and Nepal).

***Stereum rugosum* (Pers.: Fr.) Fr.,** in Eyssortier & Roux, 2011. *Le guide des champignons France et Europe.* 1018; *Neues Mag. Bot.* 1:110. 1794; Fries, *Syst. Mycol*. 1:439. 1821; Eriksson, Hjortstam &

Ryvarden, 1984, *The Corticiaceae of North Europe.*7:1429. *Stereum rugosum* (Pers.: Fr.) Fr., *Epicr.* p. 552, 1838; Phillips, 2006. (Figure 3).

Synonym: *Thelephora rugosa* (Pers.) Pers.; *Gymnoderma rugosum* (Pers.) Hoffm.: *Haematostereum rugosum* (Pers.: Fr.); *Corticium boltonii* Fr.; *Terana boltonii* (Fr.) Kuntze; *Corticium triviale* Speg., *Aleurodiscus triviale* (Speg.) Gresl.; *Terana trvialis* (Speg.) Kuntze 1891.

Taxonomic rank: Formerly, **t**his species belonged to the family Steriaceae in the order Corticiales of Basidiomycotina. Recently, it has been included in the order Russulales and the family Stereaceae.

Descriptions: Fruit body resupinate, effused reflexed, coriaceous to very hard, in orbicular patches, with narrow reflexed pileus, in dense imbricate clusters; undulate to lobate, often fused laterally, rarely more than 1 cm wide, tomentose, greyish, soon becoming glabrous and dark brown, finally black in narrow and sharp zones, margin white to pale ochraceous and rounded. Hymrnium smooth, tuberculate to undulating, pale ochraceous to buff, Hyphal system monomitic with simple-septate hyphae, thin-walled, branching frequent, cystidia of two kinds pseudocystidia thick-walled, hyaline to yellowish. Basidia clavate, spores cylindrical, slightly bent, thin-walled, smooth, amyloid.

Specimen examined: Gathered from market, Mangal Bazaar, Lalitpur, Adulterated with Haku Mhhukan, 2045/10/7 (no entry number, HRB), no 2011105, NHM, Adhikari.

Common name: Haku Mhhukan, (Newari).

Comments: *S. rugosum* is easily determined by its hard resupinate to subresupinate and orbicular fruit bodies. Perennial pileus becomes glabrous rather soon, pale yellowish brown in age, bleeding when cut or touched in fresh state, like *S. gausapatum* and *S. sanguinolentum* (the hymenium bruises to red upon injury).

Ecology: On deciduous wood of many kinds, on standing and dead trunks. A very common species.

Distributioin: North American, Alaskan, Asia including Nepal, northwestern Europe.

***Stereum striatum* (Fr.) Fr.** 1838 in Arora, 1986. *Mushrooms demystified*. 607; Lincoff, 1981. *The Audubon Society Field Guide to North American Mushroom. 497*. (Figure 4).

Synonyms: *S. sericeum.*

Taxonomic rank: Formerly, this species belonged to the family Steriaceae in the order Corticiales of Basidiomycotina. Recently, it has been included in the order Russulales and the family Stereaceae.

Description: Basidiocarp 0.5-1 cm wide, flat and round, fan-shaped, or sometimes conical with small umbo-like projection, some laterally fused, whitish, silvery, gray, sometimes concentrically zoned, covered with dry with long silky radiating hairs. Stipe rudimentary or absent. Flesh very thin and tough when fresh. Hymenial surface light buff to brown and whitish with age. Spores 6-8.5 × 2-3.5 µm. cylindrical, smooth, spore deposit white.

Specimen examined: Gathered from Mangal Bazaar, Lalitpur, 2047/10/7 HRB (No entry number), No 2011104, NHM, Adhikari.

New to Nepal.

Common name(s): Silky parchment (English), Haku Mhhukan (Newari)

Edibility: Not edible.
Comments: The silvery, silky-shiny caps are a distinctive combination. So, derivation of name Striat - means "finely furrowed" or "lined" (striate) to appearance of the upper surface.

Ecology: Habitat often fused laterally to form lines up to 10 cm long, in groups or masses on twigs and dead branches of hardwoods, especially hornbeam. All year round.

Distribution: Europe and widely distributed in central and northeastern North America.

Schizophyllum commune **Fr.: Fr.** in Eyssortier & Roux, 2011. *Le guide des champignons France et Europe.* 950; Eyssortier & Roux, 2011. *Le guide des champignons France et Europe.* 1018; Imazeki, Otani & Hongo, 1988. *Fungi of Japan*.30; Courtecuisse & Duhem, 1994 *Guide des champignons de France et D'Europe.*142; Courtecuisse, 2001 *Photo guide des champignons de France et D'Europe.* 381; Gerhardi, 2001 *Guide Vigot des Champignons. pg.* 224; Phillips, 2006. *Mushrooms.* 268. (Fig. 5).

Schizophyllum commune Fr. *Obs. Myc.* 1: 103. 1815; Lincoff, *The Audubon Society Field Guide to North American Mushroom.493*, 1981; Imazeki & Hongo, 1979, *Coloured illustrations of fungi of Japan*, 30; Lange & Hora, 1963. *Collin's guide to mushrooms & Toadstools.* 104; Arora, 1986. *Mushrooms demystified*. 590; Cooke, 1961. *Mycologia* 53(6): 575-599; Lindsey & Gilbertson, 1978. *J. Cramer: Vaduz*. 406 p; Watling & Gregory, 1989. *British Fungus Flora: Agarics and Boleti. Vol 6. Crepidotaceae and other pleurotoid agarics*.; Arora, 1986. p. 590; Jordan, 128; Miller: sp. 86.

Basionyms: *Agaricus alneus* L., 1755; *Agaricus alneus* Reichard, 1780; *Agaricus multifidus* Batsch,1786, Apus *alneus* (L.) Gray, 1821; *Merulius alneus* (L.) J.F. Gmel., 1792. *Merulius alneus* (Reichard) Schumach., 1803; *Merulius communis* (Fr.) Spirin & Zmitr., 2004; *Schizophyllum alneum* J. Schröt.,1889; *Schizophyllum alneum* (Reichard) Kuntze,1898; *Schizophyllum commune var. multifidum* (Batsch) Cooke, 1892; *Schizophyllum multifidum* (Batsch) Fr. 1875.

Taxonomic rank: Previously, it was placed under Polyporales. Recently it has been included in the order Agaaricales and the family Schizophyllaceae.

Description: Fruiting body leathery, fan-shaped bracket, 1-3.5 cm broad, ashy-grey to white when dry upper surface with dense hairy, light greyish-brown when moist, persistent, shriveling in dry weather, but reviving after rains. Stipe usually absent. Flesh thin, light grey to brown. Hymenial surface light grey consisting of well-spaced, longitudinally split gills. Spores 3-4 $\times$ 1-1.5 µm, cylindrical, smooth; spore print white.

Specimen examined: Gathered from market, Mangal Bazaar, Lalitpur, 2067/9/15, no. 2011103, MK Adhikari and KS Adhikari, sold at the rate of Rs 4000/kg); growing on *Shorea robusta* stump, Jugedi, Chitwan, 2047/8/29, (no entry number, HRB), no 2011107, NHM, Adhikari; Maruhity, Kathmandu, 2047/5/27 (no entry number, HRB), no 2011108, NHM, Adhikari; Place unknown, 6/7/1991 (no entry number, HRB), no 2011109 and 02850, NHM, Adhikari.

Common name: Mizu chyau, Kathe bagale chyau (Nepali), Chi mhhukan (Newari)), Split Gill. Sporocarp (English).

Edibility: Not edible as cited by most of literatures. Edible used by Magar and Newar ethnic casts.

Comments: Previously, recorded from Ranipauwa, Kaligandaki growing on decayed wood, (Balfour-Browne 1968), Bagarchhap (Bhandary 1991) and Pokhara (Adhikari 1996, Adhikari et al 1996). Common, inedible species. The genus name *Schizophyllum* literally means, "split leaves" referring to this character. This species possesses strong immune supporting activity and tumor retardation (Hobbs 1987 in Medicinal mushrooms. p 38). It contains schiziphyllan, which is a polysachharide active against bacterial diseases.

Ecology: It is a very common fungus growing everywhere on dead wood, branches, twigs and causes rot of woods and can also cause disease in human.

Distribution: Common species in tropical to temperate belts. Worldwide except Antartica.

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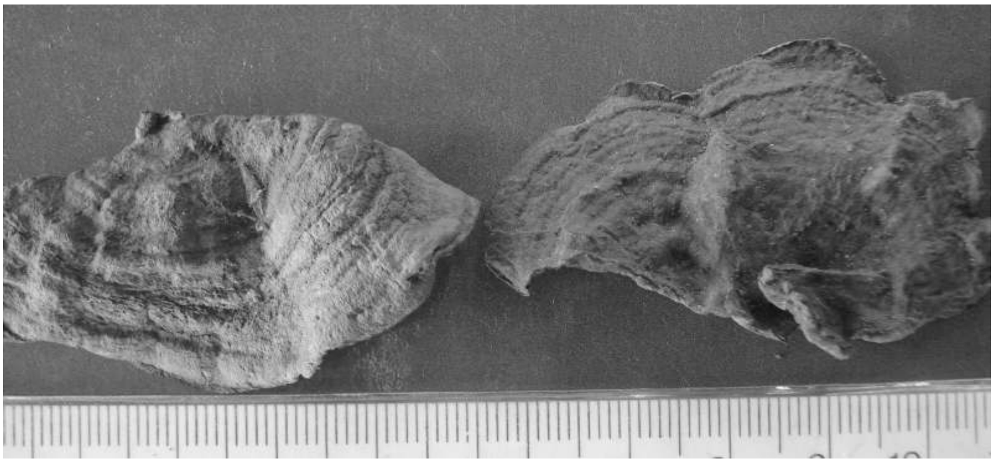

Figure 1. ***Stereum ostrea.***

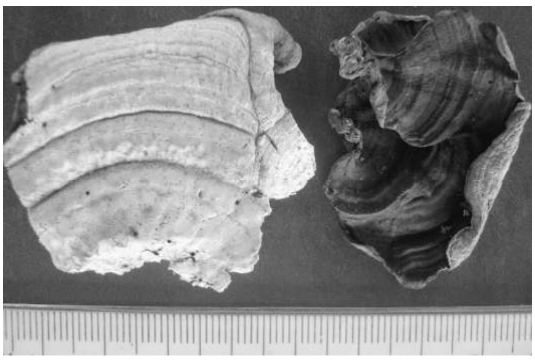

Figure 2. ***Stereum hirsutum.***

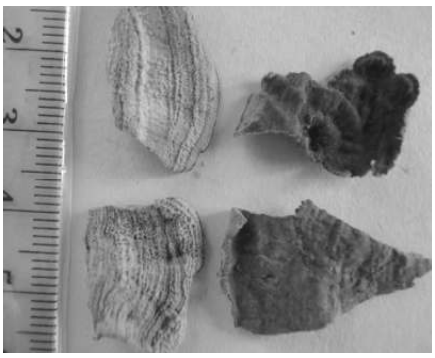

Figure 3. ***Stereum rugosum.***

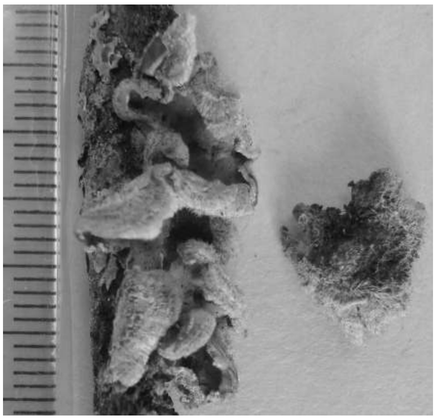

Figure 4. ***Stereum striatum.***

Figure 5. ***Schizophyllum commune.***

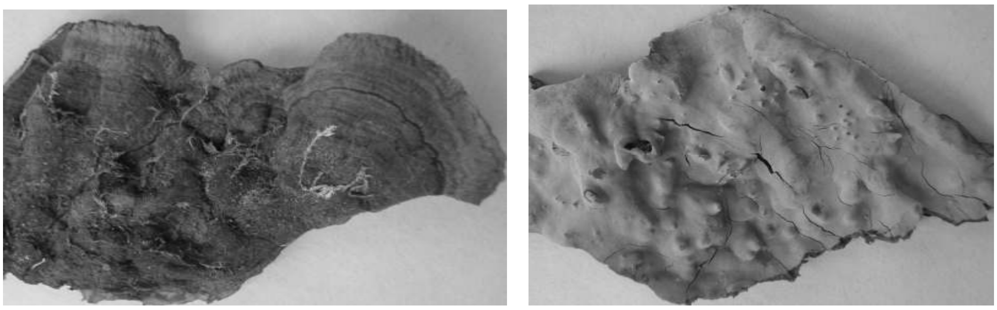

Figure 6. ***Stereum gausapatum upper and lower view.***

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RESEARCH NOTE

Chemical Management of Leaf Blight (*Bipolaris sorokiniana*) of Wheat in Central Terai of Nepal

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ABSTRACT

Foliar blight (*Bipolaris sorokiniana)* is one of the important diseases causing considerable yield loss of wheat in the terai region of Nepal. Fungicides such as Tilt (propiconazole), Polyram (metiram), Roko (thiophanate methyl) and neem products (Neem oil and Neem leaf extracts) were tested against this disease at the Regional Agricultural Research Station, Parwanipur during the year 2006/07. Area under disease progress curve (AUDPC), thousand kernel weight and percent seed infection by the pathogen were significant among the treatments. Tilt performed better in controlling the disease compared with other treatments. Neem products were not effective.

Key words: AUPDC, fungicides, leaf blight, wheat

INTRODUCTION

Commonly grown wheat (*Triticum aestivum*) varieties, UP 265, Nepal 297 and Gautam, in the terai of Nepal are heavily attacked by leaf blight disease (*Bipolaris sorokiniana*), causing considerable yield loss every year. Fungicide like Dithane M-45 (mancozeb) has been recommended as foliar spray to manage this disease. Murari and Singh (2005) reported complete inhibition of the fungus growth by Neemgold at 5 percent concentration. Dodan et al (2003) reported Tilt (propiconazole) as the most effective fungicides in reducing leaf blight incidence and increasing grain yield. Singh et al (2003) reported that Tilt alone or Econeem along with seed treatment with carboxin containing fungicide reduced leaf blight incidence and increased grain yield of wheat. This study was under taken to assess the efficacy of different fungicides and neem products in reducing leaf blight disease of wheat under the central terai conditions of Nepal.

MATERIALS AND METHODS

The experiment was carried out at agronomy block of Regional Agricultural Research Station, Parwanipur, Bara, Nepal (latitude 27^o 2’, longitude 84^o 53’ and elevation 115 m) during the year 2006/07. The plot size was 4- × 2.5-m. The seed of wheat cultivar Nepal-297 was shown on 6th December 2006 and harvested in the month of March 2007. Herbicide Pendimethylin 25 EC was sprayed @ 4 ml/lit water just after a day of wheat seeding. Plots were fertilized at the rate of 120:50:40 kg NPK/ha, of which 50 percent of nitrogen and all phosphorus and potash were applied as basal and remaining nitrogen was top-dressed just after first irrigation and at booting stage.

Three chemical fungicides: Tilt (propiconazole), Polyram (metiram), Roko (thiophanate methyl), and two neem products: Neem oil (commercial product) and Neem leaf extract (10 g of fresh neem leaf macerated in hundred ml of water and filtered thorough cleaned cloth) were included in the experiment. The experiment was designed in complete randomized block design with four replications. Fungicides and neem-based products were sprayed on 11th February, 22nd February and 6th March 2007. Two sprays of each fungicide were carried out on first two dates and three sprays were done on three dates mentioned above. The doses of fungicides used were as recommended by the manufacturers such as Polyram-70WP (Baff, India Limited), Tilt- 25% EC (Syngenta Company, India Limited) and Roko-70 WP. Disease was scored on 18th Feb, 28th Feb and 8th March. The disease scoring was done on five selected hills/plot. All leaves of the five randomly selected tillers of a selected hill were individually scored for percentage leaf blight infection. The average percentage leaf blight infection was calculated by adding percentage infection of all selected tillers of five hills divided by number of total leaves of all tillers of all selected five hills that were scored. The seed infection was determined a month after harvest at Plant Pathology Division, Khumaltar, Lalitpur. Area under disease progress curve (AUDPC) was calculated using Shanner and Finney (1998) method. Research data were analyzed using Computer-C Programme.

RESULTS AND DISCUSSION

The results showed that area under disease progress curve (AUDPC), thousand kernel weight (TKW) and percentage seed infection were statistically significant (Table 1). AUPDC was significantly lower in all chemical fungicides-treated plots compared with neem-treated and untreated control. The lowest AUPDC was in three times Tilt-sprayed plots (802), and the highest was in untreated control plot (990). Thousand kernel weight was also highest (48.5 g) in Tilt-sprayed plots followed by Polyram and Roko. Neem oil and neem leaf extracts had no effect on TWK when compared with untreated control. Also, seed infection by *B. sorokiniana* was lowest (19%) in three times Tilt-sprayed plots followed by Polyram and Roko. Neem products had no effect on seed infection. Grain yield was insignificant, however, the Tilt-treated plots gave highest grain yield of 3000 kg/ha compared with 2400 kg/ha in untreated control plots. The insignificant grain yield despite differences in the treatments could be due to poor plant stands in some of the plots because of rain just after wheat seeding. Plant height, ear length, straw yield, number of grain/ear, grain yield and harvest index were not significant (data not shown).

Table 1. Effect of fungicides and plant extracts on management of leaf blight of wheat in 2006/07

SN	Treatments	AUDPC	Yield, kg/ha	Thousand kernel weight, g	Seed infection, %
1	Tilt, -25% EC, 2 ml/lit water - two sprays	876.3 c	3049.5	47.9 ab	38.7 f
2	Tilt, 2 ml/lit water - three sprays	802.5 c	2927.5	48.5 a	19.0 g
3	Tilt, seed treatment (2 ml/kg seed) + one spray	892.8 bc	2969.3	45.9 bcdef	63.8 cde
4	Polyram -70WP, 4 g/lit water - two sprays	936.3 abc	2666.5	45.6 bcdef	54.5 e
5	Polyram, 4 g/lit water - three sprays	900.8 bc	2070.8	47.4 abc	54.0 e
6	Polyram, seed treatment (4 g/kg seed) + one spray	967.0 ab	2635.8	43.0 fgh	73.0 abc
7	Roko -70WP, 2 g/lit water - two sprays	1007 a	2413.5	46.2 abcd	59.8 d
8	Roko, 2 g/lit water - three sprays	964.0 ab	2613.8	45.8 abcde	59.5 d
9	Roko, seed treatement (2 g/kg seed) + one spray	961.8 ab	2594.3	44.8 bcdef	70.0 bcd
10	Neem oil, 5% - two sprays	986.8 a	2183.0	41.2 h	80.0 ab
11	Neem oil, 5% - three sprays	988.0 a	2321.8	41.6 gh	78.0 ab
12	Neem oil, seed treatment (5%) + one spray	933.0 abc	2308.0	42.6 efgh	76.8 ab
13	Neem leaf extract, 10% - two spray	839.0 c	2416.0	44.7 bcdefg	75.3 ab
14	Neem leaf extract, 10% - three spray	967.0 ab	2207.8	43.8 defgh	80.3 ab
15	Neem leaf extract, seed treatment (10%) + one spray	969.5 ab	2716.3	43.8 defgh	81.5 ab
16	Untreated control (no spray)	989.8 a	2410.8	42.4 fgh	82.0 a
17	F-test	**	ns	* *	* *
18	CV, %	8.40	8.64	4.39	10.95

Figures marked with same letter(s) are not statistically significantly different at 0.01 or 0.05% as judged by DMRT.

Three sprays of Polyram @ 4 g/lit water was as good as three sprays of Tilt @ 2 ml/lit of water in respect to disease control. Two sprays of Neem leaf extract @ 10 g/lit of water was as good as three sprays of Tilt @ 2 ml/lit water in respect to disease control. Thousand kernel weight was the highest in three sprays of Tilt @ 2 ml/lit of water and was at par to two sprays of Tilt @ 2 ml/lit of water, three sprays of Polyram@ 4 g/lit of water, two and three sprays of Roko @ 2 g/lit of water. Thousand kernel weight were at par in case of others treatments. Seed infection was the significantly the lowest in case of three sprays of Tilt followed by two sprays of Tilt, two and three sprays of Polyram @ 4 g/lit of water. The grain yields were not significantly different as there was rain just after wheat sowing. The results clearly showed that among the tested fungicides Tilt was the most effective in controlling leaf blight disease of wheat. These results are in line with the findings of Dodan et al (2003) and Singh et al (2003) who found Tilt effective in reducing leaf blight of wheat severity and increasing grain yield. The results clearly showed that among the tested fungicides Tilt was the most effective in controlling leaf blight disease of wheat. These results are in line with the findings of Dodan et al (2003) and Singh et al (2003) who found Tilt effective in reducing leaf blight of wheat severity and increasing grain yield. Based on these results, application of Tilt fungicide @ 2 ml/lit of water at 10-15 days interval for 2-3 times can be recommended to wheat growers to protect their crop against leaf blight disease.

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