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Molecular characterization, DNA fingerprinting and genetic diversity analysis of Nepalese rice landraces using SSR markers

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Abstract

Rice (*Oryza sativa*) is the major crop of Nepal. Genetic diversity studies in rice have been conducted extensively with collections from various parts of the world, including Nepal. However, local landraces in these collections are explored on a very limited scale for novel genetic variations. The availability of wild relatives of *Oryza sativa* has increased interest in understanding the genetic makeup of Nepalese rice landraces. This study aimed to identify the variability in 80 rice landraces using 19 simple sequence repeat (SSR) markers. The collection represented geographical regions suitable for rice farming in Nepal. DNA fingerprints of some landraces showed clear distinctions. The results indicated significant genetic differentiation among the rice landraces, with a possible formation of two distinct clusters. Among 19 SSR markers, only 12 have shown polymorphism. The lowest allele frequency was observed in the Tauli Satara landrace. The maximum heterozygosity was observed from the sample collected from Pyuthan district. The first coordinate explained 18.81% of the variation, while the second coordinate explained 13.16%. Overall, these findings will benefit rice breeders and conservationists in selecting parent material, managing conservation efforts both on-farm and ex-situ, and linking genetic diversity with geographical locations.

Keywords: Nepalese rice, SSR, genetic diversity, DNA fingerprint, district

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Introduction

Rice (*Oryza sativa*) is one of the important food crops in the world. Nepal has maintained a history of rice cultivation and is one of the most important and staple food, which also ranked first in terms of production and the area of cultivation [1]. The domestication of rice could be associated with the geographical distribution of different species and ecotypes of rice such as japonica could have domesticated in the Yangtze river valley in China; whereas indica could be around Indian sub-continent; therefore, it was concluded that there might be independent origin and domestication of this species across the world [2]. A number of species/subspecies of rice has been collected from different part of Nepal and has been evaluated for the potential use in the rice breeding program such as for the flood tolerance by [3]; bacterial leaf blight resistance by [4] and also has been utilized for generating Cytoplasmic Male Sterile lines into the hybrid breeding program [5]. The collection of wild species such as *O. nivara, O. rupipogon, O. officinalis* and *O. granulate* from Nepal indicated a potentiality of another independent source of variabilities in Nepalese rice germplasm [6,7] and the analysis using SSR markers suggested the wild relatives found in Nepal were in the same cluster with most of the landraces collected from Tarai and Inner Tarai but with a higher genetic diversity. Same report also suggested that the landraces collected from high hills were outgroup and could pose a distinct subpopulation [8]. National Agriculture Genetic Resources Center (Genebank) has collected and maintained more than 2000 rice landraces and various efforts are being made to analyze the genetic diversity in the collected rice landraces. The geographical microclimates in Nepal could be the potential factor to isolate the germplasms from Inner Tarai to the hilly regions and create variability. More recently a project on whole genome sequencing of 3010 diverse accession from Asia has included 44 rice genotypes originated from Nepal [9] and the results suggested that these genotypes represent at least 4 subpopulations, which indicated the richness of Nepalese rice landraces. Rice collected from both Tarai and hilly areas possess variability, however, these are poorly utilized in breeding program [10–12].

Morphological characterization has been carried out in some rice landraces of Nepal [13]. Molecular markers has shown a promise to differentiate germplasms; such as RAPD marker has been utilized in genetic diversity assessment where a higher genetic diversity among landraces collected from high hills were reported [14]. The availability of rice reference genome sequence has eased the use of high throughput techniques in the genetic diversity studies; however, we cannot utilize such

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techniques for all the available germplasms due to the cost associated with the generation of data; therefore, in such conditions low throughput techniques could be advantageous. A number of genome wide SSR markers have been developed to facilitate the genetic diversity studies [15]. The SSR techniques has been utilized in number of rice research activities across the world [16–18]. The reproducibility and the identification of allelic variation are important aspect of this techniques. Most of the available rice variety were developed from the landraces having specific traits; and varieties are results of accumulation of beneficial alleles from different sources [19].

The majority of traditional rice landraces have been supplanted by a limited number of improved varieties. Despite this shift, these landraces are being preserved through ex-situ conservation efforts. To expedite the utilization of this valuable gene pool, it is essential to develop and promote site-specific and geographically linked landraces. Employing DNA fingerprinting technology could serve as a valuable tool for establishing ownership documentation. Additionally, to enhance management practices both on-farm and in ex-situ environments, it is imperative to explore the genetic associations within the germplasm pool. Consequently, the assessment of genetic diversity is crucial for the improvement of rice crops, aiming not only for higher yields but also for enhanced quality of production.

Materials and methods

Figure 1. Collection site of rice accessions (red dot) used in this study (map generated by RK Basnet)

Rice landraces

A total of 80 rice landraces from National Genebank, Kathmandu were used in this study. Their passport details are given in **Supplementary Table 1**. These accessions were the collections of National Genebank during 2012 to 2013. These were collected from 37 districts with an altitude range of 70 to 2700 masl (**Figure 1**). Among 80 landraces, 64 were lowland rice and 16 were upland rice, based on the rice production systems. For lowland rice, fields are flooded during part or all of the growing season, and it includes rainfed lowland, irrigated lowland, and deep water land [20]. Upland rice is grown on level or slopy land, unbounded fields without flooding. Upland rice is called Ghaiya in Nepal and possesses drought tolerance property.

DNA extraction

Leaves were collected from young plantlets and 2 g of leaves were used for DNA extraction. DNA extraction was performed using CTAB method (Doyle and Doyle, 1990). Leaves were chopped into fine particles and transferred to microfuge tubes, each containing steel beads. Then tubes were kept on a TissueLyser (QIAGEN) at 50 oscillation per minute for 10 minutes. 400µl CTAB buffer and 10µl β-mercaptoethanol was added to the macerated leaves-tubes and the solution was incubated at 65°C for 1 hr on hot water bath. The mixture was centrifuged at 15000rpm for 10 minutes. The supernatant was transferred to fresh tube and equal volume of Chloroform: Isoamyl alcohol (24:1) was added, and tubes were inverted several times and centrifuged again at 15000rpm for 5 minutes. The supernatant was transferred to a new tube and chilled isopropanol was added for precipitation of DNA. After precipitating DNA, the tube was centrifuged at 10000rpm for 2 minutes and isopropanol was discarded. The DNA pellet was washed with 70% ethanol. Ethanol was discarded and tube was allowed to dry. Finally, 50µl TE buffer was added to the pellet and mixed well and DNA was stored at -20°C.

The concentration of each DNA sample (ng/µl) was measured by Q5000 UV-Vis Spectrophotometer (Quawell). All measured data were then exported to MS Excel for further analysis. The concentrations of these DNA samples were adjusted to 50 ng/µl by adding TE buffer to store the stock template DNA in the DNA bank whereas working solution of template DNA was prepared at a concentration of 20ng/µl.

SSR primers and polymerase chain reaction

Based on the published literature, we selected 29 SSR primers considering their values with regards to economical traits and diversity assessment (**Supplementary Table 2**). They are scattered in all chromosomes except chromosomes 9 and 10. Annealing temperature ranged from 55 to 60°C, and expected amplified fragments size ranged from 70 to 283 bp. After knowing the priming condition and their amplified products, only 19 SSR primers were used for detail genotyping and genetic diversity study.

DNA amplification was carried out in a 15 µl reaction volumes consisting of 7.5 µl of 2X GoTaq® Green Master

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mix (Promega), 1.5 µl forward and reverse primers each (10 picomole of each primer), 2.5µl nuclease free water and 2µl DNA (40 ng DNA). The amplification reaction was carried out in a Thermal Cycler (MultiGene OptiMax, Labnet International, Inc.). The basic PCR program was as follows: an initial denaturing step at 94°C for 5 min followed by 30 cycles of 30 sec denaturation at 94oC, 55-61oC annealing temperature (depending upon primer used) for 30 sec and extension at 72°C for 45 second. A final extension step at 72°C for 10 min was performed and stored at 4°C. Annealing temperature (Ta) was determined by starting below Tm by 3-5°C. Melting temperature (Tm) was calculated using Oligo calculator (http://mcb.berkeley.edu/labs/krantz/tools/oligocalc.html).

Gel electrophoresis and detection

PCR products were separated on a 2.5% agarose gel using 1X TAE buffer. Ethidium Bromide (EtBr) was used both in the gel and buffer for DNA staining. EtBr of 0.5 µg/mL concentration were added to gel after cooling gel to 60-70°C. 12µl of PCR product of each sample was loaded in well. In first lane, 2 µl of 100 bp (Bioneer) was loaded to compare the size of bands. The gels were run for 2 hrs at 90 V, 50 mA. Upon completion of run, gels were washed de-stained in water for 5 min and placed in plastic wrap. DNA fragments were visualized under UV light and photographed using Gel Doc system (UVDI, Major Science). We took 4-5 photos of each gel from Gel doc system and best one was selected for further analysis.

Gel scoring and genetic data analysis

Fragment size was estimated using PyElph 1.3 based on 1 kb and 100 bp ladder (Bioneer). Match function of PyElph was applied for detecting number of different alleles. Fragment size of all bands that fall under same match line (as per the match function) were averaged and recorded as genetic allele size and allele-size-based genotype in Excel. This size was then used for allele alignment and new allele detection in other gels. This process was applied to all primers for 80 landraces. In some gels, size and allele were double checked with visual observation in MS Picture Manager (after crop and adjustment of its brightness and contrast). In case of no bands, they were considered as null alleles.

Two-way table was prepared using samples (landraces) as row name and primer as column name. Actual bp (genetic allele size) was recorded either in homozygous or heterozygous state. Codominant coding was then formatted for GenAlEx and PowerMarker software. Null allele was scored as 1 for analyzing in GenAlEx and 0 for analyzing in PowerMarker. Different genetic parameters were estimated to characterize the markers, landraces and districts. DNA finger print of each sample with multiple markers was prepared in MS Excel based on fragment sizes and distances. Band size and allelic data were used to measure the genetic diversity along with cluster and principal coordinate analyses in GenAlEx 6.5 and PowerMarker 3.2. Dendrogram was viewed in TreeView.

Results

Characteristics of markers, landraces and collected districts

Among 19 SSR markers, seven primers did not show any polymorphism whereas other primer shown polymorphism for the 80 landraces (**Figure 2** represent sample). Altogether 74 bands were amplified from 19 SSR markers among these 67 were polymorphic in nature. The polymorphic information content ranges from 0.19 to 0.85 with a mean of 0.42. Likewise, the gene diversity ranged from 0.22 to 0.86 with an average of 0.44. The marker RM3825 was found to be effective with 10 alleles and a higher level of heterozygosity. Altogether average of 4.21 alleles were obtained from these markers for the genetic diversity analysis (**Table 3**). This suggests the markers with the higher PIC can be utilized for the genetic diversity assessment as these markers have power to differentiate among the germplasm.

Since the landraces were collected from different districts of Nepal, the gene diversity and heterozygosity were

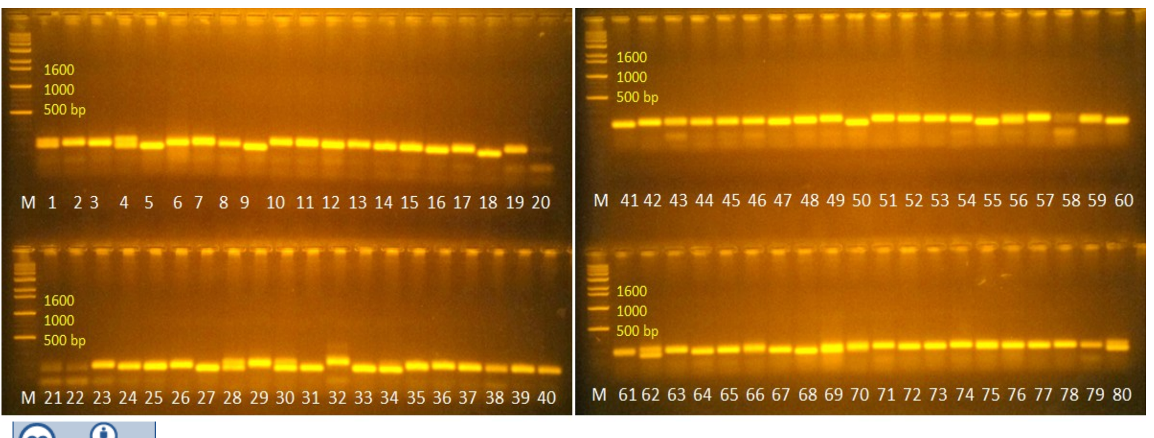

Figure 2. RM264 SSR profiles of 80 rice landraces. M, 1kb ladder; Lane number represent the name landraces of rice given in **Table 2** (correspond to first column serial number.

Table 1. Summary genetic diversity statistics of 19 SSR primers based on 80 rice landraces

SN	Marker	Major Allele Frequency	Genotype No	Allele No	No. of effective alleles (Ne)	Gene Diversity	Heterozygosity	Shannon's information index (I)	PIC
1	RM20A	0.21	8	8	6.60	0.848	0	1.95	0.83
2	RM21	0.41	6	6	3.99	0.749	0	1.57	0.72
3	RM47	0.3	7	7	4.03	0.752	0	1.55	0.71
4	RM212	1	1	1	1.00	0	0	0.00	0
5	RM264	0.29	10	8	4.74	0.79	0.08	1.70	0.76
6	RM302	0.46	8	5	3.37	0.70	0.05	1.39	0.66
7	RM401	0.29	9	7	5.35	0.81	0.05	1.80	0.79
8	RM463	0.88	2	2	1.28	0.22	0.00	0.38	0.19
9	RM536	1	1	1	1.00	0	0	0.00	0
10	RM561	1	1	1	1.00	0	0	0.00	0
11	RM1233	1	1	1	1.00	0	0	0.00	0
12	RM3825	0.206	18	10	7.24	0.86	0.48	2.08	0.85
13	RM5961	1	1	1	1.00	0	0	0.00	0
14	RM6836	0.344	8	6	3.82	0.74	0.08	1.46	0.69
15	RM7102	1	1	1	1.00	0	0	0.00	0
16	RM7575	1	1	1	1.00	0	0	0.00	0
17	RM8225	0.45	5	5	2.97	0.66	0.00	1.25	0.60
18	RM168	0.39	4	4	3.42	0.71	0.00	1.30	0.65
19	RM413	0.64	5	5	2.06	0.52	0.00	0.95	0.45
20	Mean	0.62	5.11	4.21	2.94	0.44	0.04	0.91	0.42

analyzed from the samples for district individually. The analysis of 80 rice landraces from 37 districts revealed significant variability in genetic diversity metrics. Major allele frequency ranged from 0.68 to 1, indicating varying levels of allele fixation. The allele number per locus averaged from 1 to 2.16, showing low to moderate diversity. Gene diversity, reflecting the probability of differing alleles, ranged from 0 to 0.34, with heterozygosity (proportion of heterozygous individuals) between 0 and 0.21. Polymorphism Information Content (PIC), indicating marker informativeness, varied from 0 to 0.29. The maximum gene diversity was found for the district Dadeldhura which had 6 landraces; however, the heterozygosity was 0.06 (**Supplementary Table 3**). The maximum heterozygosity was observed from the sample collected from Pyuthan district. For some landraces which had only amplified alleles the calculation for the gene diversity and heterozygosity was affected. Four markers viz. RM3825, RM264 and RM20A have shown private alleles for some landraces (Machhe Ghaiya, Dhokro, Jameli and Rajarani) which could be a unique mutation related with that particular landrace.

The genetic diversity of each rice landrace, assessed using 19 SSR markers, is detailed in **Table 2**. The major allele frequency, which indicates how common the most frequent allele is, ranged from 0.89 to 1. The lowest allele frequency was observed in the Tauli Satara landrace, suggesting it has more genetic variability at certain loci. The allele number, indicating the average number of alleles per locus, varied from 1 to 1.21, with Tauli Satara again showing the highest allele number, highlighting its genetic richness. Gene diversity ranged from 0 to 0.11. Heterozygosity, the proportion of individuals with two different alleles at a locus, ranged from 0 to 0.21, and the PIC ranged from 0 to 0.08. High levels of genetic diversity were notably observed in the landraces Pakhe Dhan, Jadamate, Pahenlo Rajmati, Bharnang, Ekle, Jhinuwa, Borang, Rajmati Dhan, and Tauli Satara, indicating these varieties possess a broader genetic base that could be valuable for breeding and conservation programs.

Genetic fingerprint of geographically isolated aromatic rice landraces

Beside the genetic diversity assessment in the collected rice landraces this study also aimed to develop the fingerprint for few landraces having economic important mainly for its fine quality grain and aroma. For this purpose, two landraces were compared and elaborated, however, all the developed fingerprints for remainder of the landraces will be act as depository for future needs beside the genetic diversity assessment in this study.

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Table 2. Summary genetic diversity statistics of rice landraces based on 19 SSR markers

SN	Landrace	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
1.	Sano Taichin	0.97	1.05	0.03	0.05	0.02
2.	Rato Anadi	1	1	0	0	0
3.	Anadi	1	1	0	0	0
4.	Chainaphor	1	1	0	0	0
5.	Jire Dhan	1	1	0	0	0
6.	Hansaraj-B	0.97	1.05	0.03	0.05	0.02
7.	Tauli	0.97	1.05	0.03	0.05	0.02
8.	Manahari Ghaiya	1	1	0.00	0.00	0.00
9.	Sali Dhan	0.97	1.05	0.03	0.05	0.02
10.	Lahare Sali	1	1	0	0	0
11.	Tauli Satara	0.89	1.21	0.11	0.21	0.08
12.	NPGR-01298	0.95	1.11	0.05	0.11	0.04
13.	Lekali	1	1	0	0	0
14.	Pokhreli Masino	1	1	0	0	0
15.	Suwa Dhan	1	1	0	0	0
16.	Seto Marsi	1	1	0	0	0
17.	Bhirtalo Dhan	0.97	1.05	0.03	0.05	0.02
18.	Dhokro	0.97	1.05	0.03	0.05	0.02
19.	Lotan Sarau	0.97	1.05	0.03	0.05	0.02
20.	Dhaudo	0.97	1.05	0.03	0.05	0.02
21.	Bageri	0.97	1.05	0.03	0.05	0.02
22.	Pakhe Ghaiya	0.97	1.05	0.03	0.05	0.02
23.	Chimathe	1	1	0	0	0
24.	Ghaiya	1	1	0	0	0
25.	Gumki Kogalya	1	1	0	0	0
26.	Rai Manowa	1	1	0	0	0
27.	Jarneli	0.97	1.05	0.03	0.05	0.02
28.	Pakhe Dhan	0.95	1.11	0.05	0.11	0.04
29.	Nepali Ghaiya	1	1	0	0	0
30.	Sunaulo	1	1	0	0	0
31.	Masino Gurda	1	1	0	0	0
32.	Gauriya	1	1	0	0	0
33.	Kale Dhan	1	1	0	0	0
34.	Shitli	0.97	1.05	0.03	0.05	0.02
35.	Jadamate	0.95	1.11	0.05	0.11	0.04
36.	Pakhe	0.97	1.05	0.03	0.05	0.02
37.	Salo Dhan	0.97	1.05	0.03	0.05	0.02
38.	Hansaraj	1	1	0	0	0
39.	Rato Dhan	1	1	0	0	0
40.	Pahenlo Rajmati	0.95	1.11	0.05	0.11	0.04
41.	Jhayale Ghaiya	1	1	0	0	0
42.	Rate Ghaiya	1	1	0	0	0
43.	Machhe Ghaiya	0.97	1.05	0.03	0.05	0.02
44.	Bharnang	0.95	1.11	0.05	0.11	0.04
45.	Pahele	1	1	0	0	0
46.	Gurdee	0.97	1.05	0.03	0.05	0.02
47.	Ekle	0.95	1.11	0.05	0.11	0.04
48.	Jhinuwa Masino	1	1	0	0	0
49.	Gurdi	0.97	1.05	0.03	0.05	0.02
50.	Jhinuwa	0.95	1.11	0.05	0.11	0.04
51.	Kamal Dhan	1	1	0	0	0
52.	Seto Marsi-K	1	1	0	0	0
53.	Sunaulo Ghaiya	1	1	0	0	0

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54.	Chhakmale Ghaiya	0.97	1.05	0.03	0.05	0.02
55.	Shyamjira	0.97	1.05	0.03	0.05	0.02
56.	Kalo Jaule Ghaiya	1	1	0	0	0
57.	Sunaulo Ghaiya-1	1	1	0	0	0
58.	Jhamma	0.97	1.05	0.03	0.05	0.02
59.	Dal Badale Ghaiya	0.97	1.05	0.03	0.05	0.02
60.	Jaule Ghaiya	0.97	1.05	0.03	0.05	0.02
61.	Bhutyal Ghaiya	0.97	1.05	0.03	0.05	0.02
62.	Jhuse Ghaiya	0.97	1.05	0.03	0.05	0.02
63.	Chainpure	1	1	0	0	0
64.	Tilki	0.97	1.05	0.03	0.05	0.02
65.	Jirmani Jau	0.97	1.05	0.03	0.05	0.02
66.	Seto Jhinuwa	0.97	1.05	0.03	0.05	0.02
67.	Rajmati Dhan	0.92	1.16	0.08	0.16	0.06
68.	Jini Dhan	0.97	1.05	0.03	0.05	0.02
69.	Patala	0.97	1.05	0.03	0.05	0.02
70.	Anga	0.97	1.05	0.03	0.05	0.02
71.	Borang	0.95	1.11	0.05	0.11	0.04
72.	Jarneli-1	0.97	1.05	0.03	0.05	0.02
73.	Bhui Pokhareli	1	1	0	0	0
74.	Bhunte Masino	0.97	1.05	0.03	0.05	0.02
75.	Ghaiya Dhan	1	1	0	0	0
76.	Basmati	0.97	1.05	0.03	0.05	0.02
77.	Kalanamak	0.97	1.05	0.03	0.05	0.02
78.	Shyamjira-B	0.97	1.05	0.03	0.05	0.02
79.	Mansara	1	1	0	0	0
80.	Rajarani	1	1	0	0	0

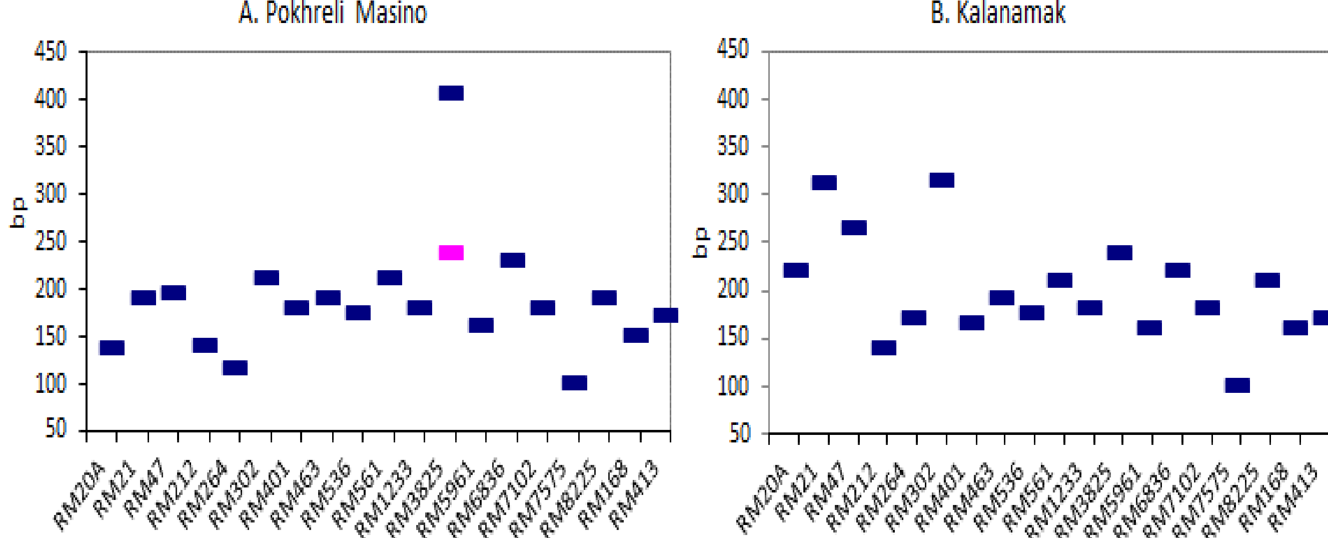

Figure 3. DNA fingerprints of two local rice landraces based on 19 SSR markers (pink mark indicates heterozygote)

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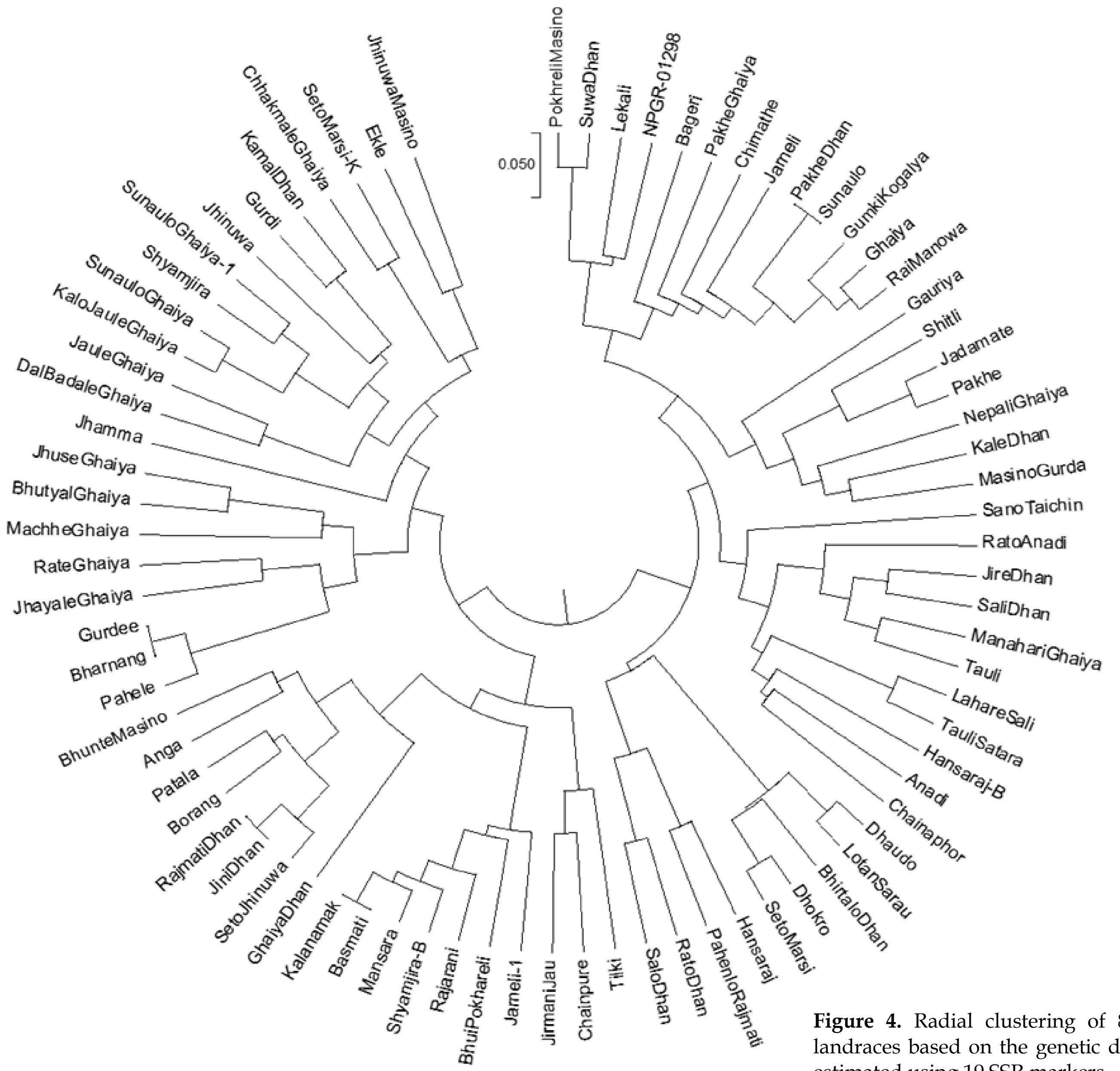

Figure 4. Radial clustering of 80 rice landraces based on the genetic distance estimated using 19 SSR markers

Pokhreli Masino is a popular landrace of Kaski district which represent midhill and has been popular for its quality grain and aroma. Likewise, Kalanamak is adapted to Rupandehi and Kapilvastu districts of Nepal which represent a Tarai region with lower altitude. Since the Tarai region represents the boarder to India which shared similar climate, the Kalanamak landrace is also cultivated in India. However, this study represents the Kalanamak landrace collected from Kapilvastu district of Nepal. The geographical isolation and climatic condition might have shaped these landraces for the adaptation although both are aromatic in nature as reflected in the **Figure 3**, where the number of markers were showing differences in allele size for these two landraces. The markers RM20A, RM21, RM47, RM264, RM302, and RM3825 were particularly of importance in distinguishing these landraces. For Pokhreli Masino the base pair lengths ranged from 100 to 405, with an average length of 185 bp. This indicates that the DNA fragments observed in this landrace varied significantly in size, but on average, they were around 185 bp. In contrast, Kalanamak showed base pair lengths ranging from 100 to 310, with a slightly higher average of 199 bp. This suggests that while both landraces have DNA fragments starting at 100 bp, the Kalanamak landrace has shorter maximum fragment lengths and a higher average fragment length compared to Pokhreli Masino.

Genetic diversity and relationship among landraces

A UPGMA based phylogenetic tree was constructed to observe the relationship among landraces. The dendrogram suggested two major groups among the studied landraces; however, a number of groups can be

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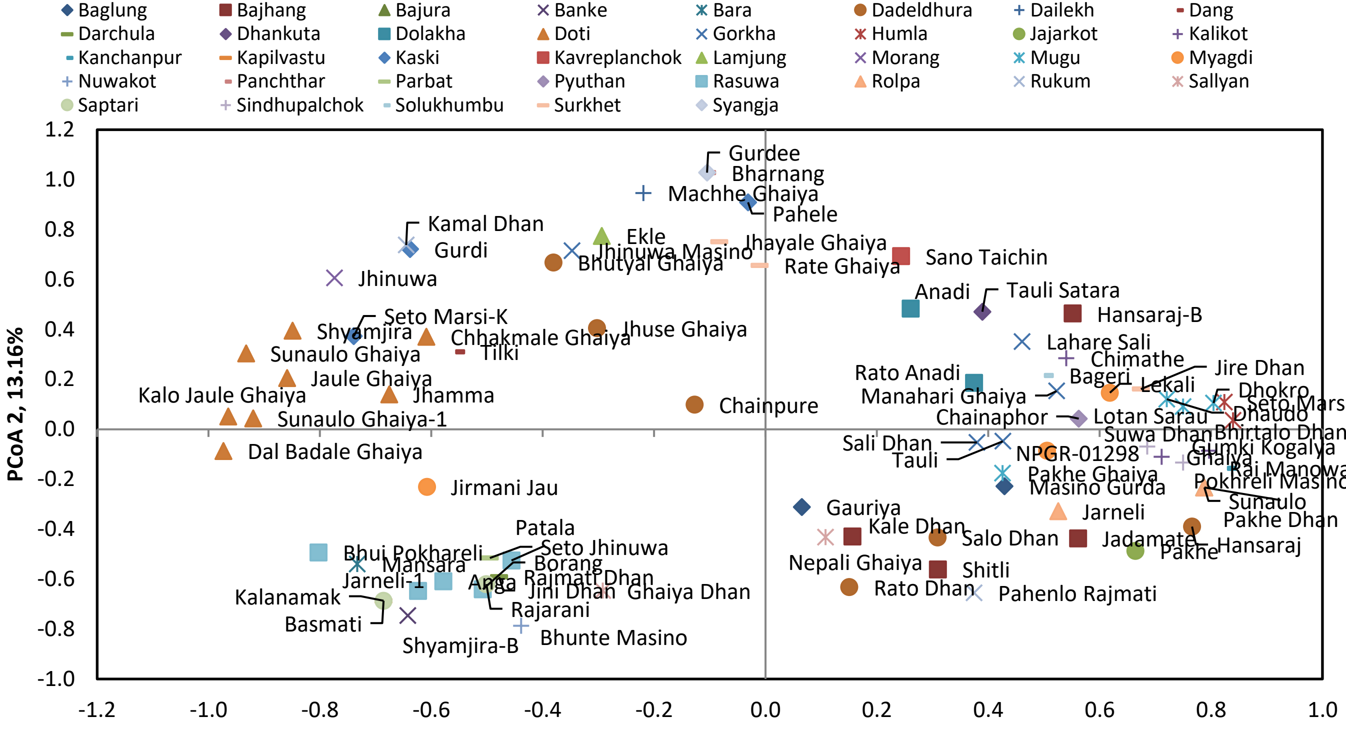

Figure 5. Plotting of 80 rice landraces based on genetic distance estimated using 19 SSR

established. Within two major clusters, nine distinct groups of rice landraces were identified. The number of landraces within these clusters varied, ranging from 3 to 15. Notably, three landraces—Tilki, Chainpure, and Jirmani Jau—formed a separate, distinct cluster. Based on the clustering analysis, several pairs of landraces were found to be similar: Gurdee and Bharnang, Rajmati Dhan and Jini Dhan, Kalanamak and Basmati, and Sunaulo and Pakhen Dhan. These pairs indicate close genetic relationships within the broader diversity of the rice landraces studied. The support value for the dendrogram with a bootstrap of 1000 well supported the relationship among the landraces. The groups were separated based on grain size as well as aroma of grain, such as Nepali Ghaiya and Pakhe represented drought tolerance landraces whereas Basmati, Kalanamk, Jarneli-1, Tilki, etc. represented aromatic rice. It showed that the aromatic rice must have a higher similarity in genomic organization as the studied SSR could not able to differentiate the aromatic landraces; however, with some exception such as Pokhreli Masino landrace (**Figure 4**).

The principal coordinate analysis also abled to differentiate the landraces in different clusters where the landraces were distributed across all the quadrant in biplot (**Figure 5**). The result suggested a higher variability among the studied landraces where the first coordinate explained 18.81% of total variation whereas the second coordinate explained 13.16 % of variation. The biplot easily able to distinguish landraces into various categories such as all the Ghaiya formed a separate cluster which were close to cluster representing aromatic rice; however, were separated by second coordinate. Sticky rice such as Anadi was distinguished from all other landraces. The scatter plot indicated that certain landraces, such as Chainpure, Jirmani Jau, Gurdee, Rato Dhan, and Pahenlo Rajmati, were positioned separately, outside the main groups of landraces. This suggests that these landraces possess unique genetic characteristics distinguishing them from the majority of the other landraces.

Discussion

Rice is one of the important crops in Nepal and it is undeniable that it is most important to have the genetic variability in the germplasm in order to improve the rice breeding program. The wild relatives as well as landraces plays important role as a source of novel variation. Theoretically, divergent parents are instrumental in defining good heterosis for the improvement of crop and in such case molecular breeding approach in defining the variability has proven efficient in comparison to highly plastic morphological variation. This study has utilized SSR markers to differentiate 80 landraces collected from different part of Nepal. The collection comprises from low land to high hills; and mostly aromatic lines were utilized along with some drought tolerant lines. The studied landraces were descended from generation to generation among farmers and could be the results of ancient selection and were highly adapted to the place of

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collection. Diversity analysis in rice has been studied by many authors across the world [21–23].

The characteristics of each primer, landrace, and district are invaluable for rice breeding, conservation, and creating dynamic populations. The primers utilized in this study are also beneficial for identifying specific trait-based landraces, as they are linked to particular economic traits. DNA fingerprinting can be employed during the submission of landraces to the National Seed Board, as DNA profiles are required [24]. This method also has the potential for monitoring landraces and ensuring they remain true to type. Some districts have shown high levels of diversity, indicating that these landraces can be repatriated to their original collection sites and used to develop location-specific varieties.

Most of the markers utilized in this study had shown polymorphism by the landraces. However, seven markers didn't show any polymorphism. Although these markers were utilized for the genetic diversity assessment [21,23) some of the markers were already tested in defining some specific trait such as brown plant hopper resistance [25), blast resistance [26–28), submergence tolerance [29), drought resistance [30,31), etc. (**Table 2**). Most of the markers related with blast resistance were monomorphic which suggested the landraces utilized in this study were mostly blast susceptible; however, the markers related with submergence tolerance had shown polymorphism indicated some of the landraces could be tolerant to flood at some instances.

The genetic relationship established among different landraces by this study will be helpful in identifying landraces in the rice breeding program. The results have shown higher variability among the landraces but there was no any particular grouping for the landraces collected from specific geographical locations. The result generated in this study might be influenced by the smaller number of markers, which would have less coverage across the genome; however, the study abled to define the genetic relationship among landraces (like by [21–23). Although two clusters were identified by dendrogram, more practically a greater number of clusters were established by principal coordinate analysis (**Figure 5**). Mansara and Jhinuwa landraces were included in the 3010 whole genome sequencing project from Asia and the result has suggested that these landraces were in different subpopulations [9); however, from our result these two landraces were clustered into same group which indicated the second cluster identified in this study has important and novel alleles which was not studied previously. Overall, this study would be useful for the rice breeders in selecting local landraces for better utilization in the overall development of rice production as well as for better conservation both on-farm and ex-situ.

Author contributions

BKJ contributed to the overall concept and planning of the research. NS, RP and RC carried out the lab works. BKJ and RC scored the gels, analyzed data and drafted the paper. All authors read and approved the final manuscript.

Competing Interests

No competing interests were disclosed

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Ethical Approval and Consent

Not applicable

Data Availability and Supplementary data

Supp. Table 1. Rice landraces and their passport used in this study

Supp. Table 2. List of SSR primers used in this study

Supp. Table 3. Summary genetic diversity statistics based on rice collection districts

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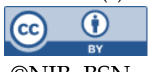

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Supp. Table 1. Rice landraces and their passport used in this study

SN	Landrace	Accession	Rice ecosystem	District	Site	Latitude (N)	Longitude (E)	Altitude (m)
1	Sano Taichin	C2716	Lowland	Kavreplanchok	Simthali-4	27.6608	85.7964	1581
2	Rato Anadi	C2731	Lowland	Dolakha	Khopachagu-3, Oligaun	27.8133	86.1017	1570
3	Anadi	C2740	Lowland	Dolakha	Babare-4, Khakalpas	27.7964	86.1186	1342
4	Chainaphor	C2962	Lowland	Pyuthan	Tiram-7, Dadakharka	28.2475	83.0189	678
5	Jire Dhan	C2999	Lowland	Surkhet	Sabiyachaur-3, Siddipur	28.985	81.4417	346
6	Hansaraj-B	C3547	Lowland	Bajhang	Banjh-8, Bagthala	29.4906	80.8981	1307
7	Tauli	NGRC01667	Lowland	Gorkha	Bakrang VDC	28.3	84.775	1494
8	Manahari Ghaiya	NGRC01670	Upland	Gorkha	Gorakhkali VDC	28.3	84.775	1707
9	Sali Dhan	NGRC01671	Lowland	Gorkha	Hanspur	28.3	84.775	2347
10	Lahare Sali	NGRC01674	Lowland	Gorkha	Kharibot	28.3	84.775	2012
11	Tauli Satara	NGRC01720	Lowland	Dhankuta	Khaireni	26.9667	87.2833	744
12	NPGR-01298	NGRC01727	Lowland	Myagdi	Tatopani	28.575	83.5083	1300
13	Lekali	NGRC01728	Lowland	Myagdi	Ghasa	28.575	83.5083	1800
14	Pokhreli Masino	NGRC01761	Lowland	Sindhupalchok	Melamchipul Bazar	27.9417	83.7917	750
15	Suwa Dhan	NGRC01772	Lowland	Sindhupalchok	Tarkeghyang	27.9417	83.7917	2400
16	Seto Marsi	NGRC01782	Lowland	Humla	Gopka	30	81.9	2100
17	Bhirtalo Dhan	NGRC01790	Lowland	Humla	Darma	30	81.9	1850
18	Dhokro	NGRC 01799	Lowland	Mugu	Tallatumma	29.55	82.1667	2350
19	Lotan Sarau	NGRC 01800	Lowland	Mugu	Karkibada	29.55	82.1667	2080
20	Dhaudo	NGRC 01803	Lowland	Mugu	Ruga	29.55	82.1667	1780
21	Bageri	NGRC 01836	Lowland	Solukhumbu	Juving	27.95	86.6917	1707
22	Pakhe Ghaiya	NGRC 01972	Upland	Mugu	Karkioda-3, Bhambada	29.55	82.1667	1960
23	Chimathe	NGRC 01975	Lowland	Kalikot	Jubitha-7, Jubitha	29.2	81.7833	1792
24	Ghaiya	NGRC 01977	Upland	Kalikot	Phatgaon-8, Piligaon	29.2	81.7833	1609
25	Gumki Kogalya	NGRC 01979	Lowland	Kalikot	Khandachakra-9, Tadi	29.2	81.7833	1768
26	Rai Manowa	NGRC 02004	Lowland	Kanchanpur	Mahendra Nagar	28.825	80.3417	195
27	Jarneli	NGRC 02012	Lowland	Rolpa	Liwang	28.325	82.6333	1270
28	Pakhe Dhan	NGRC 02015	Lowland	Rolpa	Khumil	28.325	82.6333	1500
29	Nepali Ghaiya	NGRC 02020	Upland	Sallyan	Tharmare	28.3833	83.65	1160
30	Sunaulo	NGRC 02036	Lowland	Bajura	Martadi	29.6	81.4917	2700
31	Masino Gurda	NGRC 02047	Lowland	Baglung	Kalika	28.37	82.9	957
32	Gauriya	NGRC 02052	Lowland	Baglung	Mulpani	28.37	82.9	981
33	Kale Dhan	NGRC 02062	Lowland	Bajhang	Kalukheti-8	29.875	81.2083	1737
34	Shitli	NGRC 02068	Lowland	Bajhang	Bhatekhola-5	29.875	81.2083	1829
35	Jadamate	NGRC 02069	Lowland	Bajhang	Bhatekhola-5	29.875	81.2083	1829
36	Pakhe	NGRC 02075	Lowland	Jajarkot	Rimna	28.85	82.1667	762
37	Salo Dhan	NGRC 02090	Lowland	Dadeldhura	Manara	29.2167	80.4667	1158
38	Hansaraj	NGRC 02093	Lowland	Dadeldhura	Manara	29.2167	80.4667	1128
39	Rato Dhan	NGRC 02096	Lowland	Dadeldhura	Pokhara Bagar	29.2167	80.4667	1530

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40	Pahenlo Rajmati	NGRC 02105	Lowland	Rukum	Salkhota Gaon	28.7	82.6583	812
41	Jhayale Ghaiya	NGRC 02118	Upland	Surkhet	Ganeshpur	28.7333	81.55	560
42	Rate Ghaiya	NGRC 02128	Upland	Surkhet	Ganeshpur	28.7333	81.55	560
43	Machhe Ghaiya	NGRC 02130	Upland	Dailekh	Toli	28.8917	81.7917	1300
44	Bharnang	NGRC 02138	Lowland	Panchthar	Yannam-6, Gairigaon	27.1167	87.7917	1840
45	Pahele	NGRC 02145	Lowland	Kaski	Hemja-6	28.3417	84.0083	1075
46	Gurdee	NGRC 02150	Lowland	Syangja	Yarukharka-8	28.075	83.76	1110
47	Ekle	NGRC02819	Lowland	Lamjung	Nauthar	28.2833	84.4	1402
48	Jhinuwa Masino	NGRC02821	Lowland	Gorkha	Gorakhkali VDC	28.3	84.775	1844
49	Gurdi	NGRC02875	Lowland	Kaski	Begnas tal	28.3417	84.0083	900
50	Jhinuwa	NGRC02969	Lowland	Morang	Rajghat			
51	Kamal Dhan	NGRC02987	Lowland	Rukum	Jyala	28.7	82.6583	823
52	Seto Marsi-K	NGRC02998	Lowland	Kaski	Hemja-6	28.3417	84.0083	1075
53	Sunaulo Ghaiya	NGRC03023	Upland	Doti	Sanagaun -7	29.1667	80.8833	1,218
54	Chhakmale Ghaiya	NGRC03024	Upland	Doti	Sanagaun -7	29.1667	80.8833	820
55	Shyamjira	NGRC03031	Lowland	Doti	Latamandau-9	29.1667	80.8833	572
56	Kalo Jaule Ghaiya	NGRC03033	Upland	Doti	Latamandau-9	29.1667	80.8833	572
57	Sunaulo Ghaiya-1	NGRC03038	Upland	Doti	Silgadhi-9	29.1667	80.8833	508
58	Jhamma	NGRC03040	Lowland	Doti	Silgadhi-9	29.1667	80.8833	508
59	Dal Badale Ghaiya	NGRC03041	Upland	Doti	Ballek-9, Ballek	29.1667	80.8833	610
60	Jaule Ghaiya	NGRC03045	Upland	Doti	Silgadhi-5	29.1667	80.8833	974
61	Bhutyal Ghaiya	NGRC03048	Upland	Dadeldhura	Sallaghari-10	29.2167	80.4667	1,430
62	Jhuse Ghaiya	NGRC03058	Upland	Dadeldhura	Amargadhi-2	29.2167	80.4667	1,120
63	Chainpure	NGRC03069	Lowland	Dadeldhura	Samaigee-5	29.2167	80.4667	1,140
64	Tilki	NGRC03087	Lowland	Dang	Bakhada-5, Naya basti	28.0167	85.6583	573
65	Jirmani Jau	NGRC03118	Lowland	Myagdi	Babiya Chour-5	28.575	83.5083	968
66	Seto Jhinuwa	NGRC03205	Lowland	Parbat	Khanigaun -1, Lamsal Tol	28.2083	83.6583	873
67	Rajmati Dhan	NGRC03218	Lowland	Darchula	Bramhadev-9, Kheti	29.9333	80.7417	
68	Jini Dhan	NGRC03223	Lowland	Darchula	Gokuleshwor -4, Gokuleshwor	29.9333	80.7417	
69	Patala	NGRC03227	Lowland	Rasuwa	Chilime-3, Chilime	28.1167	85.2833	2033
70	Anga	NGRC03230	Lowland	Rasuwa	Goljung-5, Goljung	28.1167	85.2833	1947
71	Borang	NGRC03234	Lowland	Rasuwa	Goljung-5, Goljung	28.1167	85.2833	1947
72	Jarneli-1	NGRC03238	Lowland	Rasuwa	Bhorle-6, Tallo Jibjibe	28.1167	85.2833	1253
73	Bhui Pokhareli	NGRC03241	Lowland	Rasuwa	Bhorle-6, Tallo Jibjibe	28.1167	85.2833	1253
74	Bhunte Masino	NGRC03246	Lowland	Nuwakot	Bidur Nagarpalika-7, Pipaltar	27.9167	85.2583	545
75	Ghaiya Dhan	NGRC03264	Upland	Sallyan	Danbang-7, Danbang	28.3833	83.65	1495
76	Basmati	NGRC03364	Lowland	Saptari	Chhinnamasta-9	26.6083	86.725	90
77	Kalanamak	NGRC03369	Lowland	Kapilvastu	Bardahawa	27.6417	82.975	100

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78	Shyamjira-B	NGRC03370	Lowland	Banke		27.05	81.8083	150
79	Mansara	NGRC03386	Lowland	Bara	Phatehpur-1, Phatehpur	27.2083	85.2333	130
80	Rajarani	NGRC03419	Lowland	Saptari	Barhibirpur-2, Hati	26.6083	86.725	103

Supp. Table 2. List of primers used in this study

SN	Primer	Forward	chr	Ta, °C	Repeat motifs	Expected size, bp	Use value	Reference
1.	RM1	F: GCGAAAACACAATGCAAAAA R: GCGTTGGTTGGACCTGAC	1	55	(GA)26	113	Sub-mergence tolerance	Matin et al 2012
2.	RM1233	F: GTGTAAATCATGGGCACGTG R: AGATTGGCTCCTGAAGAAGG	11	55	(AG)15	175	Blast resistance	Ashkani et al 2011,
3.	RM13	F: TCCAACATGGCAAGAGAGAG R: GGTGGCATTCGATTCCAG	5	55	(GA)6-(GA)16	141	Sub-mergence tolerance	Matin et al 2012
4.	RM130	F: TGTTGCTTGCCCTCACGCGAAG R: GGTCGCGTGCTTGGTTTGGTTC	3	55	(GA)10	85	Sub-mergence tolerance	Matin et al 2012
5.	RM134	F: ACAAGGCCGCGAGAGGATTCCG R: GCTCTCCGGTGGCTCCGATTGG	7	55	(CCA)7	93	Sub-mergence tolerance	Matin et al 2012, Alavi et al 2007
6.	RM168	F: TGCTGCTTGCCTGCTTCCTTT R: GAAACGAATCAATCCACGGC	3	55	T15(GT)14	116	Blast resistance	Ashkani et al 2011,
7.	RM204	F: GTGACTGACTTGGTCATAGGG R: GCTAGCCATGCTCTCGTACC	3	55	(CT)44	169	Sub-mergence tolerance, blast	Matin et al 2012, Kumbhar et al 2013
8.	RM20A	F: ATCTTGTCCCTGCAGGTCAT R: GAAACAGAGGCACATTTCATTG	12	57	(ATT)14	70-95	Diversity, drought	Umadevi et al 2014, Sun et al 2008
9.	RM21	F: ACAGTATTCCGTAGGCACGG R: GCTCCATGAGGGTGGTAGAC	11	56	(GA)18	157	Pi44, diversity	Eizenga et al., 2006, Kumar et al 2012
10.	RM211	F: CCGATCTCATCAACCAACTG R: CTTCACGAGGATCTCAAAGG	2	55	(TC)3A(TC)18	161	Sub-mergence tolerance	Matin et al 2012
11.	RM212	F: CCACTTTCAGCTACTACCAG R: CACCCATTTGTCTCTCATTATG	1	55	(CT)24	136	Drought, blast resistance	Kanagaraj et al 2010, KOIDE et al 2009
12.	RM25	F: GGAAAGAATGATCTTTTCATGG R: CTACCATCAAAACCAATGTTC	8	55	(GA)18	146	Sub-mergence tolerance	Matin et al 2012
13.	RM264	F: GTTGCGTCCTACTGCTACTTC R: GATCCGTGTCGATGATTAGC	8	56	(GA)27	178	Diversity	Kumar et al 2012
14.	RM302	F: TCATGTCATCTACCATCACAC R: ATGGAGAAGATGGAATACTTGC	1	55	(GT)30(AT)8	156	Drought resistance, blast	Kanagaraj et al 2010, Kumbhar et al 2013
15.	RM3825	F: AAAGCCCCCAAAAGCAGTAC R: GTGAAACTCTGGGGTGTTCG	1	55	(GA)21	147	MQTL1.1 (drought)	Gomez et al. 2009; Kanagaraj et al. 2010).
16.	RM401	F: TGGAACAGATAGGGTGTAAGGG R: CCGTTCACAACACTATACAAGC	4	59/55	(CT)15	283	Diversity	Umadevi et al 2014
17.	RM413	F: GGCGATTCTTGGATGAAGAG R: TCCCCACCAATCTTGTCTTC	5	55	(AG)11	79	Blast resistance, Sub-mergence tolerance	Ashkani et al 2011, Matin et al 2012
18.	RM452	F: CTGATCGAGAGCGTTAAGGG R: GGGATCAAACCACGTTTCTG	2	55	(GTC)9	209	Sub-mergence tolerance	Matin et al 2012
19.	RM463	F: TTCCCCTCCTTTTATGGTGC R: TGTTCTCCTCAGTCACTGCG	12	55	(TTAT)5	192	Brown plant hopper resistance, Sub-mergence tolerance	Li-Hong et al 2006, Matin et al 2012
20.	RM47	F: ACTCCACTCCACTCCCCAC R: GTCAGCAGGTCGGACGTC	7	56	(AG)7(AG)11	100-220	Diversity	Kumar et al 2012
21.	RM536	F: TCTCTCCTCTTGTTTGGCTC R: ACACACCAACACGACCACAC	11	59	(CT)16	243	Diversity	Umadevi et al 2014
22.	RM561	F: GAGCTGTTTTGGACTACGGC R: GAGTAGCTTTCTCCCACCCC	2	56	(GA)11	190	Diversity	Kumar et al 2012, Becerra et al 2017

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23.	RM586	F: ACCTCGCGTTATTAGGTACCC R: GAGATACGCCAACGAGATACC	6	55	(CT)23	271	Sub-mergence tolerance	Matin et al 2012, Wang et al 2011
24.	RM5862	F: TTAGTACCTCATCATAGCTG R: CTCTAATCTTCTCTCATTATCA	2	60	(ATA)28	223	Diversity	Umadevi et al 2014
25.	RM5961	F: GTATGCTCCTCCTCACCTGC R: ACATGCGACGTGATGTGAAC	11	55	(CAG)8	129	Blast resistance	Ashkani et al 2011,
26.	RM6836	F: TGTTGCATATGGTGCTATTTGA R: GATACGGCTTCTAGGCCAAA	6	55	(TCT)14	240	Pi-z (blast resistance)	Gous Meah et.al. 2014, Ashkani et al 2011
27.	RM7102	F: TTGAGAGCGTTTTTAGGATG R: TCGGTTTACTTGGTTACTCG	12	55	(AGAT)8	169	Brown plant hopper resistance	Li-Hong et al 2006
28.	RM7575	F: GGTTTGATCTCGCGTCTCTC R: GCCAGCAGCGAGAGAGATAG	2	61	(TCTA)8	118	Diversity	Umadevi et al 2014
29	RM8225	F: ATGCGTGTTCAGAAATTAGG R: TTGTTGTATACCTCATCGACAG	6	55	A11N(AAG)14	221	Blast resistance	Ashkani et al 2011,

Supp. Table 3. Summary genetic diversity statistics for district

SN	District	Major Allele Frequency	Genotype No	Sample Size	Allele No	Gene Diversity	Heterozygosity	PIC
1.	Baglung	0.84	1.37	2	1.37	0.17	0.05	0.13
2.	Bajhang	0.78	1.79	4	1.79	0.27	0.07	0.23
3.	Bajura	0.97	1	1	1.05	0.03	0.05	0.02
4.	Banke	1	1	1	1	0	0	0
5.	Bara	1	1	1	1	0	0	0
6.	Dadeldhura	0.72	2.21	6	2.16	0.34	0.06	0.29
7.	Dailekh	0.97	1	1	1.05	0.03	0.05	0.02
8.	Dang	1	1	1	1	0	0	0
9.	Darchula	0.97	1	2	1.05	0.03	0.05	0.02
10.	Dhankuta	1	1	1	1	0	0	0
11.	Dolakha	0.86	1.32	2	1.32	0.15	0.03	0.11
12.	Doti	0.82	1.68	8	1.68	0.24	0.01	0.19
13.	Gorkha	0.77	2	5	2.05	0.3	0.06	0.26
14.	Humla	0.95	1.11	2	1.21	0.07	0.05	0.06
15.	Jajarkot	0.97	1	1	1.05	0.03	0.05	0.02
16.	Kalikot	0.93	1.26	3	1.26	0.1	0.04	0.08
17.	Kanchanpur	0.97	1	1	1.05	0.03	0.05	0.02
18.	Kapilvastu	1	1	1	1	0	0	0
19.	Kaski	0.76	1.74	3	1.74	0.28	0.02	0.23
20.	Kavreplanchok	0.92	1	1	1.16	0.08	0.16	0.06
21.	Lamjung	0.97	1	1	1.05	0.03	0.05	0.02
22.	Morang	1	1	1	1	0	0	0
23.	Mugu	0.82	1.68	4	1.68	0.24	0.01	0.2
24.	Myagdi	0.8	1.63	3	1.68	0.25	0.04	0.2
25.	Nuwakot	1	1	1	1	0	0	0
26.	Panchthar	1	1	1	1	0	0	0
27.	Parbat	0.97	1	1	1.05	0.03	0.05	0.02
28.	Pyuthan	0.89	1	1	1.21	0.11	0.21	0.08
29.	Rasuwa	0.85	1.58	5	1.58	0.19	0.01	0.16
30.	Rolpa	0.86	1.26	2	1.32	0.15	0.08	0.11
31.	Rukum	0.68	1.63	2	1.63	0.32	0	0.24
32.	Sallyan	0.76	1.47	2	1.58	0.25	0.08	0.2
33.	Saptari	0.96	1.11	2	1.11	0.05	0.03	0.04
34.	Sindhupalchok	0.95	1.05	2	1.11	0.05	0.05	0.04
35.	Solukhumbu	1	1	1	1	0	0	0
36.	Surkhet	0.8	1.63	3	1.68	0.25	0.04	0.2
37.	Syangja	1	1	1	1	0	0	0

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