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RESEARCH ARTICLE

On-Farm Diversity Assessment and Participatory Varietal Evaluation of Cold-Tolerant Rice in Mid-Hills of Nepal

Ritesh Kumar Yadav[1*], Rita Gurung[2], Rajeev Dhakal[2], Achyut Raj Adhikari[2], Subash Gautam[2], Krishna Hari Ghimire[3], Bhuwon Ratna Sthapit[4†]

[1]*Faculty of Agriculture, Agriculture and Forestry University, Chitwan, Nepal*
[2]*Local Initiatives for Biodiversity, Research and Development, Pokhara, Nepal*
[3]*National Agriculture Genetic Resources Centre, NARC, Khumaltar, Nepal*
[4]*Bioversity International, Pokhara, Nepal*
[†]*Deceased 28 August, 2017*

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Abstract

Drawing upon consequentially growing food insecurity in mid-hills attributed to poor adoption levels *inter alia* inflicted by narrow range of locally adaptive farmer's preferred cold tolerant rice varietal options in national agricultural system, the present study was undertaken. We conducted *on farm* diversity assessment of 60 high altitude rice genotypes from *ex-situ* and *on farm* employing un-replicated diversity block in 2015. Subsequently, we identified eight promising locally adaptive genotypes as candidate genotypes based on the inferences of diversity block trial and evaluated them through participatory variety selection (PVS) using randomized block design in 2016 under on-farm conditions. Our studies revealed marked diversity among the Nepalese cold-tolerant rice genotypes. The UPGMA cluster analysis categorized the 60 genotypes into six distinct clusters. Strong positive correlation between grain yield and plant height; panicle length; straw yield and strong negative correlation between grain yield and 1000-grain weight was detected. PCA suggested traits viz., plant height, panicle length, days to 50% heading, and grain yield to be principal discriminatory characteristics of the cold-tolerant rice. Seto Kattike, Naulo Dhan, and Borang were most promising and adaptive genotypes whose eminence were justifiably corroborated and validated by farmer's overall evaluation. The most valued farmer's selection criteria were grain and straw yield, earliness, disease resistance, and stem borer tolerance. The findings bolster employment of novel and proven participatory plant breeding approach using diversity kits and IRD kits to expand and promote varietal choice options for immediate benefits to the farmers and facilitate rapid varietal release and registration.

Key words : Participatory varietal selection, cold-tolerant rice, diversity assessment, evaluation, Nepal

Introduction

Rice is one of the leading food crops in the world and stands in second position after wheat. It is the staple food crop of more than 60% of the world population. In Nepal, it is the principal cereal food crop and is grown in 1555,940 ha of land with the average productivity of 2.91 t/ha (AICC 2010). It nearly contributes about 20.75% to the agricultural gross domestic product (MOAC 2010) and provides more than 50% of the caloric requirements of the Nepalese people (NARC 2008). In Nepal, rice is grown in wide range of environments from flat lands in altitude less than 60 m with tropical climates to highest rice growing altitude of the world (3050 m) with temperate climate (ABD 2008). Most of the rice grown in mid to high hills of Nepal are generally cold-tolerant. However, the average productivity is comparatively very low to the average productivity of the world. Apart from numerous yield-limiting factors of rice, chilling temperature is a common production constraint in rice cultivation in mid to high hills, with potential to affect growth and development from germination to grain filling (Sthapit 1994). Rice crops are subject to chilling injury when grown at low but non-

Ritesh Kumar Yadav (✉)
Email: rkyadav@afu.edu.np

freezing temperatures in range of 10 to 20°C (IRRI 1979). Significant yield losses in rice production is also attributed to prevalence of leaf and neck blast, sheath brown rot, stem borer, Gundhi bug, poor irrigation, and nutrient management (LARC 1997). Chillig injury in rice is common in Nepal at high altitude areas of more than 1000 m above from the sea level (masl). About 26% of the rice in Nepal are grown in temperate areas of 1000-2000 masl (Sthapit 1992). Spikelet sterility due to chilling injury is a major problem above altitude of 1500 m above the sea level, restricting both the area of production and duration of crop maturity (Sthapit 1991; Sthapit et al. 1995). Very few cultivars *viz.*, Chhomrong, Machhapuchre-3, Chandannath-1, and Chandannath-3 and Palung-2 have been popular among the farmers as chilling tolerant cultivars. These cultivars have genetic background of Nepalese cold tolerance parentage (Supplementary Table 1). Conversely, many other released cold tolerant varieties are not adopted at all by the farmers principally due to lower yields, poor adaptability, and inadequacy to satisfy their needs. Yet, the adoption of the aforementioned popular chilling cultivars has been lagging in marginal environments and confined to few rice growing areas of Nepal. This anomaly in adoption trend may be attributed to indecency of the cultivars for extreme agro-ecological diversity, ethnic-specific preferences, functional production, and consumption requirements of the farmers dwelling in diverse environments, particularly, the resource-poor farmers delineating the dire need of identification, dissemination, registration, and release of location and purpose specific varieties. The growing food insecurity in various high-altitude regions of Nepal is the aftermath of poor adoption levels *inter alia* inflicted by narrow rice varietal selection options available to the farmers.

The integration of famer's participation in selection process and setting of selection criteria in breeding programs, i.e. participatory varietal selection approach has been the most effective and overriding approach to address the poor varietal adoption driven bulging food insecurity, juxtaposed with climate change by providing 'basket of choice' of locally adaptive farmer's preferred varieties, particularly in resource-poor farming communities. It is well documented that PVS approach has been promising and successful in identifying locally adaptive varieties of farmer's preferences, accelerating their wider dissemination and uptake, enhancing varietal diversity, scaling up seed production at community, and fostering rapid varietal release. (Assefa et al. 2005; Joshi and Witcombe 1996; Mulatu and Belete 2001; Mulatu and Zelleke 2002; Sthapit et al. 1996; Witcombe et al. 1996). In context of perturbing adoption levels of the released varieties and nominal varietal choice of robust and farmer preferred varieties, characterization, and participatory evaluation of genotypes from *ex-situ* and *on farm* in the end user's agro-ecology solicit earnest attention and undertaking as these valuable genetic resources with tremendous potentialities for breeding purpose and adaptation to local cultivation, have been poorly studied and utilized (Hodgkin et al. 2003). Characterization and participatory varietal evaluation of the germplasm from *ex-situ* and *on-farm*, as encompassed in the present study would bolster agro-biodiversity conservation through utilization of divergence in the germplasm and bridge the communication gap between farmers and breeders so that the farmer's concerns and preferences are well- addressed in formal breeding programs rendering enhanced adoption, swollen genetic base in community richness, and increased choices of robust and location specific varieties to farmers.

The present study is a part of GEF/UNEP funded project on Local Crops (www.himalayancrops.org) which focuses on sourcing local diversity that matches farmer's need through rapid detection, on farm-evaluation, participatory varietal selection, and dissemination of choice varieties to the local farmers. In the present study, we aimed to dissect the genetic divergence among 60 rice genotypes collected from *ex-situ* and *on farm*, identify and evaluate adaptable and high-yielding genotypes. In addition, the study also intended to identify the famer's preferred genotypes and their selection criteria via participatory varietal selection (PVS) approach for cold-tolerant rice in the study area. The information thus obtained would broaden the varietal selection choices to local farmers by providing set of promising farmer preferred varieties fostering agro-biodiversity and food security and complement the formal breeding system.

Materials and Methods

Diversity block

The material used in this study consisted a total of 60 genotypes (54 landraces and 6 released varieties) collected from different high altitude agro-ecology of Nepal (*ex-situ* and *on-farm*) as depicted in Supplementary Table 1. In the first year, we aimed to assess polymorphism among the rice germplasm and select high-yielding and cold-tolerant candidate genotypes for including them in on-farm yield trial directed to further evaluation and validation. An *on-farm* diversity block using un-replicated rod row design was laid out in Ghanpokhara village development committee (VDC)-1 (Bhache) of Lamjung district at N28˚17.440' latitude and E84˚19.289' longitude and altitude of 1565 masl in 2015. Diversity block is an experimental block of farmers' varieties managed by local institution for research and development purposes (Tiwari et al. 2006). The block is not only used for measuring and analyzing agro-morphological characteristics but also used to validate farmers' descriptors by inviting farmers to watch the diversity block in the field and determine whether farmers are consistent in naming and describing varieties. The i-button installed above experimental field estimated the mean precipitation during the crop season (May-November) to be 2112 mm and the minimum monthly air temperature during the crop season ranging from 14.2 to 17.4°C, which was quite ideal for screening of genotypes for chilling tolerance. Each genotype was grown in 1.2 m^2 plot of two rows with a distance of 20 cm between each row and 15 cm

Table 1. List of cold-tolerant rice genotypes included in yield trial along with their pedigree and source.

S.N.	Genotypes	Type/Parentage	Chilling tolerance	Source
1	Borang	Local landrace	Unknown	Lamjung
2	Lekali-1	Released variety; Banjaiman/Chhomrong)	Cold tolerant adapted in Jumla (2000m)	ABD*
3	Lekali-3	Released variety; Yunlen-5/Chhomrong	Cold tolerant adapted in Jumla (2000m)	ABD
4	Lumle-2	Promising variety; IR-36/Chhomrong	Cold tolerant adapted in Lumle (1500-1700m)	LI-BIRD**
5	Machhapuchre-3	Released variety; Fuji 102/Chhomrong	Cold tolerant adapted in Lumle (1500-2000m) (Joshi et al. 1997)	NAGRC^
6	Naulo Dhan	Local variety	Unknown	Lamjung
7	NR10695-2-2	Elite breeding line; Fuji 102/Yunlen 1	Cold tolerant	ABD
8	Seto Kattike	Local landrace	Unknown	Lamjung

*Agricultural Botany Division, Nepal Agricultural Research Council, Khumaltar, Nepal
^National Agriculture Genetic Resources Center, Nepal Agricultural Research Council, Khumaltar, Nepal
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between each plant. All agronomic and management practice were followed as per farmer's practice. Well rotten farmyard manure @ 6-8 t ha^{-1} was applied in the field during land preparation. No chemical fertilizer and pesticides were used. Two hand weedings were performed 30 and 60 days after transplanting. The data on panicle exsertion, a better indicator of cold tolerance (Cruz et al. 2008) and overall phenotypic performance was recorded as per the descriptors of *Oryza* species (Bioversity International, IRRI and WARDA 2007) while grain yield (g $plot^{-1}$), at harvest, was determined for each genotype and later extrapolated to $kgha^{-1}$ for statistical analysis.

The UPGMA cluster analysis based on Euclidean distance using JMP 13 was performed to categorize the entries into different clusters deeming traits such as yield performance, panicle exsertion and overall phenotypic performance into account. The candidacy of genotypes for inclusion in further evaluation studies were determined by visualizing the results of the Cluster analysis. The genotypes to be included in the yield trial were identified from clusters depicting preferable value of considered traits.

Yield trial

The plant material used in this study consisted a total of eight genotypes, of which six genotypes were identified and selected from diversity block trial for their eminence in terms of yield, overall phenotypic performance and panicle exsertion while two promising genotypes viz. Lumle-2 and NR10695-2-2 available in national agricultural research system were included (Table 1). In order to evaluate the agronomic performance of the identified candidate genotypes, *on farm* experiment using a randomized block design with three replicates was set up in Ghanpokhara VDC-1 of Lamjung district located at 28˚17.445' North latitude and 84˚19.271' East longitude and altitude of 1551 masl in 2016. The i-button installed over the experimental field revealed the mean precipitation during the crop season to be 2340 mm and the minimum monthly air temperature during the crop season ranging from 13.4 to 17.2°C. Each genotype was grown in 6 m^2 plot of 10 rows with a distance of 20 cm between each row and 15 cm between each plant. Local farmer's practice was followed to raise the crop as described earlier. The data were recorded for eight yield attributing traits such as days to 50% heading, days to 80% maturity, plant height (cm) at maturity, panicle length (cm) at maturity, grain yield (kg $plot^{-1}$), thousand grain weight (g), and straw yield at 12% moisture content (kg $plot^{-1}$) as per the descriptors of *Oryza* species. Data on grain and straw yield were extrapolated to $kgha^{-1}$ for statistical analysis.

Statistical analysis

UPGMA Cluster analysis of the 60 genotypes in diversity block trial based on Euclidean distance was performed using JMP 13 to assess the degree of divergence and relatedness among the germplasm and group the genotypes into distinct cluster by traits viz. yield, overall phenotypic performance and panicle exsertion. This was followed by computation of descriptive statistics of each trait for different clusters. The data of yield trial were subjected to analysis of variance under Randomized Block Design using statistical software R and the significance of differences between the means were compared using Least Significant Difference (LSD) at 5 percent level of significance. In addition to these, Pearson's correlation coefficients between each pairs of traits and principal component analysis (PCA) was conducted using statistical software R and Minitab 15.0 in order to estimate the extent of association among the traits, and relative importance and contribution of traits to the overall variation.

Participatory varietal evaluation

Qualitative data on eight cold tolerant rice genotypes included in the yield trial was collected at maturity stage deploying participatory approach of direct matrix and pairwise ranking methods. A total of 22 local famers (12 male + 10 female) pertaining to diversity field school (DFS) and seed producer groups were selected randomly hinged principally on their rice-growing experience, willingness to participate in research, and considering role of gender. DFS is a community-based action designed to create a platform for learning and sharing of crop diversity-related knowledge with emphasis on agency, participation and empowerment and development of both knowledge base and leadership potential of local farmers (www.himalayancrops.org). Initially, focus group discussion (FGD) was held to assess the challenges and constraints of rice production in the mid hills of Lamjung. Farmers were then allowed to prioritize the challenges and set their own selection criteria and demands to combat the aforementioned constraints. Both male and female participants prioritized and jointly agreed on eight decisive traits viz., grain yield, straw yield, earliness, disease resistance, stem borer tolerance, cold tolerance, shattering resistance, and lodging resistance. These traits were tabulated in a matrix scoring table, and each selection criterion was compared with another in a pair-wise fashion. These rank assignments were determined from frequency of times each selection criterion was preferred by the group (Lelo et al. 1995). A direct matrix table was prepared for the cold tolerant rice genotypes listed in the row and traits preferred by farmers in the column. Each genotype was assigned with scores based on the selection criteria (5=excellent, 4=very good, 3=good, 2=poor, and 1=very poor). In this study, cards of five different colors were used to score farmers' decisive selection criteria using symbolic representation given as follows; excellent (5) represented by green card, very good (4) by blue card, good (3) by yellow card, poor (2) by pink card, and very poor (1) by red card. During the direct matrix ranking, farmers assigned rating of importance (relative weight) from 1 to 3 (3=very important, 2=important, and 1=less important) pertaining to a selection criterion and the rating of performance of a genotype for each selection criterion (farmer preferred traits) was given based on their level of importance taking consensus of evaluators into consideration. The score of each genotype was multiplied by the relative weight of a particular character to obtain the final result and the, summed up with the results of other traits to determine the total score of a given genotype. Scoring and ranking were conducted on consensus. The differences were resolved by discussion as suggested by de Boef and Thijssen (2008).

Results

Pattern and degree of divergence in the cold tolerant rice genotypes

The UPGMA cluster analysis of 60 rice genotypes by three decisive traits viz. grain yield, overall phenotypic performance and panicle exsertion revealed six distinct clusters based on Euclidean distance (Fig. 1). The descriptive statistics of the distinct cluster are depicted in Table 2. The number of genotypes belonging to different clusters varied from 2 in Cluster IV to 18 in Cluster VI. The largest cluster VI comprising of 18 genotypes (30%) exhibited higher mean value for yield and was superior in terms of cold tolerance and overall phenotypic performance. The Cluster I comprising of 16 genotypes, Cluster II comprising of seven genotypes, and Cluster III comprising of 11 genotypes also exhibited higher mean value for yield. However, these clusters showed poor phenotypic performance and cold tolerance. The selection

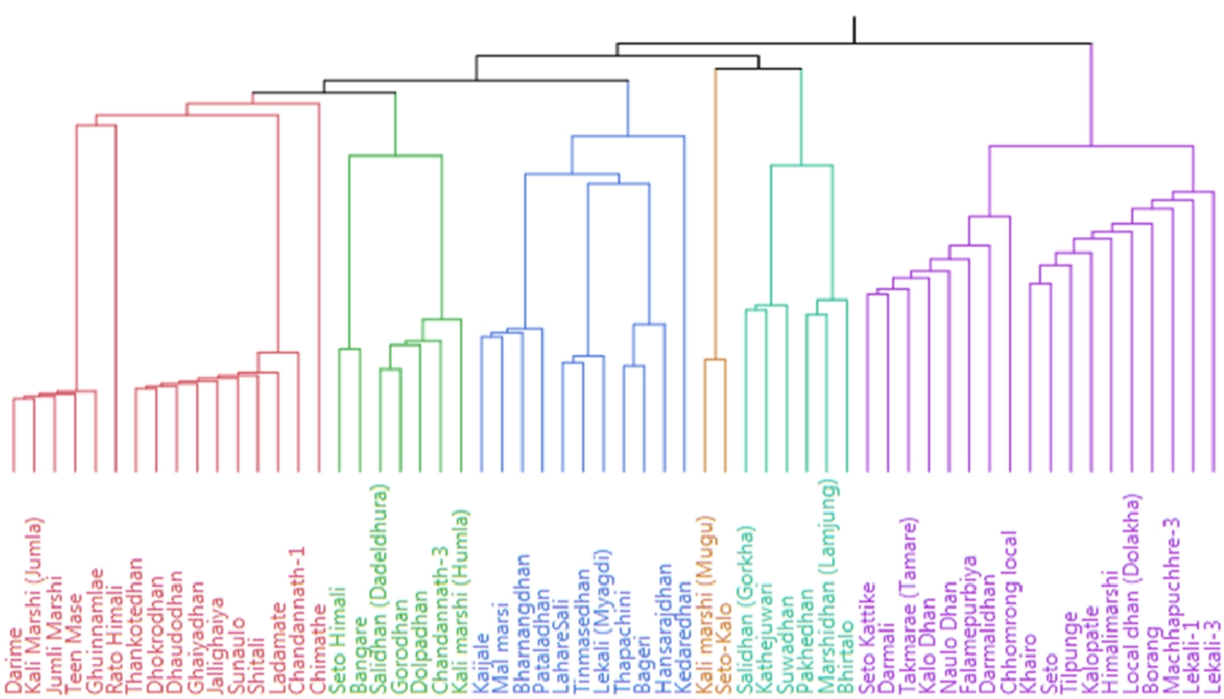

Fig. 1. UPGMA clustering of 60 rice genotypes included in diversity block based on average linkage and Euclidean distance.

of the robust and adaptive genotypes from Cluster VI holds more justifiable and rational. Thus, six genotypes embraced in Cluster VI *viz.*, Seto Kattike, Naulo Dhan, Borang, Machhapuchre-3, Lekali-1, and Lekali-3 for portraying reasonably superior performance under Lamjung conditions were opted for further evaluation studies and validation.

Performance evaluation and dissection of structure of variability in the identified pool of promising cold tolerant genotypes

The analysis of variance revealed significant variation among the genotypes for all the agronomical traits ($P \leq 0.01$) and panicle length ($P \leq 0.05$) (Table 3). The highest plant height was recorded by genotype Seto Kattike (138.47±3.30 cm) followed by Borang (130.33±4.98 cm) and Naulo Dhan (128.00±7.55 cm) which were statistically at par with each other while the lowest plant height was recorded by genotype Lumle-2 (96.67±0.88 cm). Panicle length is one of the important yield-components in rice. In general, the higher the panicle length, the higher will be the number of grains/ panicle attributing higher yield. Among the test genotypes, Borang was found to record the longest panicle length (24.00±0.00 cm) followed by Seto Kattike (23.67±0.88 cm) and Naulo Dhan (23.00±1.00 cm) which were statistically at par with each other. The highest 1000-grain weight was recorded by genotype Machhapuchre-3 (27.40±0.12 g) followed by Lekali-2 (26.2±0.06 g) and Lekali-1 (26.1±0.06 g) while, the lowest 1000-grain weight was recorded by Seto Kattike (21.17±0.09 g) and Naulo Dhan (22.40±0.06 g). Despite being inferior with respect to 1000-grain weight, Seto Kattike was found to be superior with grain yield (2463.44±117.93 kgha^{-1}) and straw yield (5166.67±346.94 kgha^{-1}) followed by Borang (2074.06±136.16 kgha^{-1}and 3277.78±242.16 kgha^{-1}) and Naulo Dhan (1879.89±235.46 kgha^{-1} and 2444.44±547.16 kgha^{-1}). The genotype Lekali-1 recorded the lesser days to 50% heading followed by Lumle-2 (124.67±2.33 days) and NR10695-2-2 (124.67±0.88 days), being statistically at par with each other. Similarly, the lesser days to 80% maturity was recorded by the genotype Lumle-2 (163.67±0.33 days) followed by Lekali-1(165.33±0.33 days) and NR10695-2-2 (165.67±0.33 days) being at par with each other.

Concerning the association of yield and agronomical traits (Supplementary Table 2), highly significant positive correlations between grain yield and plant height (r= 0.8019), panicle length (r= 0.6381) and straw yield (r= 0.8743). Days to 50% heading exhibited significant positive correlation (r= 0.5074 and 0.4480) with the grain yield. A highly significant negative correlation (r= -0.6334) was detected between the grain yield and 1000-grain weight.

Supplementary Table 6 presents the principal component and percentage of contribution of each component to the total variation in the tested rice genotypes. The first principal component accounted for 72.77% of the total variation in the population. Plant height, panicle length, grain yield, and days to 50% heading contributed more to the variation in negative direction while the 1000-grain weight contributed positively to the first component. Second principal component contributed to 18.05% of the total variation. The agro-morphological traits that contributed more to the second component included straw yield, days to 50% heading and 80% maturity. The first two principal components with Eigen value ≥1 accounted for 90.82% of the total variation. The present findings indicated that indirect selection based on traits such as plant height and panicle length can be worthwhile for identifying high-yielding cold tolerant genotypes of rice. High level of variability existing within the genotypes and the traits will make room for further improvement of the cultivars in the breeding programs.

Farmer's evaluation of the promising cold tolerant genotypes

Decisive selection criteria in the study area were based on ample discussion and consensus. The selection criteria determined by the consensus of the famer's group were grain yield, straw yield, earliness, disease resistance, stem borer tolerance, shattering resistance, and lodging resistance. Pair-wise matrix ranking of the selection criteria was used to

Table 2. Number of genotypes in each cluster along with mean agronomic and phenotypic performance.

Cluster	Number genotypes	Yield (kg ha^{-1})	Overall phenotypic performance	Panicle exsertion (cm)
I	16	1740.25 (769.66-2030.66)	9	4.79 (3-7)
II	7	1749.83 (312.50-2328.5)	8.43 (5-9)	5.25 (1-5)
III	11	1588.33 (743.33-2140.83)	7.97 (5-9)	3.48 (1-5)
IV	2	1460.5 (1335.83-1585.33)	8 (7-9)	4.69 (1-5)
V	6	1052.92 (964.16-2086.66)	6 (5-7)	4.30 (1-5)
VI	18	2038.35 (1778.5-2256.6)	3.11 (1-5)	2.57 (1-3)

Figures in the parenthesis represent the range of values of traits for each cluster

Table 3. Mean values ± Standard Error (S.E.) of agro-morphological traits of cold tolerant rice genotypes in yield trial in 2016.

Genotypes	Plant height (cm)	Panicle length (cm)	Grain yield (kgha^{-1})	Test weight (g)	Straw yield (kgha^{-1})	Days to 50% heading	Days to 80% maturity
Borang	130.33±4.98a	24.00±0.00a	2074.06±136.16b	23.17±0.09d	3277.78±242.16b	135.33±0.67ab	180.33±6.67 a
Lekali-1	107.33±2.67b	21.00± 0.58bc	1527.33±54.45cde	26.10±0.06b	1888.89±200.31cd	124.00±1.73d	165.33±0.33b
Lekali-3	109.33±4.18b	20.67±0.67c	1702.22±65.35bcd	26.20±0.06b	2333.33±333.330.20c	125.67±0.88d	166.00±0.00b
Lumle-2	96.67±0.88b	21.33±0.33bc	1427.50±95.16de	22.70±0.06e	2333.33±346.940.21c	124.67±2.33d	163.67±0.33b
Machhapuchre-3	102.47±4.22b	21.33±0.88bc	1224.28±162.06e	27.40±0.12a	1666.67±166.67d	129.67±0.67c	167.33±2.40b
Naulo Dhan	128.00±7.55a	23.00±1.00ab	1879.89±235.46bc	22.40±0.06f	2444.44±547.16c	137.67±0.88a	187.67±2.67a
NR10695-2-2	103.67±1.76b	20.67±0.33c	1592.50±128.85cde	24.00±0.06c	1888.89±200.31cd	124.67±0.88d	165.67±0.33b
Seto Kattike	138.47±3.30a	23.67±0.88a	2463.44±117.93a	21.17±0.09g	5166.67±346.94a	132.67±0.33bc	166.67±0.67b
p-value	<0.0001	0.0165	0.0002	<0.0001	<0.0001	<0.0001	0.0002
SE.m±	5.93	1.00	176.75	0.11	280.35	1.79	3.93
LSD (p ≤0.05)	12.73	2.14	379.08	0.23	601.28	3.85	8.44
CV (%)	6.35	5.58	12.47	0.55	13.08	1.70	2.83

Mean values in columns with different letters are significantly different ($p \leq 0.05$) according to Fisher's LSD test

Table 4. Pairwise ranking of farmer's selection criteria of cold tolerant rice.

Selection criteria	Grain Yield (GY)	Straw Yield (SY)	Earliness (E)	Disease Resistance (DR)	Stem borer tolerance (STR)	Cold tolerance (CT)	Shattering resistance (SR)	Lodging resistance (LR)	Total Score	Rank
Grain Yield	X	GY	GY	GY	GY	GY	GY	GY	7	1st
Straw Yield		X	E	SY	SY	SY	SY	SY	5	2nd
Earliness			X	E	E	E	SR	SR	4	3rd
Disease Resistance				X	DR	CT	DR	DR	3	4th
Stem borer tolerance					X	CT	SBT	SBT	2	5th
Cold tolerance						X	CT	CT	4	3rd
Shattering Resistance							X	SR	3	4th
Lodging Resistance								X	0	6th

Table 5. Direct matrix ranking evaluation of cold tolerant rice varieties performance rating value of each genotype.

Criteria	Grain Yield	Straw Yield	Earliness	Disease Resistance	Stem borer tolerance	Cold tolerance	Shattering resistance	Lodging resistance	Total Score	Rank
Relative weight	3	3	3	2	3	3	2	2	-	-
Borang	4 (12)	4 (12)	2 (6)	4 (8)	4 (12)	4 (12)	4 (8)	3 (6)	76	1st
Lekali-1	2 (6)	2 (6)	3 (9)	1 (2)	3 (9)	4 (12)	3 (6)	3 (6)	56	6th
Lekali-3	3 (9)	2 (6)	3 (9)	3 (6)	3 (9)	4 (12)	2 (4)	3 (6)	61	5th
Lumle-2	2 (6)	3 (9)	4 (12)	2 (4)	3 (9)	4 (12)	3 (6)	4 (8)	66	4th
Machhapuchre-3	1 (3)	2 (6)	3 (9)	2 (4)	2 (6)	4 (12)	3 (6)	3 (6)	52	8th
Naulo Dhan	3 (9)	3 (9)	2 (6)	4 (8)	4 (12)	4 (12)	3 (6)	3 (6)	68	3rd
NR10695-2-2	2 (6)	2 (6)	3 (9)	3 (6)	2 (6)	4 (12)	4 (8)	2 (4)	53	7th
Seto Kattike	4 (6)	4 (12)	2 (6)	4 (8)	4(12)	4 (12)	4 (8)	3 (6)	70	2nd

determine and prioritize the order of farmer's selection criteria. Apart from grain yield and straw yield, traits such as earliness, disease resistance, and stem borer tolerance were proposed as crucial criteria in descending order as depicted in Table 4. Neck blast and leaf blast were reported to be the major disease in rice fields, preceding drastic decline in the production. The direct matrix ranking evaluation of the cold tolerant rice genotypes unveiled total score ranging from 52 to 76 as depicted in Table 5. Borang secured the 1st rank with total score of 76 followed by Seto Kattike (70) and Naulo Dhan (68). These three genotypes demonstrated pre-eminence for most of the farmer's preferred trait except earliness and lodging resistance. These top three genotypes despite their pre-eminence for most of the selection criteria, were relatively

Table 6. Farmer's pair-wise ranking of the cold tolerant genotypes in Lamjung.

Genotypes	Borang	Lekali-1	Lekali-3	Lumle-2	Machhapuchre-3	Naulo Dhan	NR10695-2-2	Seto kattike	Total Score	Rank
Borang	X	Borang	Borang	Borang	Borang	Borang	Borang	Borang	7	1st
Lekali-1		X	Lekali-3	Lekali-1	Lekali-1	Naulo Dhan	NR10695-2-2	Seto Kattike	2	6th
Lekali-3			X	Lekali-3	Lekali-3	Naulo Dhan	Lekali-3	Seto Kattike	4	4th
Lumle-2				X	Lumle-2	Naulo Dhan	NR10695-2-2	Seto Kattike	1	7th
Machhapuchre-3					X	Naulo Dhan	NR10695-2-2	Seto Kattike	0	8th
Naulo Dhan						X	Naulo Dhan	Seto Kattike	5	3rd
NR10695-2-2							X	Seto Kattike	3	5th
Seto Kattike								X	6	2nd

inferior in terms of earliness and lodging resistance. Nevertheless Lumle-2, the 4th rank holder, was assigned with relatively higher scores for earliness and lodging resistance while moderate scores for other criteria. Similarly, the pair-wise preference ranking of the cold tolerant genotypes also inferred Borang, Seto Kattike, and Naulo Dhan to be the top three genotypes based on farmer's preference in descending order as indicated in Table 6. Farmer's overall evaluation of the cold tolerant rice genotypes based on both direct and pair-wise ranking procedure identified the genotypes viz. Borang, Seto Kattike, and Naulo Dhan as the most preferred and Machhapuchre-3 as the least preferred.

Discussion

Assessment of genetic diversity is crucial in rice breeding from the perspective of selection, conservation and proper utilization (Mohammadi-Najad 2008). The morphological and agronomic characterization of a crop is an important step in the management of genetic diversity (Manzano et al. 2001; Radhouane 2004; Yobi et al. 2002). It is also a pre-requisite towards the selection of improved varieties (Fraleigh 1987; Smith et al. 1991). Evaluation and characterization of rice is important due to increasing needs of varietal improvement. High level of variation among the 60 rice genotypes in terms of yield, overall phenotypic performance, and panicle exsertion was revealed signifying tremendous potentialities for its exploitation in improvement and breeding of cold-tolerant rice. Cold tolerance at the reproductive period has been associated with the degree of panicle exsertion, which could be used as a selection criterion (Nanda and Seshu 1979). In addition to this, panicle exsertion is a better indicator of cold tolerance under field conditions than spikelet sterility, which may also be affected by other climatic factor (Pereira da Cruz et al. 2008). The dearth of promising and robust cold-tolerant varieties has been a major constraint for up surging the production and area coverage, leading to food insecurity in mid-hills. The dire concern of ensuring food security propelled the present investigation with the aim of identification of adaptive high-yielding cold-tolerant rice varieties and its further registration and release in national agricultural system.

The UPGMA cluster analysis of the 60 rice genotypes based on yield, panicle exsertion, and overall phenotypic performance categorized the genotypes into six clusters implying ample variability for the studied traits and enabled the selection of candidate genotypes with high value of desirable traits for further evaluation. Several studies on variability assessment for rice based on cluster analysis are reported (Gahalain 2006; Hien et al. 2007; Mathure et al. 2011; Naik et al. 2006; Roy et al. 2012; Roy et al. 2014; Sarawgi and Bhisne 2007). The clustering pattern of the rice genotypes did not comply with the geographical origin of genotype. Similar trend has been reported in other studies (Mathure et al. 2011; Ratho 1984; Roy et al. 2014). The six genotypes from Cluster VI *viz.*, Seto Katike, Naulo Dhan, Borang, Machhapuchre-3, Lekali-1, and Lekali-3 for revealing superior overall performance along Lumle-2 and NR10695-2-2 procured from National agricultural research system were opted for their agro-morphological evaluation.

Significant variation among the eight genotypes selected from the diversity block for the agro-morphological traits reflected distinct genetic backgrounds. The genotypes responded differently for different agro-morphological traits implying their prominence in identifying the best genetic make up for a specific environment and farmer's need. Seto Kattike, Borang, and Naulo Dhan were found promising and adaptive to the farming community for exhibiting higher panicle length, grain, and straw yield. Borang and Naulo Dhan, being quite late-maturing varieties, may not be appealing to the farming community preferring early varieties. Nevertheless, Seto Kattike is likely to gain popularity and be better-adopted in multi-cropping system for its earliness and higher yield. The findings bolster the large-scale testing of IRD kits for popularizing the variety (Witcombe et al. 2016). In addition to diversifying the choices among the agrarian community, the wider dissemination and adoption of these cold-tolerant varieties will assure augmented production and area coverage of rice in the mid-hills warranting food security in the region.

Strong positive correlations between grain yield and plant height, panicle length and straw yield detected in the present investigation corroborates the findings of Ojha et al. (2010), Ojha (2010), Ojha (2009), Ojha (2007), Sharma et al. (2007), and Ojha and Sharma (2005). The strong inter-correlation between plant height, panicle length, straw yield, days to heading and days to maturity suggests that rice growth at vegetative stage is crucial for the physiological process that determines the output of its performance in terms of yield and other physiological attributes contributing to its development. Significant positive correlation between days to heading and grain yield was detected, which is in accordance with findings of Ul-qamar et al. (2005) and Manuel and Palananisamy (1989). A highly significant negative correlation was detected between the grain yield and 1000-grain weight. The results substantiate the findings of Khan et al. (1999) and Venkateswarlu et al. (1981) while contradicts with the findings of Al-Salim et al. (2016), Maji and Shaibu (2012), and Ogunbayo et al. (2012). This type of situation was encountered because there is hardly any compensatory system operating between thousand grain weight and grain number in the late maturing cultivars (145-165 days) with 1000-grain weight ranging from 15-30 g (Venkateswarlu et al. 1981).

Principal component analysis (PCA) is designed to transform the original variables into new uncorrelated variables called 'components', which are linear combinations of the original variables. It is used to explain the observed variances and to understand the interrelationship among different parameters (Rencher 2002). The first two principal components with Eigen value ≥ 1 accounted for 90.82% (72.77% and 18.05) of the total variation among the test genotypes for the selection of the diverse parents. If the initial components accumulate a relatively high percentage of total variation, generally above 80%, they satisfactorily explain the variability among individuals (Madia et al. 1979). The genetic divergence among the rice genotypes on the basis of their phenological traits plays a crucial role in the selection of the diverse genotypes for the further improvements of the varieties through breeding (Shahidullah et al. 2009). Characters with high variability are expected to provide high level of gene transfer during breeding programs (Aliyu et al. 2000; Gana 2006). The results of PCA suggested traits such as plant height, panicle length, days to 50% heading, and grain yield to be the principal discriminatory characteristics of the cold-tolerant rice genotypes. The present finding indicated that the indirect selection based on traits such as plant height and panicle length can be advisable for ascertaining robust and high-yielding cold tolerant genotypes of rice.

Participatory varietal selection (PVS) typically employs intensive systems of *on-farm* participatory evaluation that involve frequent interactions between researchers and farmers to assess their preferences in new varieties (Joshi and Witcombe 1996; Witcombe et al. 1996). Farmers' revelation for the evaluation of new varieties is an essential to exploit their indigenous knowledge and expertise of selecting adaptive varieties that meet their needs and interests. Farmers' participation in the variety selection help to include farmers' selection traits which are often overlooked by researchers during conventional variety development and remarkably cut off the costs of variety development and speed up the variety release process. Apart from grain yield and straw yield, the most prioritized farmer's selection criteria for cold tolerant rice in the mid-hills were earliness and disease resistance and stem borer tolerance. This endorsed the findings of Karki et al. (2010) who reported the grain and straw yield, milling recovery and resistance to diseases and pests as major factors that determine the fate and adoption rate of cold tolerant varieties.

Conclusion

The present findings revealed preponderance of genetic diversity in the studied rice genotypes pertaining to agronomic traits and chilling tolerance reflecting their diverse genetic background which could be utilized in crop improvement and breeding programs. The genotypes viz., Seto Kattike, Naulo Dhan, and Borang were most promising and adaptive among the tested ones in terms of yield components and chilling tolerance based on evaluation studies and participatory varietal selection approach. The eminence of the most promising genotypes as revealed in the evaluation studies were justifiably corroborated and validated by farmer's overall evaluation hinged on both direct and pair-wise ranking procedure and thus manifests a participatory approach, a proven and effective practice to rapidly identifying locally adaptive and robust genotypes of farmer's preference and needs, expediting their wider expansion, rendering swollen genetic base of the community richness, and enabling rapid varietal release. The most valued decisive selection criteria of farmers were grain yield and straw yield, earliness, disease resistance and stem borer tolerance in descending order. The information thus generated complements the robust breeding program of cold tolerant genotypes of end user's preference in different environment. In addition, the findings also bolster employment of innovative and proven participatory plant breeding approach using diversity kits and IRD kits to expand and promote the varietal choice options for expeditious benefits to the farmers, fostering agro-biodiversity and food security in the mid-hills. Community seed banks can play a pivotal role in popularizing these robust landraces at community level via establishment of demonstration plots and distribution of IRD and diversity kits and, ensuring the accessibility of quality seeds by incorporating them in its routine conservation and seed multiplication activity. The wider dissemination and adoption of these robust cold-tolerant varieties will assure augmented production and area coverage of rice in the high-altitude areas warranting food security in the region.

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Supplementary Table 1. List of genotypes of rice included in the diversity block trial in 2015.

Entry	Local Name	District	Source	Entry	Local Name	District	Source
1	Darime	Jumla	Farmers' field	31	Bhirtalo	Humla	Genebank
2	Kali Marshi	Jumla	Farmers' field	32	Kali marshi	Mugu	Genebank
3	Jumli Marshi	Jumla	Farmers' field	33	Kali marshi	Humla	Genebank
4	Teen Mase	Jumla	Farmers' field	34	Dhokrodhan	Mugu	Genebank
5	Ghuinnamlae	Humla	Farmers' field	35	Dhaudodhan	Mugu	Genebank
6	Seto Kattike	Lamjung	Farmers' field	36	Ghaiyadhan	Mugu	Genebank
7	Darmali	Lamjung	Farmers' field	37	Thapachini	Bajur	Genebank
8	Takmarae	Lamjung	Farmers' field	38	Bageri	Solukhumbu	Genebank
9	Seto-Kalo	Lamjung	Farmers' field	39	Jallighaiya	Udaypur	Genebank
10	Khairo	Lamjung	Farmers' field	40	Chimathe	Kalikot	Genebank
11	Seto	Lamjung	Farmers' field	41	Sunaulo	Bajura	Genebank
12	Kalo Dhan	Lamjung	Farmers' field	42	Darmalidhan	Kaski	Genebank
13	Naulo Dhan	lamjung	Farmers' field	43	Shitali	Bajhang	Genebank
14	Kaijale	Dolakha	Farmers' field	44	Ladamate	Bajhang	Genebank
15	Seto Himali	Dolakha	Farmers' field	45	Bharnangdhan	Panchthar	Genebank
16	Rato Himali	Dolakha	Farmers' field	46	Salidhan	Dadeldhura	Genebank
17	Tilpunge	Dolakha	Farmers' field	47	Gorodhan	Mugu	Genebank
18	Kedaredhan	Baitati	Genebank^	48	Tinmasedhan	Mugu	Genebank
19	Salidhan	Gorkha	Genebank	49	Himalimarshi	Dolakha	Genebank
20	Falamepurbiya	Gorkha	Genebank	50	Local dhan	Dolakha	Genebank
21	LahareSali	Gorkha	Genebank	51	Pataladhan	Rasuwa	Genebank
22	Pakhedhan	Lamjung	Genebank	52	Borang	Rasuwa	Genebank
23	Marshidhan	Lamjung	Genebank	53	Dolpadhan	Dolpa	Genebank
24	Mal marsi	Humla	Genebank	54	Hansarajdhan	Bajhang	Genebank
25	Kathejuwari	Kaski	Genebank	55	Chhomrong local	-	ABD*
26	Bangare	Kaski	Genebank	56	Machhapuchhre-3	-	ABD
27	Kalopatle	Kaski	Genebank	57	Chandannath-1	-	ABD
28	Lekali	Myagdi	Genebank	58	Chandannath-3	-	ABD
29	Suwadhan	Sindhupalchok	Genebank	59	Lekali dhan-1	-	ABD
30	Thankotedhan	Sindhupalchok	Genebank	60	Lekalidhan -3	-	ABD

*Agricultural Botany Division, Nepal Agricultural Research Council, Khumaltar, Nepal
^National Agriculture Genetic Resources Centre, Nepal Agricultural Research Council, Khumaltar, Nepal

Supplementary Table 2. Correlation matrix between different agro-morphological traits of cold tolerant rice.

Agro-morphological traits	Plant height	Panicle length	Grain yield	1000 grain weight	Straw yield	Days to 50% heading
Panicle length	0.844**					
Grain yield	0.8019**	0.6381**				
1000 grain weight	-0.5699**	-0.5420**	-0.6334**			
Straw yield	0.7612**	0.6350**	0.8743**	-0.6496**		
Days to 50% heading	0.7142**	0.6660**	0.5074*	-0.4513*	0.4153*	
Days to 80% maturity	0.5151**	0.4915*	0.3381^{ns}	-0.3198^{ns}	0.0799^{ns}	0.7627**

**Correlation coefficient is significant at $p \leq 0.01$ (2-tailed)
*Correlation coefficient is significant at $p \leq 0.05$ (2-tailed)
^{ns}Correlation coefficient is non-significant at $p \leq 0.05$ (2-tailed)

Supplementary Table 3. Eigen values, factor scores and contribution of first three principal component axes to the variation in cold tolerant rice genotypes

Agro-morphological traits	PC1	PC2	PC3
Eigen value	5.0939	1.2635	0.4242
Variance (%)	72.77	18.05	6.06
Cumulative variance (%)	72.77	90.82	96.88
Plant height	-0.4274	-0.0088	0.3411
Panicle length	-0.4239	0.0821	0.0604
Grain yield	-0.4034	-0.2944	0.1742
1000 grain weight	0.3408	0.2566	0.8742
Straw yield	-0.366	-0.4757	0.1966
Days to 50% heading	-0.3784	0.4268	0.1026
Days to 80% maturity	-0.2856	0.6574	-0.1905