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Diversity among different populations of a locally common rice (*Oryza sativa* L.) landrace from north-western Indian Himalayas

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Abstract

Genetic structure of five populations of a locally common rice (*Oryza sativa* L.) landrace Jaulia from parts of Uttarakhand state of India was studied using sequence tagged microsatellite site (STMS) markers. Of these, four populations were on-farm managed, assembled from different niche environments, and one population was conserved *ex situ* and represented static conservation. The 16 STMS primer pairs fully differentiated the inter- and intrapopulation diversity. A total of 72 alleles were recorded with a mean of 4.5 alleles per locus. Population wise, the total number of alleles ranged from 21 to 41, with maximum number of alleles for population IC 548358 and minimum number of alleles for population IC 100051 representing static conservation. A greater number of alleles specific to populations under farmer management could be recorded. Changes in yield parameters also seemed to be affected under farmer management besides other environmental adaptations for qualitative morphological characters. The marker diversity using STMS primer pairs indicates the genetic differentiation among populations resulting from joint effects of several evolutionary forces operating within the historical and biological context of the crop landrace. The variations in adaptations, on the other hand, indicate the degree to which populations are adapted to their environments and their potential for continued performance or as donors of characters in plant breeding. Both biotic and abiotic aspects of the environment are involved.

Keywords: *in situ* conservation on farm; landrace population structure; *Oryza sativa* L; rice; static conservation

Introduction

Farmers' landraces under continuous on-farm cultivation may undergo evolutionary change. Such a change might occur if farmers keep growing their traditional seeds while cultivation is being intensified. Brown (2000) reviewed and critically discussed the *in situ* conservation on farm of crop genetic resources with proposed advantages. Soleri and Smith (1995), working with maize varieties, demonstrated loss of diversity in the material maintained *ex situ* in gene bank compared with the same material maintained *in situ* on farm. The differences were interpreted to be due to genetic drift or shift, and were not associated with crop evolution under specifically changed environments. Allowing for continued evolution, however, has been mentioned as one of the advantages of such a conservation method (Jana and Khangura, 1986; Le Boulc'h *et al.*, 1994). Tin *et al.* (2001) studied the diversity and adaptedness of the same-named farmers' varieties of rice under static (*ex situ*) and dynamic (*in situ*) management. No significant

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change in allelic frequencies of isozymes was observed except in the adaptedness, which is at risk under on-farm conservation. Natural and intentional selection will normally not remain constant over time. Consequent genetic changes included loss of adaptation to old conditions and adaptation to new conditions.

Measuring the average diversity of a field and differences in allele frequencies and in levels of polymorphism among populations will help in devising a rational conservation plan on farm. Diversity analysis will provide information on the genetic distinctiveness of farmer-named varieties and genetic similarity/distinctness among populations of the same-named landrace grown by different farmers in specific niche environments (Kumar *et al.*, 2010). Diversity analysis at different spatial scales will enable comparisons at community and regional levels, such as whether the crop population of one village represents all the genetic diversity in the region. Some recent studies have reported considerable crop genetic diversity being maintained on farm in the form of traditional crop varieties, as is the increase in genetic diversity of rice even when the number of varieties decreases in traditional productions (Jarvis *et al.*, 2008; Steele *et al.*, 2009).

The present research aimed at investigating the potentials and consequences of on-farm management of different populations of a locally common upland rice landrace Jaulia in marginal agro-ecosystems of north-western Indian Himalayas in Uttarakhand state. The major objectives of the study were to understand the dynamics of farmer management of different populations of the same-named landrace in specific niche environments through a study of inter- and intrapopulation molecular diversity at microsatellite loci and morphological characterization for environmental adaptations. In fact, two measures of diversity in rice landrace populations have been studied. The first measure is marker diversity using sequence tagged microsatellite site (STMS) primer pairs, largely revealing information as to the ancestry or breeding history of the populations and other population genetic parameters. They are indicators of the recency of bottlenecks in population size, the prevalence of outcrossing, the ease with which genes are recombined, the level of gene flow etc. The second measure is variations in adaptations. This set comprises indicators of the degree to which populations are adapted to their environments and of their potential for continued performance or as donors of characters in plant breeding.

Materials and methods

The experimental materials comprised five populations of a locally common landrace Jaulia from parts of Uttarakhand state in north-western Himalayas. Of these, one population represented static (*ex situ*) conservation (collected and conserved since 1991), and was taken from the National Gene Bank at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. The remaining four populations were collected directly from farmers' fields during 2006 cropping season for the present study, and represented dynamic conservation on farm in specific niche environments.

The on-farm managed populations were collected from four specific niche environments spanning about 4000–5000 km^2 geographical area of Uttarakhand state, with the altitudes ranging from 1000 to 1800 masl. The impact of improved modern varieties, in general, is low in the region, and replacement of local landraces seldom exceeds 20–30% even in valleys under assured irrigation. In these mountainous regions, no formal *in situ* (on-farm) programmes are initiated; yet, largely traditional agriculture is practised. Proper sampling strategies were followed for collecting the four on-farm managed populations, whereas limited information is available on the sampling strategies of one population conserved *ex situ* in the gene bank at NBPGR, which was used in the present study.

The DNA extracted from 30 individual plants per population was used for STMS analysis. Genomic DNA was extracted using the CTAB method (Saghai-Maroof *et al.*, 1984). DNA samples were diluted to a working concentration of approximately 10 ng/μl. Each 25-μl PCR mixture contained 3.0 mM MgCl$_2$, 1 U *Taq* DNA polymerase, 200 μM dNTP, 0.2 μM STMS primers and 30 ng genomic DNA in 10 mM Tris–HCl and 50 mM KCl, pH 8.3. The amplification regime as detailed in the Cornell University website www.gramene.com was followed. The gels were stained with ethidium bromide, and were viewed under UV light. Patterns were scored for the presence of each allele in an accession.

Thirty STMS primers were screened to identify suitable primers for detailed molecular diversity analysis of rice landrace populations. The primers were selected from the sequence information obtained from the Cornell University website. Of the 30 primers screened, 16 informative primer pairs were used in the present study for the final STMS analysis. Frequency of an allele in each population was calculated. The statistical analysis was performed using POPGENE 32 (Yeh *et al.*, 2000).

The landrace populations were also grown for agro-morphological diversity in an on-station field experiment at the NBPGR Regional Station, Bhowali (Uttarakhand), situated at about 1800 masl. All five populations of the landrace were planted on 4-m^2 plots in a complete randomized block design with three replications during the rainy season of 2007 and 2008 following standard agronomic practices. Five control varieties were also included in on-station trials for comparison. The control varieties included VL Dhan 206, VL Dhan 207, VL Dhan 208, VL Dhan 209 and Majhera 7. These varieties have been developed under public institutional programmes by Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, for Uttarakhand state for upland spring rice cultivation. On each plot, 20 plants were randomly chosen for morphological characterization. Observations on 22 characters, 15 quantitative and 7 qualitative, were recorded. Data on quantitative traits were statistically analysed using INDOSTAT statistical software developed at the INDOSTAT Services, Hyderabad.

Results

Primer screening and properties of STMS markers

All the 16 primers were polymorphic. A total of 72 alleles were detected in five populations of Jaulia using 16 STMS markers. The number of alleles per locus and predominant allele size, individual population wise and overall, are presented in Table 1. The number of alleles ranged from 2 to 8, with an average of 4.5 alleles per locus. The overall size of the amplified products ranged from 100 bp (RM228) to 250 bp (RM19).

Genetic structure of different populations

The allelic structure of all the five populations is presented in Supplementary Table S1 (available online only at http://journals.cambridge.org). A maximum of eight alleles were recorded for STMS loci RM250 followed by RM206 (seven alleles) and RM216 (six alleles). A total of 72 alleles were recorded, of which, 66 were common and 6 were rare. Population wise, the total number of alleles ranged from 21 to 41, with maximum number of alleles for population IC 548358 and minimum number of alleles for population IC 100051 representing static conservation. Similarly, population wise, the number of common alleles varied from 20 (IC 100051) to 35 (IC 548358). The number of rare alleles varied from 1 (IC 100051) to 6 (IC 548363). Population genetic parameters of the five populations of the landrace are presented in Table 2. The genetic diversity in terms of Shannon's information index was highest (0.54) for the population IC 548358 and was lowest (0.07) for the population IC 100051, as was the expected heterozygosity, which was highest (0.30) for the former and lowest (0.03) for the

Table 1. The number of alleles per locus, their allele size range and predominant alleles in different populations

			Allele size (bp)					
			Predominant allele size (bp)					
Locus	No. of alleles	Range	P1	P2	P3	P4	P5	Overall
RM206	7	130–200	150	155	150	160	160	160
RM19	5	200–250	230[a]	250	230	230	210	230
RM21	4	120–150	140[a]	140	140	140	140	140
RM255	3	145–165	155	155	145	155	155	155
RM235	3	105–140	120	120	120	120	120	120
RM217	4	140–170	165[a]	140	165	165	165	165
RM249	2	125–145	125[a]	125	125[a]	125	125	125
RM216	6	120–200	140[a]	200	135[a]	135	130	135
RM228	5	100–130	130[a]	110	110	110	102	110
RM234	5	130–160	140[a]	150[a]	150	150	130	150
RM247	5	140–160	140[a]	150	150	145	160	150
RM250	8	130–180	140	150	150	150	175	150
RM263	3	190–200	190[a]	195[a]	200	190	195	195
RM201	3	150–165	165[a]	160[a]	160	150[a]	150	160
RM104	5	120–150	140[a]	145[a]	150[a]	135[a]	120	140
RM3262	4	160–180	170	170	170	175[a]	175[a]	175
Total	72	–						–
Mean	4.5	–						–

Population P1 (IC 100051) represents static conservation, whereas populations P2–P5 (IC 548358, IC 548363, IC 548639 and IC 548668, respectively) are on-farm managed.
[a] Allele sizes represent monomorphic loci.

Table 2. Summary diversity analysis of five rice landrace populations based on STMS primers

Landrace populations	Total no. of alleles detected	Na	Ne	I	Ho	He	No. of polymorphic loci	Polymorphic loci (%)
IC 100051[a]	21	1.31 ± 0.47	1.04 ± 0.06	0.07 ± 0.11	0.01 ± 0.04	0.03 ± 0.06	5	31.25
IC 548358	41	2.56 ± 1.26	1.69 ± 0.84	0.54 ± 0.46	0.02 ± 0.04	0.30 ± 0.25	12	75.00
IC 548363	38	2.25 ± 0.85	1.46 ± 0.46	0.42 ± 0.32	0.04 ± 0.06	0.26 ± 0.21	13	81.25
IC 548639	33	2.06 ± 0.68	1.29 ± 0.26	0.34 ± 0.25	0.00 ± 0.00	0.19 ± 0.16	13	81.25
IC 548668	29	2.37 ± 0.71	1.34 ± 0.25	0.42 ± 0.23	0.00 ± 0.00	0.23 ± 0.14	15	93.75

Na, observed no. of alleles; Ne, effective no. of alleles; I, Shannon's information index; Ho, observed heterozygosity; He, expected heterozygosity.
[a] Populations representing static (*ex situ*) management since 1991 (taken from the National Gene bank of NBPGR, New Delhi).

Table 3. Variations among rice landrace populations for important quantitative traits

Landrace populations	DF	PHT	FLL	FLW	PL	GPP	TPP	DM	GL	GW	GWT	YPP	SYP
IC 100051	135.7	136.9	28.5	1.69	15.7	110.7	8.67	175.0	0.65	0.29	3.57	10.33	81.0
IC 548358	136.0	149.0	36.9	1.59	18.7	77.7	7.67	176.7	0.55	0.28	3.33	8.80	59.0
IC 548363	134.0	166.1	35.3	1.66	23.1	128.9	7.40	174.7	0.47	0.36	3.48	5.33	75.0
IC 548639	134.0	137.0	30.1	1.56	26.6	91.1	9.20	172.0	0.64	0.26	3.25	7.67	92.3
IC 548668	134.7	166.8	31.2	1.63	16.3	123.9	8.93	174.0	0.67	0.34	3.69	15.00	78.3
VL Dhan 206 (control)	133.3	129.1	27.1	1.46	18.8	124.1	9.13	169.1	0.71	0.28	3.93	11.23	81.2
VL Dhan 207 (control)	135.7	123.9	33.4	1.55	21.0	122.4	7.8	171.0	0.74	0.34	3.25	13.21	67.6
VL Dhan 208 (control)	139.3	123.5	31.5	1.61	19.3	114.3	9.13	175.3	0.66	0.29	3.32	10.56	81.2
VL Dhan 209 (control)	132.3	135.7	31.1	1.60	19.7	124.9	7.0	171.0	0.70	0.25	3.75	12.23	62.2
Majhera 7 (control)	131.3	128.9	28.9	1.49	22.6	138.2	12.2	172.3	0.70	0.28	3.92	15.12	78.2
Overall mean	134.6	139.7	31.4	1.58	20.2	115.6	8.71	173.1	0.65	0.30	3.55	10.95	75.6
C.V. %	0.92	8.28	5.25	6.90	6.52	3.93	7.14	0.59	5.89	6.59	4.34	13.98	3.89
C.D. (5%)	NS	35.57	3.20	NS	2.45	7.40	1.13	1.93	0.07	0.04	0.28	2.48	5.65

DF, days to flowering; PHT, plant height (cm); FLL, flag leaf length (cm); FLW, flag leaf width (cm); PL, panicle length (cm); GPP, no. of grains/panicle; TPP, no. of tillers/plant: DM, days to 80% maturity; GL, grain length (cm); GW, grain width (cm); GWT, 100 grains weight (g); YPP, yield/plant (g); SYP, straw yield/plant (g).

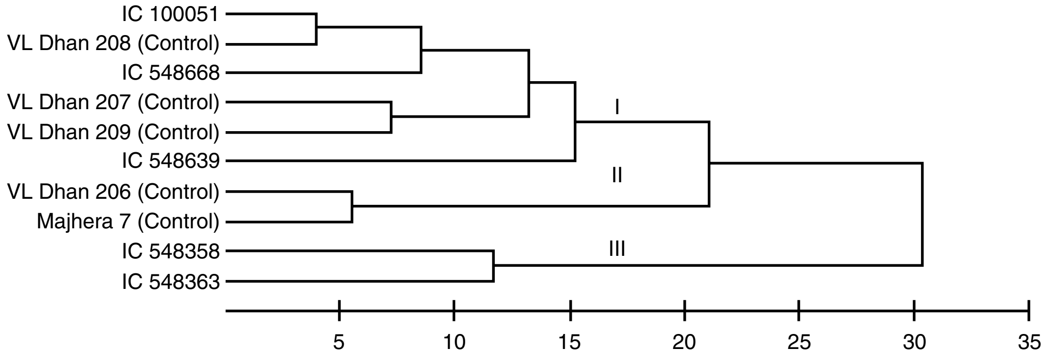

Fig. 1. Ward's minimum variance dendrogram of rice landrace populations and control varieties based on quantitative data.

latter. A maximum of 11 loci were monomorphic for IC 100051 representing static conservation.

The four on-farm managed populations represented 63 alleles at the 16 STMS loci analysed. Of these, 16 alleles were present in all the four on-farm managed populations, and could be categorized as the common and widespread alleles. A total of 11 and 10 alleles were present in three and two populations, respectively. There were a total of 26 unique alleles which were present in any one of the four populations. In the landrace population under static management, there were a total of 21 alleles for all 16 loci, of which, 19 were still present in the on-farm managed populations with varying frequencies, whereas 2 alleles were not recorded in any of the four on-farm managed populations. A total of 38 alleles were new and specific only to the on-farm managed populations. The frequency of rare alleles was more for on-farm managed populations.

The clustering pattern revealed that the population under static management (IC 100051) was relatively closer to IC 548363 and IC 548639, and almost equidistant to IC 548668 and IC 548358 (Supplementary Table S2, available online only at http://journals.cambridge.org). Populations IC 548358 and IC 548363 were the closest and grouped together (Supplementary Fig. S1, available online only at http://journals.cambridge.org).

On-station trials for adaptive variations

The range of variations for important quantitative traits is presented in Supplementary Table S3 (available online only at http://journals.cambridge.org). Wide variations for various agronomic traits such as grain yield per plant, panicle length, number of grains per panicle, straw yield per plant, grain length and width were recorded among different populations of Jaulia. Least variations were observed for days to flowering and maturity. Except for one population (IC 548668), significant reduction in yield-related traits was recorded for the other three remaining on-farm managed populations compared with the population under static management, IC 100051 (Table 3). All control varieties were superior in yield compared with the landrace populations except one on-farm managed population, IC 548668, which was at par or even better than the controls. Barring the presence of aroma and presence of awn, not much variation was recorded for other qualitative traits viz. panicle exertion, seed coat colour, husk colour and threshability.

The clustering pattern revealed that the populations IC 100051, IC 548668 and IC 548639 formed a separate cluster together with three control varieties (Fig. 1). These three populations, including the population representing static conservation, could be characterized with high yield potential. Populations IC 548358 and IC 548363 together forming a separate cluster could be characterized as dual-purpose types with relatively greater straw yield (Supplementary Table S4, available online only at http://journals.cambridge.org).

Discussion

In situ conservation on farm has been considered as a back-up and complementary strategy to *ex situ* conservation. On-farm conservation is now considered as an enhanced plant genetic resource (PGR) utilization at the local level, and is consistent with agricultural development in marginal environments and small-farm situations (Brush, 1991, 2004, Worede and Mekbib, 1993; Maxted *et al.*, 1997, 2002; Bellon *et al.*, 1997; Worede *et al.*, 1999; Feyissa, 2000; Jarvis *et al.*, 2004; Bisht *et al.*, 2006, 2007; Jarvis and Hodgkin, 2008; FAO, 2009). The recent FAO State of the World Reports on PGR for Food and Agriculture (FAO, 2009) has documented that scientific principles do exist for *in situ* conservation on farm, but they need to be adapted at a more wider scale. Smale (2006) and Smale *et al.* (2004) have discussed methods for identifying least cost sites where significant crop genetic diversity, high environmental heterogeneity and high local socio-cultural conditions exist. The highland areas worldwide are, therefore, considered to be the ideal sites for *in situ* conservation on farm. *Ex situ* and *in situ* conservation on-farm strategies are, however, yet to be systematically researched, and the complementary roles of these strategies are yet to be fully understood and developed. Often, the baseline data on the original composition of landraces are not available to assess the trend of genetic change in space and time.

All the 16 STMS loci were variants for 30 individuals in each of the five populations of the landrace analyzed (Table 1). Low allelic richness was, however, recorded for the landrace population under static management compared with on-farm managed populations. Methodologically, homonymous populations collected from the same farm and pair-wise comparison for allelic composition made between these populations for the two collection dates would have been desirable. Due to the fact only one population was available under *ex situ* management for comparison in the present study and the on-farm managed populations were from different farms of the region, the analysis could only reveal overall increase in intra-accession genetic diversity over time.

The temporal evolution of genetic diversity is, however, not conclusive. The results must be considered as an indication of low diversity of the populations under static management. Substantially high number of alleles in on-farm managed populations may probably be due to sampling variation, as we cannot expect generation of new allelic variations in a short duration of 15–16 years in the evolutionary time scale. Increase in intra-varietal diversity over continuous cultivation has, however, been reported in several other studies (Bezancon *et al.*, 2005, 2008; Bary *et al.*, 2008). Rice genetic diversity from high altitude region of Nepal was predicted to increase even when a number of local landraces are displaced by modern varieties (Steele *et al.*, 2009). Partial replacement of landraces increased genetic diversity if the modern varieties were adopted on up to 65% of the area. Only above these levels did overall diversity decline. High allelic richness and diversity under dynamic on-farm management of rice landraces have also been reported from Indian Himalayas (Kumar *et al.*, 2010).

The landrace populations differed significantly among themselves with respect to yield potential (Table 3). Not much variation was, however, recorded for qualitative traits. The yield potential of one population representing static conservation was relatively high compared with the majority of the on-farm managed populations except IC 548668. No change with respect to grain yield was, however, reported in some previous investigations (Tin *et al.*, 2001). The control varieties, in general, were superior in grain yield than majority of the on-farm managed Jaulia populations, depicting their wider environmental adaptation. Jaulia landrace population IC 548668 was at par with all other control varieties developed in the institutional crop improvement programmes for the Himalayan region. The findings indicate that though there may be new adaptive variations under farmer selection for different populations of the same-named landrace, selections, most of the time, are definitely not for yield. Except for one population (IC 548668), significantly low level of yield was recorded for the other three remaining on-farm managed populations compared with the population under static management, IC 100051. High yield potential of one population suggests that farmers often make conscious selection for yield also. In hilly areas, rice straw is an important source of fodder. Thus, farmers normally prefer dual-purpose varieties having good straw along with grain yield.

The clustering pattern revealed a greater similarity between STMS diversity and variations due to environmental adaptations. It is important for us to understand how farmers make use of their crops' agro-morphological characteristics in different capacities, particularly selecting among the plants in the crop populations to maintain the desirable characteristics and to increase the prevalence of other valued traits in the population over time (Jarvis *et al.*, 2000). Gathering this information requires investigations and discussions with farmers at different stages of plant growth throughout the growing season.

Genetic diversity and divergence, in fact, require assessment for two sets of attributes, analogous to the characterization and evaluation data of genetic resources (Brown, 2000). The first set is marker diversity or the extent of differences between individual copies of genes. This set of attributes is informative as to the ancestry or breeding history of the populations. They are indicators of the recency of bottlenecks in population size, the prevalence of outcrossing, the ease with which genes are recombined and the level of gene flow between populations. The second set is variation in adaptation. This set comprises indicators of the degree to which populations are adapted to their environment and of their potential for continued performance or as donors of characters in plant breeding. Both biotic and abiotic aspects of the environment are involved.

The population genetic parameters can therefore be helpful in measuring the average genetic diversity of a field/village/region, the differences in allele frequencies among different populations and the differences in the level of polymorphism among populations. The differences in different populations of the same-named landrace highlight the role of various evolutionary forces and provide us baseline data to monitor the future loss of diversity in space and time. Furthermore, the selection process of seeds for the next generation is one important force that will direct or maintain characteristics of the population over time. When new genetic diversity becomes apparent in a population, whether from within a population (by hybridization, introgression or mutation) or outside a population (by migration as farmers introduce new seeds), farmers may select for or against the new characteristics, depending on their level of desirability. Farmer selection may be confounded with environment selection over time. Since plants with traits best adapted to the specific environment have better chance of survival, the seeds for the next crop will contain a larger proportion of these adapted and preferred traits than the seeds used for the previous crop. Indeed, one of the challenges of *in situ* (on-farm) conservation research is to evaluate how economic development is affecting farmer maintenance of diversity so as to account for this process in the implementation of conservation initiatives. Appropriate policy support is required to support farming systems maintaining high diversity if the opportunity costs of conservation become too high for farmers to continue cultivating diverse landraces. However, the level of actual genetic diversity being maintained by farming systems must be evaluated before attempting such a costly endeavour.

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