

Research Article**Assessment of genetic diversity among rice (*Oryza sativa* L.) landrace populations under traditional production using microsatellite (SSR) markers**

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Abstract

Despite the surge of support for on farm conservation of plant genetic resources on global scale, no agreed set of scientific principles yet exists for its effective implementation. Farming communities in traditional agroecosystem have been playing an important role in conserving agricultural diversity and assessment at genetic level is a prerequisite for understanding detrimental evolutionary patterns and devising suitable strategies for their conservation and sustainable use. The present investigation was undertaken with the objectives of understanding farmer management of population structure of rice landraces in traditional farming systems as well as inter- and intra-population molecular diversity at microsatellite loci. The microsatellites (STMS) markers were used for analysing selected eleven rice landrace populations from various parts of Uttarakhand state in north-western Himalayas. A total number of 98 alleles were recorded, of which 91 were common and seven were rare. The mean number of alleles per locus was 6.13 and for different groups of rice landrace populations, namely eight populations of common landrace and three populations of rare landraces were 4.96 and 4.37, respectively. The study also compared genebank-conserved (*ex situ*) and on-farm-managed (*in situ*) landrace populations of same named common landraces Jaulia and Thapachini, and revealed greater number of alleles per locus for on-farm-managed populations as compared to the populations under static management. Significant number of alleles specific to populations under dynamic management could also be recorded. Changes in yield parameters also seemed affected under dynamic farmer management for same rice landrace populations. Further, the rare landraces included in the present study were more diverse than the common landrace populations. The rare landraces were distinct entities largely representing locally common alleles. Genetic differentiation results from the joint effects of various evolutionary forces, which in turn must operate within the historical and biological context of the crop. The present investigation about the population genetic structure is therefore helpful in monitoring diversity loss over time and space, and also for devising a rational conservation plan, particularly on-farm management of farmer landraces under traditional production.

Key words: On-farm conservation, Rice, landrace population structure, Microsatellite markers, genetic diversity

Introduction

Dynamic (*in situ*) and static (*ex situ*) conservation of biodiversity for agriculture and forestry are complementary approaches. The emphasis on *in situ* and *ex situ* approaches will, however, depend on the conservation context-the object, aims and location of conservation. Keeping in view for each situation, an understanding and assessment of the crop genetic diversity is a prerequisite as well as fundamental step (Bisht et al.2006). Conservation *in situ*, with the help of local communities and

farmers ensures that resources remain directly in the hands of primary users. Individual farmers and farming communities in traditional agroecosystem have been playing an important role in conservation of crop genetic diversity. They are not only the custodian but also managers of the agricultural diversity and also they maintain the dynamic process of crop evolution as well as their adaptation. Diversity in farmer varieties is thought to be related to population size and heterogeneity in the environment conditions where they are grown. Farmer's landraces under continuous on-farm cultivation may undergo evolutionary change. Such change might occur if farmers keep growing their traditional seeds while cultivation is being

intensified. Over the last three to four decades, large scale *ex situ* build up of plant genetic resource (PGR) collection has been clearly seen across the globe. The genebanks have been helpful in successfully conserving crop seed viable and safeguarding against genetic erosion resulting from technological changes and agro-ecological destruction. However, soon after the inception of *ex situ* conservation both biological and social scientists have started questioning the adequacy of *ex situ* conservation strategy mainly on grounds of being static and for detaching the collection from local knowledge systems. As a result, *in situ* conservation has been considered as back-up and complementary strategy to *ex situ* conservation and models for its implementation has also been suggested (Maxted et al. 1997, 2002). *In situ* conservation has now been considered as enhanced PGR utilization at the local level and consistent with agricultural development (Worede and Mekbib, 1993; FAO 1998; Worede et al., 1999). As it stands now there seems to be a *de facto* conservation of landraces that farmers have been practicing for centuries as part of their farming system (Seboka and van Hintum, 2006). Despite the surge of support for on-farm conservation of PGR on global scale, no agreed set of scientific principles yet exists for its implementation.

The north-western Indian Himalayan regions are the reservoir for a large number of crop genetic resources because of the preponderance of locally developed traditional crop varieties owing to high agro-climatic heterogeneity and local social cultural diversity. Traditional agroecosystem in the Himalayan region are very diverse and crop husbandry, animal husbandry and forests constitute complex and interlinked production system. However, there has been gradual reduction of traditional crop diversity in this region during the last three to four decades which require adequate attention of researcher and policy makers for its safe conservation and sustainable utilisation for agricultural production (Singh et al. 1984, Bisht et al. 2007). 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 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 as well as regional levels. Some recent studies have reported considerable crop genetic diversity being maintained on farm in the form of traditional crop varieties, as in the increase in the genetic diversity of rice even when the number of varieties decreases in traditional production (Jarvis et al. 2008; Steele et al. 2009).

The present research investigation was undertaken aimed at understanding farmer management of population structure of rice landraces in traditional farming systems as well as inter- and intra-population molecular diversity at microsatellite loci and morphological characterisation. This study also compared gene bank conserved (*ex situ*) and on-farm managed (*in-situ*) landraces population of same named landraces.

Materials and Methods

The experimental materials comprised of eleven rice landraces populations from different parts of Uttarakhand state in north western Himalayas. Out of eleven, five populations represent locally common upland landraces Jaulia, three populations of a common irrigated landrace Thapachini and three different locally grown rare landraces population. Of these, one population of Jaulia and Thapachini represented static conservation (collected and conserved since 1991) and was collected from the National Gene Bank at the National Bureau of Plant Genetic Resources (NBPGR) whereas remaining population were directly collected from farmer's field in 2006. These populations represented dynamic conservation on farm in specific niche environments. Proper sampling strategies were followed for collecting the on-farm managed populations. 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 $10\text{ng}\mu\text{l}^{-1}$. Each $25\mu\text{l}$ PCR contained 3.0mM MgCl_2 , 1U *Taq* DNA polymerase, $200\mu\text{M}$ dNTP, $0.2\mu\text{M}$ STMS primers and 30ng genomic DNA in 10mM Tris-HCl, 50mM KCl pH8.3. The amplification regime included an initial denaturation step ($95^\circ\text{C} / 5\text{min}$), followed by 40 cycles of $94^\circ\text{C} / 1\text{min}$, $T_a / 1\text{min}$ and $72^\circ\text{C} / 1\text{min}$, and a final extension step of $72^\circ\text{C} / 8\text{min}$. PCR products were mixed with $2.5\mu\text{l}$ gel loading dye (6X dye: 0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol in water) and electrophoresed on a 3.8% metaphor agarose gel at 120 V in 1X TAE buffer. A 100bp DNA ladder was used as molecular size standard. The

gels were stained with ethidium bromide and viewed under UV light. Patterns were scored for 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 website of Cornell University (Hong-Liang *et al.*, 2004). The properties of rice STMS primers, primer sequence, repeat motif, their chromosomal location and annealing temperature are present in Table 1. Of the thirty primers screened, sixteen informative primer pairs were used in the present study for final STMS analysis. Frequency of an allele in each population/group was calculated. The statistical analysis was performed using POPGENE-32 (Yeh *et al.*, 2000). All eleven landraces populations were also grown for morphological characterisation in an on-farm field experiment at NBPGR Regional Station, Bhowali (Uttarakhand). All populations of the landrace were planted in four square meter plots in a complete randomized block design with three replications during the rainy season of 2007 following standard agronomic practices. Data on quantitative traits were statistically analyzed using INDOSTAT statistical software.

Results and Discussion

Primer screening and properties of Microsatellite (STMS) markers

Thirty primers were screened to identify polymorphic primer for molecular diversity of rice landraces population. Out of the thirty, sixteen informative primer were identified as polymorphic. A total of 98 alleles were detected in eleven populations of different landraces by using 16 STMS markers. The number of alleles per locus and their allelic size ranges are represented in Table 2. The number of alleles ranged from four to eleven with an average of 6.13 alleles per locus. The overall size of amplified products ranged from 100bp (RM 228) to 250bp (RM 19). A few representative amplification profiles are given in Fig.1. The STMS analysis of the rice landraces indicated enough polymorphism to fully differentiate the inter and intra-population diversity (Wu and Tanksley, 1999 and Temnykh *et al.* 2001). All the sixteen STMS loci showed variation for 30 individuals of each landraces.

Genetic structure of common and rare landrace populations

The genotypes of thirty individuals of each landrace populations were not identical at sixteen studied STMS loci. The mean number of alleles per locus was 6.13 for different population which is presented in Table 1. For different group of rice landraces, namely eight populations of common landraces and three distinct rare landraces, the average number of alleles per locus were 4.96 and 4.37 respectively (Table 3). The number of alleles per locus varied from 4 to 11 with maximum number of alleles recorded for STMS loci RM 206 followed by RM 250 (10 alleles) and RM 247 (8 alleles). A total of 98 alleles were recorded, of which, ninety one were common and seven were rare. Out of the eleven landrace populations, the maximum number of 41 alleles was recorded for the population IC 548358 of common landrace Jaulia followed by the population IC 548398 (a rare landrace). Population-wise, total number of alleles ranged from 21 to 41 with maximum number of alleles (41) for population IC 548358 and minimum 21 alleles for population IC 100051, both of common landrace Jaulia. Similarly population-wise, the number of common alleles varied from 19 (IC 548386) to 35 (IC 548358) where as the number of rare alleles varied from 1 (IC 100051) to 7 (IC 548398). The genetic diversity analysis using 16 STMS primers of eleven rice landraces population indicated that rare landrace populations were more diverse than the common landrace populations with relatively greater values for effective number of alleles per loci observed and expected heterozygosity and Shannon's information index.

Population genetic structure of landrace populations under static and dynamic management: Overall comparison

Genetic diversity of two named rice landrace populations, Jaulia (rainfed) and Thapachini (irrigated) were compared under static (*ex situ* in genebank) and dynamic (on-farm) management. One population each of these two common landraces maintained *ex-situ* in genebank were compared with four on-farm managed populations of Jaulia and two populations of Thapachini individually and in combinations. The results of overall population genetic structure of rice landrace populations under static (one population each of Jaulia and Thapachini) and dynamic management (six populations, four populations of Jaulia and two of Thapachini) is presented in Table 4. The on-farm managed population's revealed greater diversity with more number of effective alleles, greater Shannon's information index and greater

expected heterozygosity as compared to populations under static management.

The population differentiation between the two populations under static management was, however, greater ($F_{ST} = 0.84$) as compared to six populations under dynamic management ($F_{ST} = 0.65$). The six on-farm managed populations represented 66 alleles, out of a total of 98 alleles recorded in all 11 rice landrace populations analyzed in the present study, of which 8 alleles were categorized as common and widespread which was present in all six populations. There were 30 unique alleles present only in either of the on-farm managed populations. For the upland common landrace Jaulia, the mean number of alleles per locus and per population was 4.37 with 1.31 for the single population IC 100051 under static management and an average of 4.18 alleles per locus for the four on-farm managed populations. The same trend was also recorded for the Shannon's information index and expected heterozygosity. All the four on-farm managed populations of Jaulia landrace represented 63 alleles out of the total 98 alleles present in 11 rice landrace populations at 16 STMS loci analyzed in the present study. Of these, 16 alleles were present in all four on-farm managed populations and could be categorized as the common and widespread alleles. There were a total of 26 unique alleles which were present only in either of the four populations. In the landrace population under static management, there were a total of 21 alleles for all sixteen loci of which 19 were still present in the on-farm managed populations with varying frequencies whereas two alleles were not present. A total of 38 alleles were new and specific to the on-farm managed populations. The frequency of rare alleles was more for on-farm managed populations.

High population differentiation could be noted between two populations under static management ($F_{ST} = 0.84$) as compared to six populations under dynamic management ($F_{ST} = 0.65$). Differences of genetic differentiation may have been associated with differences in sampling method and accessions handling. Genetic differentiation between samples maintained in a genebank and samples collected in farmers' fields could be explained by differences in collection methods during the periods compared. On-farm managed populations for the present study were all derived from unsorted bulk seed in farmers' granaries. However, it was unknown whether the samples under static management from the genebank were

obtained via the same process or if they were derived from seeds shed from panicles that had been screened for morphotype during the survey.

On-farm experiment for morphological characterization

The range of variation and mean performance for important quantitative traits of 11 rice landrace populations are presented in Table 5 and Table 6 respectively. The range of variation for rice landrace populations revealed that maximum variations were recorded for grain yield, straw yield per plant, number of tillers per plant, panicle length and grain length. Least variation was observed for the traits such as days to 75% panicle emergence, days to maturity and flag leaf width. Highly significant differences for all quantitative traits among landraces were recorded. The quantitative traits such as number of tillers per plant, grain yield per plant and grain length revealed greater diversity than other traits. The population quantitative parameters revealed high grain yield per plant for population IC 548668 of Jaulia followed by IC 548390 (rare landrace) and IC 548396 (Thapachini). Population IC 548668 of Jaulia also showed maximum 100-grain weight followed by IC 548390 (rare landrace). Among nine on-farm managed rice landrace populations, the maximum straw yield per plant was recorded for population IC 548639 followed by IC 548668 of Jaulia.

These morphological variations indicated that landraces significantly varied for yield related traits under farmer management. Even different populations of the same landrace Jaulia and Thapachini varied for yield traits.

Summary

Genetic diversity continues to meet farmers' needs and play an important role in traditional agroecosystem. Assessing diversity of available crop genetic resources at the field as well as molecular level is a prerequisite step for understanding detrimental evolutionary patterns and developing on farm conservation and its sustainable utilisation strategies. The present study demonstrated farmer management of crop population structure and temporal evolution of rice genetic diversity in traditional production systems. Thirty microsatellite (STMS) primers were screened to identify ideal primers for detailed genetic diversity analysis of 11 rice landrace populations comprising five populations of rainfed common landrace Jaulia; three populations of an

irrigated common landrace Thapachini, and three different rare landrace populations. The molecular analysis of rice landrace populations indicated enough polymorphism to fully differentiate the inter- and intra-population diversity. Genetic differentiation between samples maintained in a genebank and samples collected in farmers' fields could be explained by differences in collection methods during the periods compared. Changes in yield parameters also seemed affected under dynamic farmer management for same named rice landrace populations. The study also compared genebank-conserved and farmer-managed population of the same named landraces Jaulia and Thapachini and revealed greater number of alleles per locus for on-farm managed population as compared to population under static management. The rare landrace populations were more diverse than the common landrace populations with relatively greater values for effective number of alleles per loci, observed and expected heterozygosity, and Shannon's information index. The present investigation about the population genetic structure is therefore helpful in monitoring diversity loss over time and space, and also for devising a rational conservation plan, particularly on-farm management of farmer landraces under traditional production. Further, identification of genetically important crop populations would assist in investigating different options for "adding value" to these populations in a given social, economic and ecological context.

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Table 1: Details of STMS Primers, their chromosomal location, sequence and annealing temperature

Primer name	Chromosome location	SSR motif	Primer Sequence 5'-3'	A* (°C)
RM206(F)	11	(GA) ₂₁	5'CCCATGCGTTTAACTATTCT3'	48
RM206(R)			5'CGTTCCATCGATCCGTATGG3'	
RM19(F)	12	(ATC) ₁₀	5'CAAAAACAGAGCAGATGAC3'	46
RM19(R)			5'CTCAAGATGGACGCCAAGA3'	
RM21(F)	3	(GA) ₂₄	5'TGGTCAAACCAAGGTCCTTC3'	52
RM21(R)			5'GACATACATTCTACCCCGG3'	
RM255(F)	4	(GA) ₁₆	5'TGTTGCGTGTGGAGATGTG3'	50
RM255(R)			5'CGAAACCGCTCAGTTCAAC3'	
RM235(F)	12	(GA) ₂₄	5'AGAAGCTAGGGCTAACGAAC3'	50
RM235(R)			5'TCACCTGGTCAGCCTCTTTC3'	
RM217(F)	6	(GA) ₂₀	5'ATCGCAGCAATGCCTCGT3'	46
RM217(R)			5'TAGAAAGATGGAAGGAATGGTGAT3'	
RM249(F)	5	(GA) ₁₄	5'GGCGTAAAGGTTTTGCATGT3'	50
RM249(R)			5'ATGATGCCATGAAGGTCAGC3'	
RM216(F)	10	(GA) ₁₈	GCATGGCCGATGGTAAAG	48
RM216(R)			TGTATAAAAACACACGGCCA	
RM228(F)	10	(GA) ₃₆	CTGGCCATTAGTCCTTGG	48
RM228(R)			GCTTGC GGCTCTGCTTAC	
RM234(F)	7	(GA) ₁₇	ACAGTATCCAAGGCCCTGG	52
RM234(R)			CACGTGAGACAAAGACGGAG	
RM247(F)	12	(GA) ₁₆	TAGTGCCGATCGATGTAACG	48
RM247(R)			CATATGGTTTTGACAAAGCG	
RM250(F)	2	(GA) ₁₀	GGTTCAAACCAAGCTGATCA	48
RM250(R)			GATGAAGGCCTCCACGCAG	
RM263(F)	2	(GA) ₃₄	CCCAGGCTAGCTCATGAACC	50
RM263(R)			GCTACGTTTGAGCTACCACG	
RM201(F)	9	(GA) ₁₇	CTCGTTATTACCTACAGTACC	50
RM201(R)			CTACCTCCTTTCTAGACCATA	
RM104(F)	1	(GA) ₉	5'GGAAGAGGAGAGAAAGATGTGTCC3'	50
RM104(R)			5'TCAACAGACACACCCGCCACCG3'	
RM3262(F)	8	(CT) ₁₃	5'TTATAGCTCTCTCGCCGAGG3'	50
RM3262(R)			5'TGACCTCACTTCACTTCCCC3'	

A*=annealing temperature

Table 2: The number of alleles per locus and their alleles size range.

Locus	No. of Allele	Allelic size (bp)	
		Range	Difference
RM206	11	130-250	120
RM19	7	175-250	75
RM21	5	100-150	50
RM255	5	120-165	45
RM235	5	100-140	40
RM217	6	120-170	50
RM249	4	110-160	50
RM216	6	120-200	80
RM228	6	100-140	40
RM234	6	130-160	30
RM247	8	130-170	40
RM250	10	130-180	50
RM263	4	180-200	20
RM201	5	150-170	20
RM104	6	120-150	30
RM3262	4	160-185	25
Total	98	-	-
Mean	6.13	-	-

Table 3: Group-wise summary diversity analysis for all loci of common and rare rice landrace populations

	Observed no. of alleles	Effective no. of alleles	Shannon's information index	Observed heterozygosity	Expected heterozygosity	Nei's expected heterozygosity	Fixation index (F_{ST})
Group I	4.90±1.59	2.62±1.10	1.03±0.42	0.01±0.02	0.54±0.20	0.54±0.20	0.72
Group II	4.37±1.45	2.90±0.63	1.15±0.23	0.06±0.10	0.64±0.09	0.63±0.09	0.66
Average	6.06±1.91	3.36±1.27	1.35±0.33	0.02±0.02	0.66±0.12	0.66±0.12	0.74

Group1 (common landrace populations) and Group2 (rare landrace populations)

Table 4: Group-wise summary diversity analysis for all loci of rice landrace populations under static and dynamic management

	Observed no. of alleles	Effective no. of alleles	Shannon's information index	Observed heterozygosity	Expected heterozygosity	Nei's expected heterozygosity	Fixation index (F_{ST})
Jaulia							
Group I	1.31±0.47	1.04±0.06	0.07±0.11	0.01±0.04	0.03±0.06	0.03±0.06	0.00
Group II	4.18±1.37	2.28±0.76	0.94±0.30	0.01±0.02	0.52±0.13	0.52±0.13	0.52
Mean	4.37±1.45	2.60±1.04	1.03±0.36	0.01±0.01	0.55±0.17	0.55±0.17	0.63
Thapachini							
Group I	1.43±0.62	1.07±0.18	0.10±0.19	0.00	0.05±0.11	0.05±0.11	0.00
Group II	2.31±0.79	1.57±0.50	0.47±0.32	0.00	0.33±0.22	0.30±0.22	0.81
Mean	2.75±0.68	1.79±0.62	0.61±0.32	0.00	0.37±0.21	0.37±0.2	0.85
Overall static vs. dynamic							
Group I	2.12±0.71	1.55±0.48	0.44±0.31	0.006±0.02	0.29±0.23	0.29±0.23	0.84
Group II	4.31±1.41	2.40±1.03	0.98±0.37	0.01±0.01	0.53±0.17	0.52±0.17	0.65
Mean	4.56±1.59	2.62±1.10	1.03±0.42	0.01±0.01	0.54±0.20	0.54±0.20	0.72

Group I (static management) and Group II (dynamic management)

Table 5: Range of variations for important quantitative traits of rice landrace populations

S. No.	Characters	Minimum	Maximum	Mean	C.V.
1	Days to 75% panicle	132	144	136.9259	2.5049
2	Plant height (cm)	115	193	147.244	15.3683
3	Flag leaf length(cm)	23.86	38.62	32.5707	12.2942
4	Flag leaf width (cm)	1.30	1.78	1.5663	8.2962
5	Panicle length (cm)	15.92	27.00	20.8681	17.7075
6	No. of grains / panicle	75	127.8	100.3630	15.1567
7	No. of tillers / plant	7	14.60	9.3111	22.3250
8	Days to maturity	122	179	173.6296	6.0661
9	Grain length (cm)	0.45	0.96	0.6291	16.6286
10	Grain width (cm)	0.26	0.38	0.3215	10.6610
11	100 grain wt (g)	2.58	3.86	3.1981	11.5092
12	Grain yield / plant (g)	4.00	17.00	10.2370	32.2247
13	Straw yield / plant (g)	45.00	95.00	63.7778	22.4678

Table 6: Mean variations among rice landrace populations for important quantitative traits

Population	Days to 75% panicle emergence	Plant height (cm)	Flag leaf length (cm)	Flag leaf width (cm)	Panicle length (cm)	No. of grains/panicle	No. of tillers/Plant	Days to maturity	Grain length (cm)	Grain width (cm)	100 grain wt (g)	Grain yield/Plant (g)	Straw yield/plant (g)
IC 100051	135.7	136.9	28.5	1.7	15.7	100.7	8.7	175.0	0.7	0.3	3.6	10.3	81.0
IC-548358	136.0	149.0	36.9	1.6	18.7	77.7	7.7	176.7	0.6	0.3	3.3	8.8	59.0
IC-548363	134.0	166.1	35.3	1.7	23.1	109.0	7.4	174.7	0.5	0.4	3.5	5.3	75.0
IC-548639	134.0	137.0	30.1	1.6	26.3	91.1	9.2	172.0	0.6	0.3	3.3	7.7	92.3
IC-548668	134.7	166.8	31.2	1.6	16.3	120.9	8.9	174.0	0.7	0.3	3.7	15.0	78.3
IC 113843	136.3	120.9	28.0	1.4	17.0	86.4	8.1	171.3	0.6	0.3	2.5	10.3	45.7
IC-548386	135.7	123.5	30.5	1.4	18.3	84.9	8.2	158.7	0.6	0.4	2.8	8.3	50.0
IC-548396	142.0	126.0	35.7	1.5	18.3	110.0	13.2	178.0	0.6	0.3	2.9	13.0	57.0
IC-548401	143.0	116.1	24.3	1.5	18.1	85.7	9.0	176.3	0.6	0.3	2.9	8.0	52.3
IC-548390	136.0	170.2	34.0	1.6	24.7	110.9	11.9	176.7	0.7	0.3	3.7	13.3	56.7
IC-548398	137.0	170.6	35.1	1.7	24.3	113.3	8.3	175.7	0.8	0.3	2.8	12.7	52.7
	136.8	143.9	31.8	1.6	20.1	99.1	9.1	173.6	0.6	0.3	3.2	10.3	63.7
	0.9	6.0	4.9	5.8	6.6	3.1	11.4	5.6	9.7	5.8	4.2	10.7	5.1
	2.1	14.6	2.6	0.2	2.3	5.3	1.8	16.7	0.1	0.0	0.2	1.9	5.5

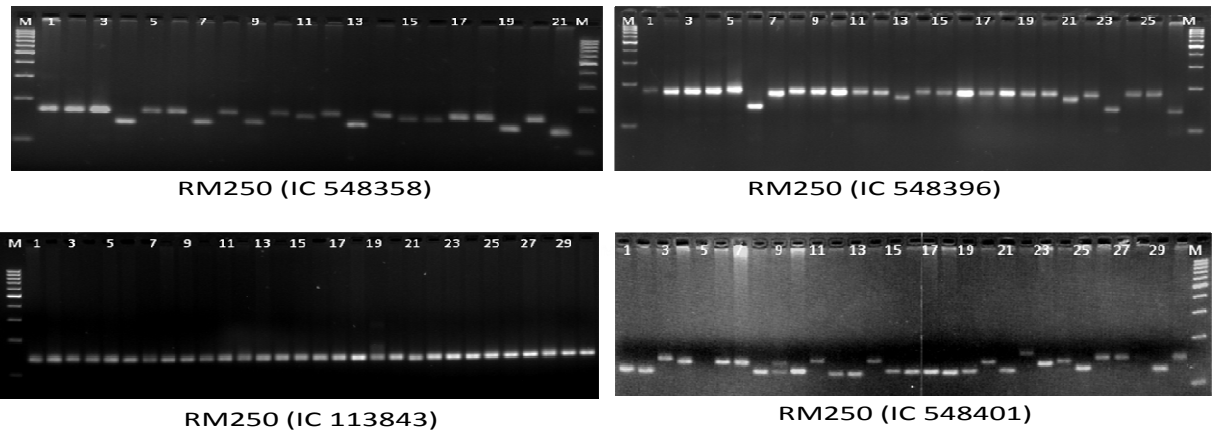


Figure 1: STMS profile of population IC 548358 of Jaulia (In situ), IC 548396 of Thapachini (In- situ), IC113843 of Thapachini (Ex-Situ) and IC 548401 of rare landraces indicating comparison of polymorphism between common and rare landraces as well as dynamic to static management for the STMS Markers with the primer RM250.