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Mehmood, Shiraz

2021-11

Mehmood , S , Din , I U , Ullah , I , Mohamed , H , Basit , A , Khan , M N , Shah , S S H & Rehman , A U 2021 , ' Agro-morphological and genetic diversity studies in Rice (Oryza sativa L.) germplasm using microsatellite markers ' , Molecular Biology Reports , vol. 48 , pp. 7179-7192 . https://doi.org/10.1007/s11033-021-06710-5

http://hdl.handle.net/10138/348310
https://doi.org/10.1007/s11033-021-06710-5

unspecified
acceptedVersion

AGRO-MORPHOLOGICAL AND GENETIC DIVERSITY STUDIES IN RICE (ORYZA SATIVA L.) GERMPLASM USING MICROSATELLITE MARKERS

Shiraz Mehmood[1], Israr Ud Din[1], Izhar Ullah[2*], Syed Saad Hussain Shan[1], Abdul Basit[3], Ateeq ur Rahman[4,5]

[1]Institute Of Biotechnology and Genetic Engineering, The University Of Agriculture, Peshawar-Pakistan
[2]Department of Horticulture, Ondokuz Mayis University,Samsun-Turkey
[3]Department of Horticulture, The University of Agriculture, Peshawar-Pakistan
[4]Natural Resources Institute (Luke), Toivonlinnantie 518, FI-21500 Piikkiö, Finland
[5]Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, FI-00790, Helsinki, Finland

Corresponding author:izharhorticons361@gmail.com

ABSTRACT

Knowledge of the genetic diversity and population structure of germplasm collections is an important foundation for crop improvement. Rice production across a broad range of rice-growing environments resulting in the diverse array of local rice varieties. Due to the loss of biodiversity, many rice varieties have missed grown in Pakistan. To protect the biodiversity of rice varieties, an experiment was carried out to check the genetic and morphological variations between 8 exotic and 7 local rice genotypes, using 5 different SSR markers i.e RM3, RM259, RM341, RM520, and RM11943.The analysis of morphological and quality traits of rice observed significant variation across genotypes. The results revealed that genotype Irri-Pak attained the highest plant height and primary branches plant^{-1}, while genotype Mushkan produced a higher number of productive tillers and obtained a higher fertility factor (%). Similarly, the highest value for panicle length was observed for genotype Faker-e-Malakand, 1000-grains weight in genotype Calmochi, and maximum days to maturity was noticed in genotype Swati-2014. Moreover, the genotype Brio attained the highest value of stem diameter, while maximum seed length was noted in genotype Sug Dasi. The highest number of primary branches plant^{-1} in genotype Ibge-I and secondary branches plant^{-1} in genotype Calmochi were noticed. A higher concentration of sodium and potassium was observed for genotype Marte, while the genotype Muskan attained maximum content of Copper. Moreover, the highest concentration of Iron in genotype Originario, Zinc in genotype JP-5, and Cadmium content were noticed in genotype Ibge. Similarly, the dendrogram analysis for quantitative parameters showed three clusters at 74.13 % similarities. Whereas all the genotypes of European origin formed a separate cluster. A set of 5 SSR primers covering four chromosomes, amplified a total of 14 alleles and showed 100% polymorphism with an average PIC value ranged from 0.39 to 0.91. The UPGMA cluster analysis separated the 15 rice genotypes into 3 main groups based on 32.5% similarities and the highest genetic distance (45.1%) was observed between two genotypes (Fakher Malakand and Musa), having different geographical origin. There was no genetic distance between genotype Marte and Brio, irrespective of having the same origin.

Keywords: Agro-morphological traits, Genetic diversity, Microsatellite Markers, Rice (*Oryza sativa* L)

INTRODUCTION

Rice (*Oryza sativa* L) is a self-pollinated crop, belongs to the family *Graminae* and is the most important staple food grains that occupies one-fifth of the total land covered. (Chakravarthi and Naravaneni, 2006). Rice was originated in china about 10000 years ago and presently two species i.e. *Oryza sativa* L. (Asian Rice) and *Oryza glabberrima* L. (African rice) are cultivated around the world, which were independently domesticated from wild Asian rice, *Oryza rufipogon* and wild African rice, *Oryza barthii* respectively (Chang, 1976; Oka, 1988; Khush, 1997; Cheng et al., 2003; Yamanaka et al., 2004). Rice is the second leading cereal crop of Pakistan after wheat, which is cultivated on an area of 2.3 million hectares with a net production of 5.5million tones (Junaid and Ali, 2015). In the global market, Pakistan is one of the main exporters of aromatic rice. Annual rice export of Pakistan stands at about 0.84 million tons for aromatic basmati and 2.85 million tons for non-aromatic indica rice, yield a total of Rs 69325 million to the Pakistan's economy (Rabbani et al., 2010). Pakistan, being a leading exporter of Basmati rice since 1970. However, as a result of green revolution, and introduction of high yielding semi dwarf varieties, many landraces of Basmati grown in Pakistan eradicated. Many improved rice genotypes have been released by rice research institutions for cultivation in different geographic regions of Pakistan that have narrow genetic bases because of higher homozygosity (Rabbani et al., 2008).

As the loss of global biodiversity context, many rice varieties have been missed to grown in Pakistan. Therefore, it is not only important to protect the land race genotypes but also very necessary to examine the gene pool element of aromatic rice for breeding purposes (Rabbani et al., 2008).The rice germplasm is a rich reservoir of useful genes that researchers can harness for rice improvement programme. Genetic variability exists among rice germplasm leaving a wide scope for crop improvements. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. It contributes to monitoring germplasm and can also be used to predict potential genetic gains (Mandel et al., 2011). Classification of different rice varieties are very closely linked together in groups which need time for characterization and showed ineffective diversity by enzymatic, morphological, and physio-chemical method. The most appropriate and reliable method of molecular characterization is the use of DNA based markers (Sarao et al., 2010). DNA markers are less expensive, less naturally conditioned, fast, accurate, and reproducible with high authenticity and can be useful in identifying and registering the plant varieties, seed quality and purity monitoring (Cirillo et al., 2009).

Simple Sequence Repeats (SSR) markers have been widely used to evaluate the genetic variations and its associations with subspecies among the rice varieties and advantages of allele specificity and co-dominance (Kobayashi et al., 2006, Chuan-Guang and Zhang, 2010, Latif et al., 2011). Currently, SSR markers are used for molecular characterization i.e. highly reproducible, reliable, co-dominant, scattered throughout the whole genome, variable, abundant and multiallelic in nature (Salgotra et al., 2015). SSRs microsatellite markers has become best choice for a wide range studies of evolution, genetics and population study on the basis of variation in the simple sequences DNA repeats (Jarne and Lagoda, 1996; Powell et al., 1996). The evolution of second generation genetic maps have been significantly done with highly polymorphic markers in many kind of species including human beings (Dib et al. 1996) and plants like potato (Milbourne et al., 1998), wheat (Bryan et al., 1997; Röder et al., 1998), soyabean (Akkaya et al., 1995; Cregan et al., 1999). Maize (Chin et al., 1996; Taramino and Tingey, 1996). Keeping these views the importance of microsatellite markers, an experiment was planned to evaluate the agro-morphological traits and genetic diversity of the rice genotypes using microsatellite markers.

MATERIAL AND METHODS

Plant Material and field evaluation of morphological traits

The present study was executed at the division of Plant Tissue Culture, Genetic Resources & Conservation, Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar during kharif season 2019. The research was carried out on 8 exotic and 7 local short grain rice genotypes in Randomized Complete Block Design with one factor (Genotypes) replicated five times (Table 1). All these genotypes were analyzed for morphological, agronomic, and genetic diversity. Rice seeds were soaked in 45ml of distilled water in 50 ml falcon tubes and incubated at 26 ºC for 48 hours. Seeds were grown in pots in green house to establish seedlings nurseries. The pots were checked regularly and watered as per requirement. After five weeks the seedlings were shifted to the field (plot size of 6×2.5 ft^2) and then transplanted to rows at distance of 1ft apart. For DNA extraction young leaves were collected from 25 days old seedlings and immediately stored at -20°C till further analysis.

Table 1. List of local and exotic rice genotypes used in this study

S.NO	Genotype	Origin
1	Swati-2014	Pakistan
2	Originario	Italy
3	Brio	Italy
4	Ibge-2	China
5	Onice	Italy
6	Marte-4	Italy
7	Ibge-1	Pakistan
8	Musa-7	Italy
9	Mushkan-340	Pakistan
10	IIRI-Pak	Pakistan
11	Begami/ JP-5	Pakistan

12	Calmochi	Italy
13	Ibge-3	China
14	Fakher-e-malakand	Pakistan
15	Sug-Dasi/DR-83	Pakistan

Observations were recorded on plant height (cm), panicle length (cm), fertility factor (%), days to maturity, number of seed plant^{-1}, number of productive tillers, 1000-grains weight, primary branches and secondary branches plant^{-1} of rice genotypes. Similarly the micronutrients and heavy metal contents in seed samples were measured after acid digestion as describe by Benton et al. (1991). Dry seed sample (0.5 g) was taken in 50 ml Pyrex flask and 10 ml of nitric acid was added to it and the sample was kept overnight. Perchloric acid (4 ml) was added and the mixture was heated on hot plate till the appearance of white and cleared fumes. Diluted the digest by 100ml of distilled water in volumetric flask upto the mark. Potassium and sodium was determined using flame photometer, while micronutrients content in the extract was determined using atomic absorption spectrophotometer.

DNA extraction and PCR amplification

DNA extraction from rice seedlings was carried out with CTAB method previously described by Doyle et al. (1987). A leaf sample of 0.5g was taken and crushed in liquid nitrogen into powder. One ml of Cetyl Tri Methyl Ammonium Bromide (CTAB) buffer was added immediately and transferred to 2 ml of Eppendorf tubes. The samples were incubated in water bath at 60°C for 30 min and then vortexed. A centrifugation step was performed at 12000 rpm for 15 minutes. After centrifugation the upper layer was transferred to new Eppendorf tube and 500 µl of Phenol, Chloroform and Isoamylalcohol solution (P:C:I, 25:24:1) were added followed by centrifugation at 12000 rpm for 12 minutes to separate the sample into distinct phases. The upper layer was taken and transferred to a new tube and 28 µl ice cold Na acetate and 600 µl pre-cooled isopropanol was added for the DNA precipitation. Sample was centrifuged again at 12000 rpm for 12 min and supernatant was removed without affecting the pellet. The pellet was washed two times with 500µl of 70% ethanol. The ethanol was discarded and the pellet was dried at room temperature. Then DNA was treated for 1 hr with 40 micro-gram RNase-A at 37ºC. The DNA pellet was suspended in 50µl TE buffer (10Mm Tris, 1mM EDTA, PH 8). The overall genomic DNA concentration, quality and optical density was measured at a wavelength of 260/280 nm by Nano Drop for all the samples and the samples were stored at -20ºC for further analysis.

Molecular characterization

To study the genetic diversity among rice genotypes, five SSR markers were used covering 4 chromosomes as shown in Table 2.

Table 2. List of SSR primers and their sequences

S.No	Primer	Primer sequence (5´-3´)	Annealing temp(ºC)	Chr.No	Product Size
1F	RM 3	ACACTGTAGCGGCCACTG	55^{0}C	6	118bp
1R	RM 3	CCTCCACTGCTCCACATCTT			
2F	RM259	TGGAGTTTGAGAGGAGGG	55^{0}C	1	172bp
2R	RM259	CTTGTTGCATGGTGCCATGT			
3F	RM341	CAACAAACCTCAATCCGAGC	57^{0}C	2	137bp
3R	RM341	CTCCTCCCGATCCCAATC			
4F	RM520	AGGAGCAAGAAAAGTTCCCC	55^{0}C	3	247bp
4R	RM520	GCCAATGTGTGACGCAATAG			
5F	RM11943	CTTGTTCGAGGACGAAGATAGGG	55^{0}C	1	77bp
5R	RM11943	CCAGTTTACCAGGGTCGAAACC			

Polymerase Chain Reaction (PCR)

PCR reaction was performed as described by Prasad et al. (2000), with a small modification in thermal profile. A 10 µl PCR reaction containing 1 µl template DNA, 2 µl SSR primer, 1.8 µl nuclease free water, 0.2 µl Taq polymerase and 5 µl of master mix. PCR conditions for the amplification were set. The early denaturation step of 3 min at 94^0 C followed by 35 cycles each comprised of a denaturation 30 sec step at 94^0 C, annealing step of 45 sec at 55^0 C and an extension step of 45 sec at 72^0C. BIO-RAD T100 thermal cycler was used.

Gel Electrophoresis

The amplified PCR products were confirmed on 2% Agarose gel, which was prepared in 1X TBE buffer and then melted in microwave oven for 2 min and 2 µl ethidium bromide were added. After cooling at room temperature for 20 min, the gel was kept in the gel tank, DNA ladder (50 bp) and PCR product was loaded and ran for 45 min at 100 V. After electrophoresis the gel was stained with UV in a gel documentation system.

Statistical analysis

For statistical analysis, the amplified bands of the same size were marked as a single allele and of different size were scored as different alleles. The Simple Sequence Repeats (SSR) data was analyzed by Microsoft excel and Pop gene version 3.25. The alleles were marked as present or absent. Genetic diversity among genotypes were calculated using Unweight Pair Group Method with Arithmetic Mean (UPGMA) procedure to construct a dendrogram by Pop gene. Principal component analysis (PCA) was calculated by using XL Stat for simplifying the complexity in high-dimensional data while retaining trends and patterns.

RESULTS

Morphological characterization

Different morphological attributes were significantly influenced by different exotic and local genotypes of rice (Table 3). The taller plant was noticed for IRRI-Pak followed by genotype Sug-Desi 130 cm, while a shorter plant was observed for genotype Onice. Overall the local varieties were taller than exotic varieties. Maximum panicle length was observed for genotype Fakhr-e-Malakand and the highest fertility factor was noted for genotype Mushkan, while minimum panicle length and minimum fertility factor were observed for genotype Onice. Similarly, the highest number of productive tillers panicle-1 and number of seeds per panicle were produced by genotype Mushkan, while the lowest number of productive tillers panicle-1 was recorded for genotype Begami/JP-5, and the total number of seeds per panicle were noted for genotype Originario. Maximum days to maturity were taken by genotype Swati-2014 and minimum days to maturity were recorded for genotype Marte. The highest 1000 grains weight was noted for genotype Calmochi as compared to other genotypes. The maximum seed length was attained by genotype Sug-Dasi followed by genotype Mushkan and Calmochi. Whereas genotypes Brio and Marte received minimum seed length. Among the 8 exotic and 7 local rice varieties, the maximum seed diameter was noted for genotype Brio and minimum seed diameter was recorded for genotype Musa and Mushkan respectively. The highest number of primary branches plant^{-1} for Ibge-I and secondary branches plant^{-1} was produced by genotype Calmochi, while the lowest number of primary branches plant^{-1} was recorded for genotype Calmochi and secondary branches plant^{-1} was observed for genotype Brio.

Quality attributes

Data regarding quality attributes of rice was significantly influenced by different exotic and local genotypes. The highest concentration of sodium and potassium content were noted for genotype Marte and lowest concentration of sodium was noted for genotype Brio and Mushkan (Figure 1.a,b). The maximum copper content was noted for genotype Mushkan followed by genotype Brio. While the minimum concentration of copper was attained by genotype Ibge-1 (Figure 1.c). Similarly the genotype Originario attained higher content of Iron over other rice germplasms (Figure 1.d). The maximum concentration of Zinc was noticed in JP-5 followed by Mushkan and Onice and the minimum concentration of Zinc was observed in Originario (Figure 1.e). The highest concentration of cadmium was noted for genotype Ibge-and the lowest concentration of cadmium was recorded in Swati-2014 (Figure 1.f).

Principle Component Analysis (PCA)

To determine the variability between rice germplasm on the basis of morphological traits, Principle Component Analysis (PCA) was performed using XLSTAT software. Observation of PCA for the first two principle components have Eigen values greater than 1 accounting for 74.13% of total variation (Figure 5). The first and second components factor (F1) and factor (F2) were considered that is 54.94% and 19.19% of total variations, respectively (Figure 4, 5). Except days to maturity all the traits vary together according to PCA1. These

traits were total seeds and 1000 grains weight, while days to maturity show negative value. According to PCA2, other traits such as plant height, number of productive tillers and panicle length vary together. Similarly, in PCA scattered plot, genotype Originario, Musa, Ibge-2 and Swati-2014 showed negative scores on both axis. Genotype Begami/Jp-5, Ibge-1, Sug-Dasi and Mushkan displayed positive scores for first two axis and showed negative score for second axis. Genotype Onice, Marte, Calmochi, and Brio displayed negative value for first axis, while attained positive score for second axis. Genotype ibge-3, FM, Irri-pak showed positive for both axis (Figure 5).

Molecular Characterization

Five SSR markers covering 4 chromosomes were used to characterize and evaluate genetic diversity among fifteen diverse rice genotypes. Based on SSR analysis of 5 microsatellite loci, a total of 14 alleles were detected using five pairs of SSR primers. The average polymorphism for all the markers observed was 100% (Table 4). A significant level of genetic variability for five microsatellite loci was detected among these fifteen rice genotypes.

SSR Marker RM 3

Among 15 different rice genotypes, amplified with 5 SSR primer pairs, we observed 15 allelic bands with 3 band patterns with a band size of 118-150bps. All the observed band patterns were polymorphic. The overall frequency scored for allele A, B and C were 0.4000, 0.3333 and 0.1333 respectively. PIC value for RM 3 was found to be 0.64 (Figure 6.a)

SSR Marker RM 259

The 15 rice genotypes amplified with RM 259 showed 14 allelic bands with 2 band patterns with a band size of 172 bps. All the observed band patterns were polymorphic. The overall allelic frequency was scored for allele A and B, which was 0.2143 and 0.5714, respectively. PIC value for RM 259 was found to be 0.39 (Figure 6.b).

SSR Marker RM 341

For RM341, we observed a total of 16 allelic bands with 4 bands patterns among 15 rice genotypes. All the observed band patterns were polymorphic with a band size of 150-200bps. The overall frequency showed by allele A, B, C, and allele D was 0.0714, 0.2857, 0.0714 and 0.2143 respectively. PIC value for RM 341 was found to be 0.83 (Figure 6.c).

SSR Marker RM 520

DNA fingerprinting of 15 rice genotypes using RM 520 primers produced a total of 15 allelic bands with 3 band patterns with a band size of 247-270bps. All the observed bands patterns were polymorphic. The overall allelic frequency recorded for allele A, B and allele C was 0.0667, 0.0667 and 0.2667, respectively. PIC value for RM 520 was found to be 0.91 (Figure 6.d).

SSR Marker RM 11943

Molecular characterization of15 rice genotypes by RM 11943 primers depicted 15 allelic bands with 2 band patterns with a band size of 77bps. Both the band patterns were polymorphic. The overall allelic frequency scored for allele A and B was 0.3333 and 0.2000, respectively. PIC value for RM 11943 was found to be 0.83 (Figure 6.e).

Overall allelic frequency

Genetic variability assessment of 15 rice genotypes, produced a total of 14 allelic bands utilizing 5 SSR primers (Table 5). For RM3, the highest allelic frequency has shown by allele A (0.4000), followed by allele B (0.3333) and the lowest allelic frequency was recorded for allele C (0.1333). Using RM 259, the highest frequency was recorded for allele B (0.5714), while the lowest frequency was noted for allele A (0.2143). Similarly, with RM 341, maximum allelic frequency was obtained for allele B (0.2857) followed by allele D (0.2143), while minimum allelic frequency was noted for allele A and C, i.e. 0.0714. For RM 520, the highest allelic frequency was noted for allele C (0.2667) and lowest allelic frequency was noted for allele A and B, i.e. 0.0667. Moreover, RM 11943 displayed maximum allelic frequency for allele A (0.3333), while minimum allelic frequency was noted for allele B (0.2000) (Table 6, 7).

Genetic relationship of the rice germplasm

In this study, the genetic relationship was evaluated by constructing dendrogram, using Unweight Pair Group Method with Arithmetic Means(UPGMA) cluster analysis, which was based on Nei's Genetic distance Modified from NEIGHBOR procedure of PHYLIP Version 3.5 Pop-Gene software. All the genotypes of rice were divided into three main groups at 32.5% similarities based on dendrogram results. There were seven varieties in group A, i.e. FM, DR-83, Mushkan, Ibge-3, Calmochi, Irri-pak and JP-5. In group B, there were five varieties, i.e. Onice, Brio, Marte, Swati-2014 and Originario. While Musa, Ibge-2 and Ibge-3 were classified in group C. The rice genotypes used for genetic distances and grouping

arrangements are presented in Table 8. The maximum genetic distances were noted for genotype, Faker-e-Malakand and Musa, i.e. 45.1% as the origin of the genotypes are different. While the minimum genetic distance, i.e. 12.0 % was shown by genotype, Marte and Once, where both are from the same origin. Furthermore, there was no genetic distance between genotype Marte and Brio i.e. 0% having the same origin (Table 8).

DISCUSSION

The development of a small number of standards, short duration, fertilizer responsive elite varieties of rice, because of the utilization of high yielding genotypes in a breeding program, has led to genetic erosion and consequently a remarkable loss of traditional heterogeneous cultivars (Hamid et al., 2015; Jones et al., 2008). For the development of a diverse population structure of rice, it is important to explore the genetic diversity within the population and among populations. The utilization of genetically diverse parents in the breeding program has the potential to develop verities with higher yields per unit area (Roy et al., 2015). The study of genetic diversity based on phenotypic diversity is the primary step for the evaluation of the germplasm (Nawaz et al., 2015). The major aim of studying genetic diversity and the identification of a certain valuable trait is to enhance the grain production of a particular crop. A lot of work has been done on agro-morphological characterization that has led to the identification of phenotypic variability in rice (Moukoumbiet al., 2011; Ogunbayoet al., 2011). In the present study, eight exotic and seven local rice genotypes have shown a significant diversity in both quantitative and qualitative traits including plant height, days to maturity, panicle length, fertility factor, 1000 grains weight, and seed minerals content.

To measure the contribution and importance of each trait to the total variance, the Principal Component Analysis (PCA) is very useful. It can be applied to evaluate the independent impact of a specific trait on the total variation, while each coefficient of proper vectors identifies the degree of contribution of each original trait with which each principal component is related. They will be more effective in discriminating between genotypes when the value of coefficients is higher, irrespective of the direction whether negative or positive (Vishnu et al., 2014). In the present study, PCA for the first two principal components has shown a total variation of 74.13% (Figure 4). The first and second components factor (F1) and factor (F2) were considered which is 54.94% and 19.19% of total variations respectively. Except for days to maturity, all the traits vary together according to PCA1. According to PCA2, other traits such as plant height, number of productive tillers, and panicle length vary together.

Molecular characterization of germplasm is important both for the protection of species and for the improvement of the crop (Ludivineet al.,2015). Previously, various classical and molecular markers have been used to provide a comparatively unbiased estimate of genetic diversity and efficiently link phenotypic and genotypic variations in plants. These markers include (1) biochemical markers (allozymes and other protein markers), (2) morphological markers, and (3) genetic or molecular markers (DNA markers) (Wijerathna, 2015). Genetic markers are further categorized as (i) single nucleotide polymorphisms (SNPs) based on genome sequencing (ii) PCR-based markers i.e. random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), or microsatellites, inter simple sequence repeats (ISSRs) and (iii) Restriction fragment length polymorphisms (RFLPs) (Rajwantet al., 2011; Varshney et al., 2014). Genetic markers improve and speed up the plant breeding program by genome-wide association mapping and indirect selection linked to both simple and quantitative traits of interest (Heibaet al. 2016). Various types of molecular markers are available and used for genetic diversity analysis, where each type differs in genome abundance, genome coverage, expression/inheritance, and level of polymorphism (Nguyen et al., 2015). Microsatellite or SSR markers are the most used genetic markers that show a high level of polymorphism as compared to other markers and are preferred in almost all aspects of genetic research and breeding in most plant species (Kashianiet al.,2012). Furthermore, microsatellite markers are more abundant, ubiquitous in presence, hypervariable in nature, highly reproducible, require low amounts of DNA, easy to apply, having low cost, less laborious can be exchanged between laboratories, transferable between populations, and exhibit a high level of polymorphism even between closely related genotypes (Senanet al., 2014). The use of SSR or microsatellite markers for genome-wide association mapping, genetic variability, and species identification in rice and many other crop plants has been previously reported (Babak et al., 2015; Brondaniet al.,2006; Jayamaniet al., 2007; Khatabet al.,2015; Madhav et al., 2015; Mollaet al.,2008). In this study, 5 SSR loci were assessed to find out the genetic diversity in 8 exotic and 7 local varieties. RM341 was the most effective marker, which produced the highest number i.e. 4 polymorphic alleles, whereas RM259 gave the least

number of polymorphic alleles. The polymorphism observed for all 5 SSR markers used, was 100%. The average PIC value ranged from 0.39 to 0.91. In previous studies Wang et al., 2013 reported the same results and in this result PIC value with an average of 0.52 for 47 SSR markers ranged from 0.24 to 0.92. The number of band patterns observed in this study corresponded well with a previous study by Hassan et al. (2012). PIC value represents the information of a marker, allele frequency, and diversity between various varieties (Sajib et al., 2012). The high PIC value and greater allele frequencies in contrast with previous studies support the aim that the genotype contains unique variations. The UPGMA cluster analysis separated the 15 rice genotypes into 3 main groups based on 32.5% similarities and the highest genetic distance (45.1%) was observed between two genotypes (Fakhre Malakand and Musa) having different geographical origin.

Conclusion

Based on quantitative morphological traits, the local and exotic genotypes were well separated into three clusters, where all the genotypes of European origin were grouped into a separate cluster. Based on molecular markers assessment, all the genotypes of rice were divided into three main groups at 32.5% similarities, where maximum genetic distances were noted for genotype, Faker-e-Malakand and Musa having a different origin, while minimum genetic distance was shown by genotype, Marte and Onice, from the same origin. Furthermore, there was no genetic distance between genotype Marte and Brio, having the same origin. The SSR markers used in this study were all polymorphic and amplified 14 alleles with variable allelic frequencies.RM 520 was highly informative with PIC value of 0.91. The highest number of alleles, i.e. 4 alleles were obtained for RM 341. Evaluation of local and exotic rice genotypes at different locations under variable climatic conditions is recommended for better exploitation of germplasm. The exotic genotypes can be checked for cooking and taste qualities if these qualities have to be introgressed in local varieties to improve seed quality. The narrow genetic base of local rice cultivars is probably because of high selection pressure for good grain quality and repeated use of the same origin parents in the breeding program, resulting in significant genetic erosion of the local rice gene pool. It is recommended that representative exotic varieties having better traits should be chosen for inclusion in breeding programs aimed at varietal improvement.

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Table 3. Mean performance of 15 rice genotypes for various morpho-yield traits in Peshawar during 2019 crop season.

Genotypes	Plant height (cm)	Panicle length (cm)	Fertility factor (%)	Daysto maturity (no.)	Total number of seed plant^{-1}	No.of productive tillers	1000grain weight (g)	Primary branches plant^{-1}	Secondary branches plant^{-1}
Swati-2014	85.2 f	21.88 de	93.55 ab	126.8 a	100.2 e	38.2 c	26.9 de	10.2 ef	18.4 fgh
Fakher-e-malakand	104.8 d	32 a	86.23 de	123.4 ab	188.8 b	30 d	25.41 ef	8 gf	14.2 hi
Sug-dasi	130 b	26.1 b	88.93 cd	122.4 ab	85.4 efg	29.8 d	21.65 f	10.8 de	23.4 de
Irri-pak	151.6 a	26.54 b	87.91 cd	120 abc	187.2 b	46.6 b	24.42 ef	12.2 bcd	14 hi
Calmochi	98.64 de	21.54 de	82.58 ghi	118 abcd	128 cd	27.4 d	34.64 a	7 h	10.4 i
Jp-5	107.6 d	26.6 b	84.82efgh	118 abcd	126 cd	13.6 f	26.84 de	9.6 efg	19.2 efg
Musa	72.2 g	14.1 gh	86.52 def	118.6 abcd	90 ef	17 ef	28 cde	12.6 bc	21.4 ef
Ibge-3	87.8 f	25.1 bc	85.66 def	118 abcd	186 b	18.2 ef	25.45 ef	9.6efg	16.8 gh
Mushkan	121.8 bc	23.3 cd	95.45 a	117 abcd	218.6 a	52 a	25.09 ef	11 cde	33.8 a
Ibge-1	118 c	25.1 bc	90.72 bc	117 abcd	145 c	14 f	22.09 ef	14.4 a	28.4 bc
Originario	68 gh	16.1 fg	81.72 hij	114.6 bcd	65 g	21.6 e	30 bcd	11.6 cde	21 efg
Onice	61.8 h	13.1 h	75.61 k	114.2 bcd	69.6 fg	21.6 e	31.41 abc	10 ef	27 cd
Brio	90.66 ef	21.16 de	79.07 j	112 bcd	146 c	31.4 d	33.01 ab	12.2 bcd	35 a
Ibge-2	102.8 d	20.16 e	81.14 ij	110.2 cd	107.6 de	14.6 f	27.6 cde	13.2 ab	32.8 ab
Marte	71.06 g	17.62 f	84.13 fghi	107.6 d	125.4 cd	21 e	26.32 dc	9 fg	12 i
LSD $_{0.05}$	8.99	2.3	3.13	11.54	24.08	4.97	4.1	1.71	4.42

Means values followed by different letters shows significant variation at 5% level of significance

Table 4. Shows polymorphic bands, number of band patterns and polymorphism of each marker between 15 rice genotypes

Primers	Band Pattern	Polymorphic Bands	Polymorphism %
RM 3	3	3	100%
RM 259	2	2	100%
RM 341	4	4	100%
RM 520	3	3	100%
RM 11943	2	2	100%
Total	14	14	100%

Table 5. Shows Overall allelic frequency

Primer	Locus/Allele	Frequency
RM 3	A	0.4000
RM 3	B	0.3333
RM 3	C	0.1333
RM259	A	0.2143
RM259	B	0.5714
RM 341	A	0.0714
RM 341	B	0.2857
RM 341	C	0.0714
RM 341	D	0.2143
RM 520	A	0.0667
RM 520	B	0.0667
RM 520	C	0.2667
RM11943	A	0.3333
RM11943	B	0.2000

Table 6. Shows multi-populations Descriptive Statistics

Locus	Na	Ne	Obs.Hetro	Exp.Hetro	PIC value
Primer RM259	2.0000	1.3902	0.0000	0.6032	0.391
Primer RM 11943	2.0000	1.7108	0.0000	0.6529	0.539
Primer RM341	4.0000	4.9000	0.1429	0.8254	0.833
Primer RM520	3.0000	2.1690	0.0000	0.7080	0.914
Primer RM 3	3.0000	2.2609	0.0000	0.7172	0.642

*** na = Observed number of alleles**
*** ne = Effective number of alleles [Kimura and Crow (1964)]**
*** PIC= Polymorphic information content [Botstein et al (1980)]**

Table 7. Overall allele frequency

Allele/locus	Primer RM259	Primer RM11943	Primer RM341	Primer RM520	Primer RM3
A	0.2143	0.3333	0.0714	0.0667	0.4000
B	0.5714	0.2000	0.2857	0.0667	0.3333
C			0.0714	0.2667	0.1333
D			0.2143		

Table 8. Estimation the genetic diversity among 8 exotic and 7 local rice germplasm with five SSR primer sets

	Swati-2014	Originario	Brio	IBGE-2	Onice	Marte-4	IBGE-1	Musa	Mushkan-340	IIRI-Pak	JP-5	Calmochi	IBGE-3	Faker Malakand	Sug-Desi
Swati-2014	0.0														
Originario	13.5	0.0													
Brio	13.5	17.0	0.0												
IBGE-2	19.1	17.0	24.1	0.0											
Onice	19.1	20.9	12.0	20.9	0.0										
Marte-4	13.5	17.0	0.0	24.1	12.0	0.0									
IBGE-1	33.0	29.5	29.5	17.0	20.9	29.5	0.0								
Musa	23.3	26.9	20.9	20.9	17.0	20.9	20.9	0.0							
Mushkan-340	26.9	24.1	24.1	29.5	20.9	24.1	29.5	26.9	0.0						
IIRI-Pak	35.6	31.9	31.9	31.9	24.1	31.9	26.9	29.5	12.0	0.0					
JP-5	35.6	31.9	31.9	31.9	24.1	31.9	26.9	29.5	12.0	0.0	0.0				
Calmochi	33.0	29.1	29.1	28.6	20.3	29.1	23.0	31.1	17.1	12.1	12.1	0.0			
IBGE-3	26.9	22.6	26.4	29.0	22.6	26.4	32.0	29.0	1.2	13.5	13.5	19.1	0.0		
FM	42.6	40.0	43.4	43.4	38.1	43.4	40.0	45.1	20.9	17.0	17.0	21.5	24.1	0.0	
Sug-Desi	42.6	31.9	36.1	26.9	29.5	36.1	20.9	29.5	20.9	17.0	17.0	20.3	22.6	24.1	0.0

F

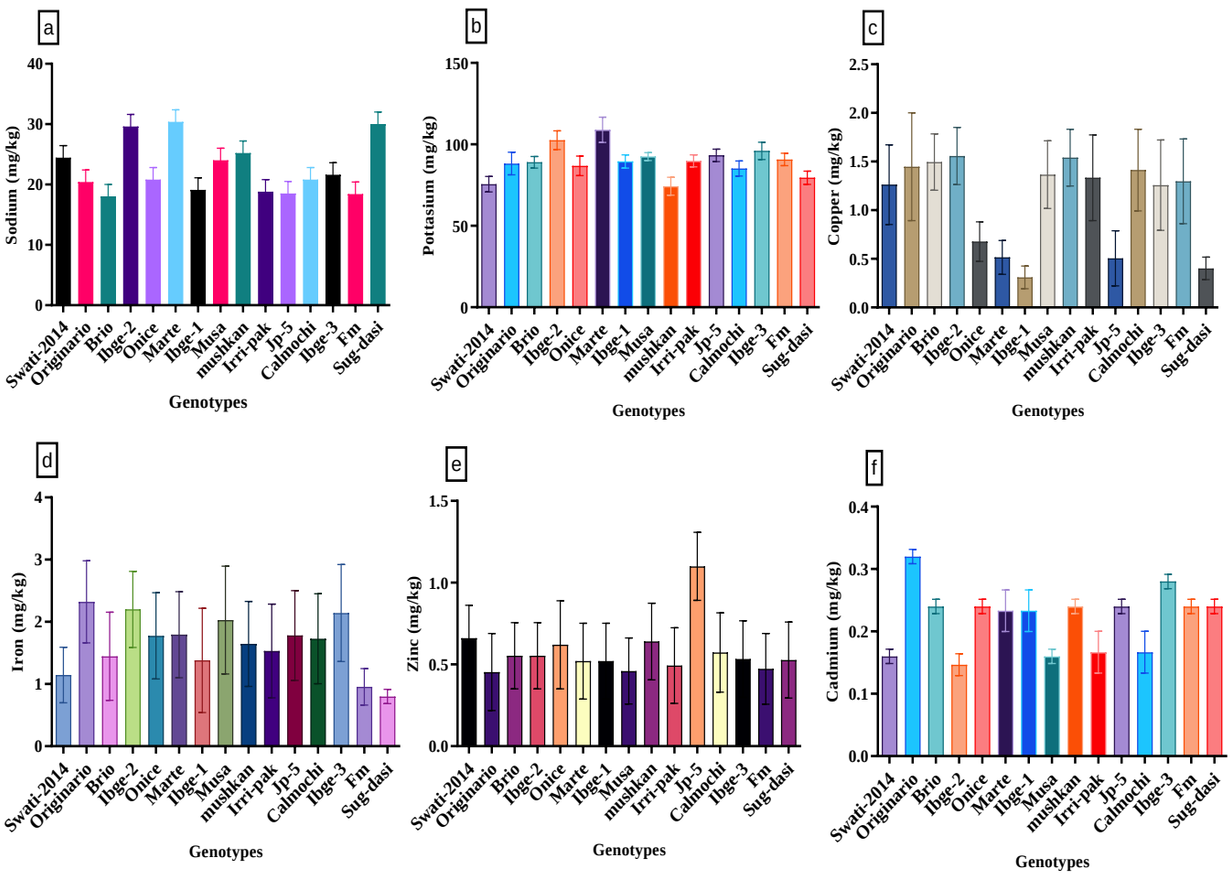

Figure 1.Quality attributes of local and exotic genotypes of rice

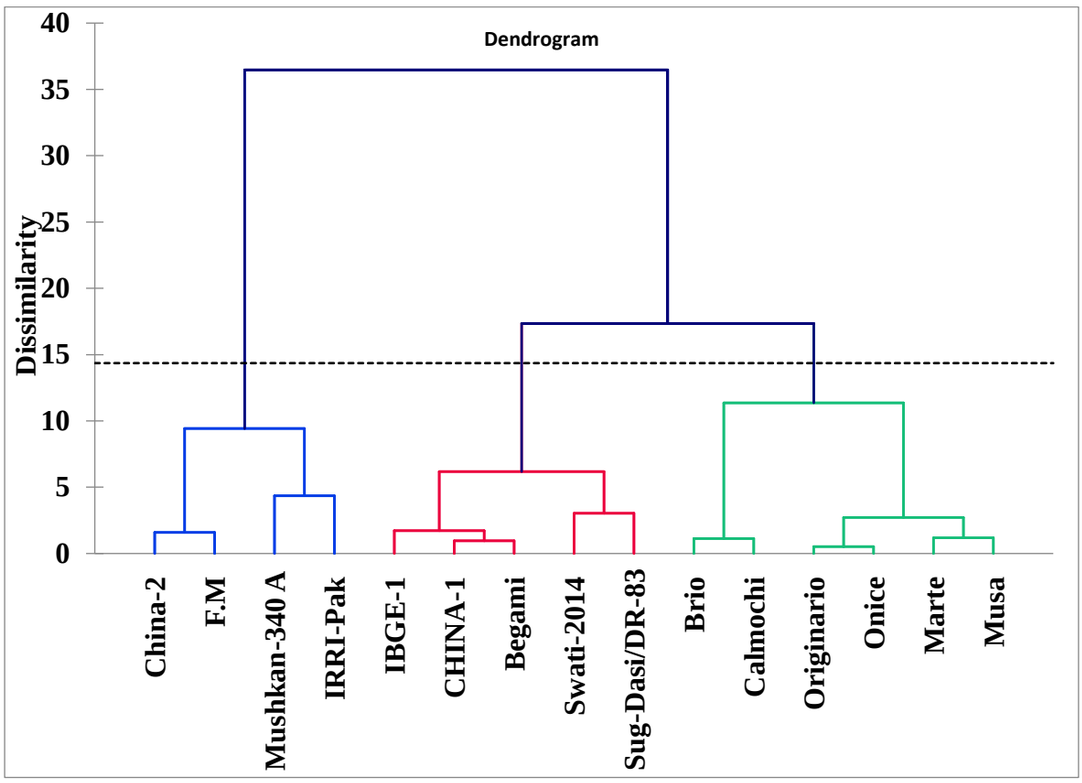

Figure 2. Morphological Dendrogram of 15 rice genotypes

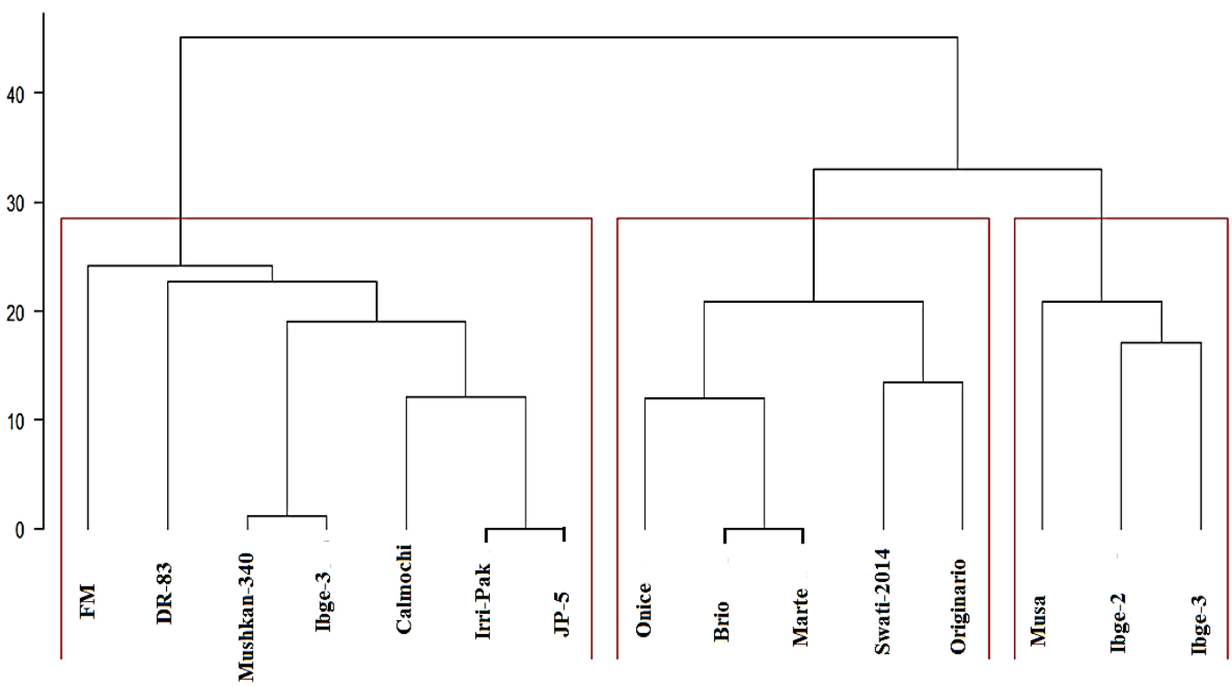

Figure 3. Dendrogram constructed for 15 rice germplasm by Pop-gene version 3.5 based on genetic similarities using five set of SSR primers.

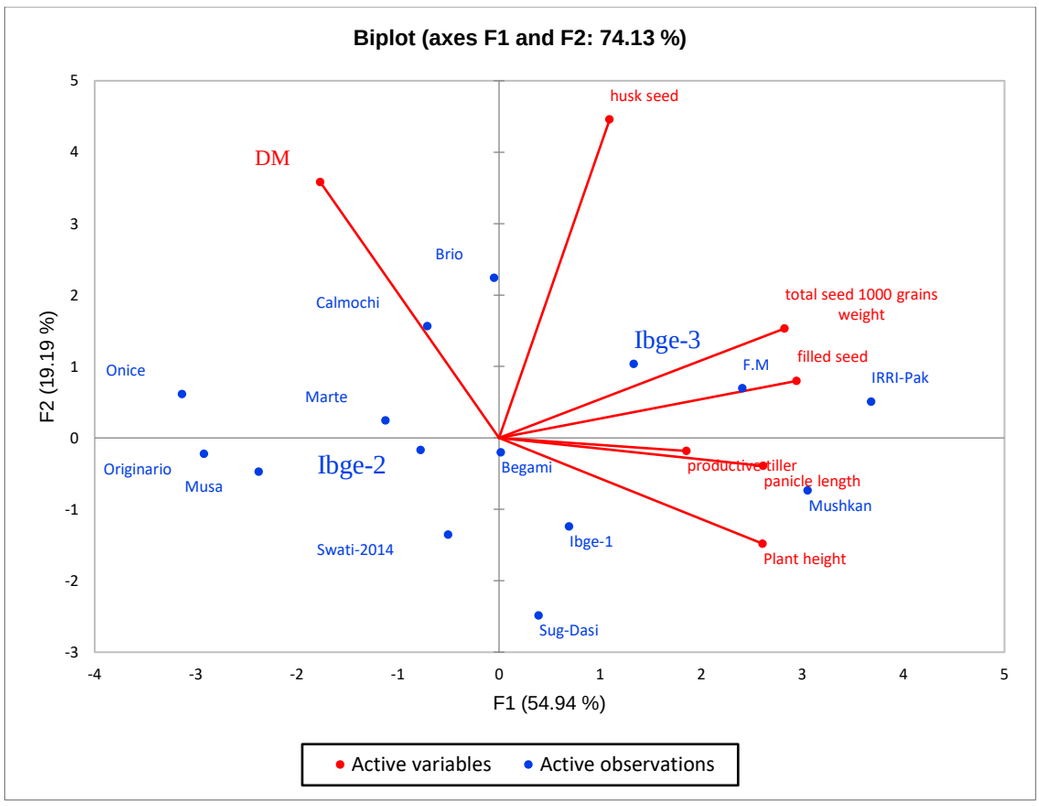

Figure 4. Principle component analysis (PCA), based on agro-morphological traits

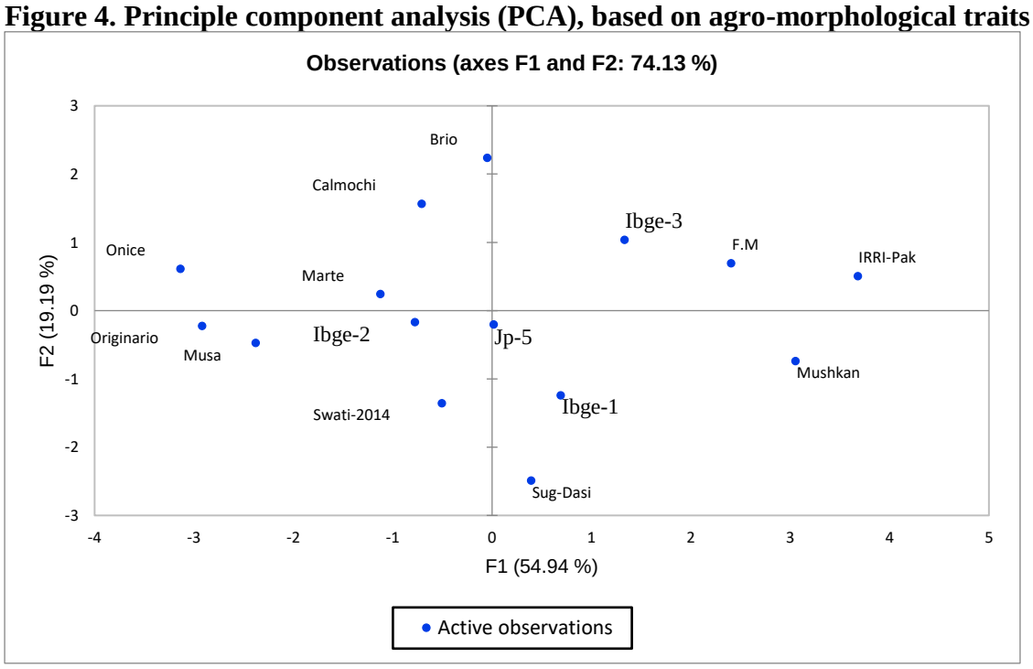

Figure 5. PCA scattered plot constructed based on agro-morphological traits

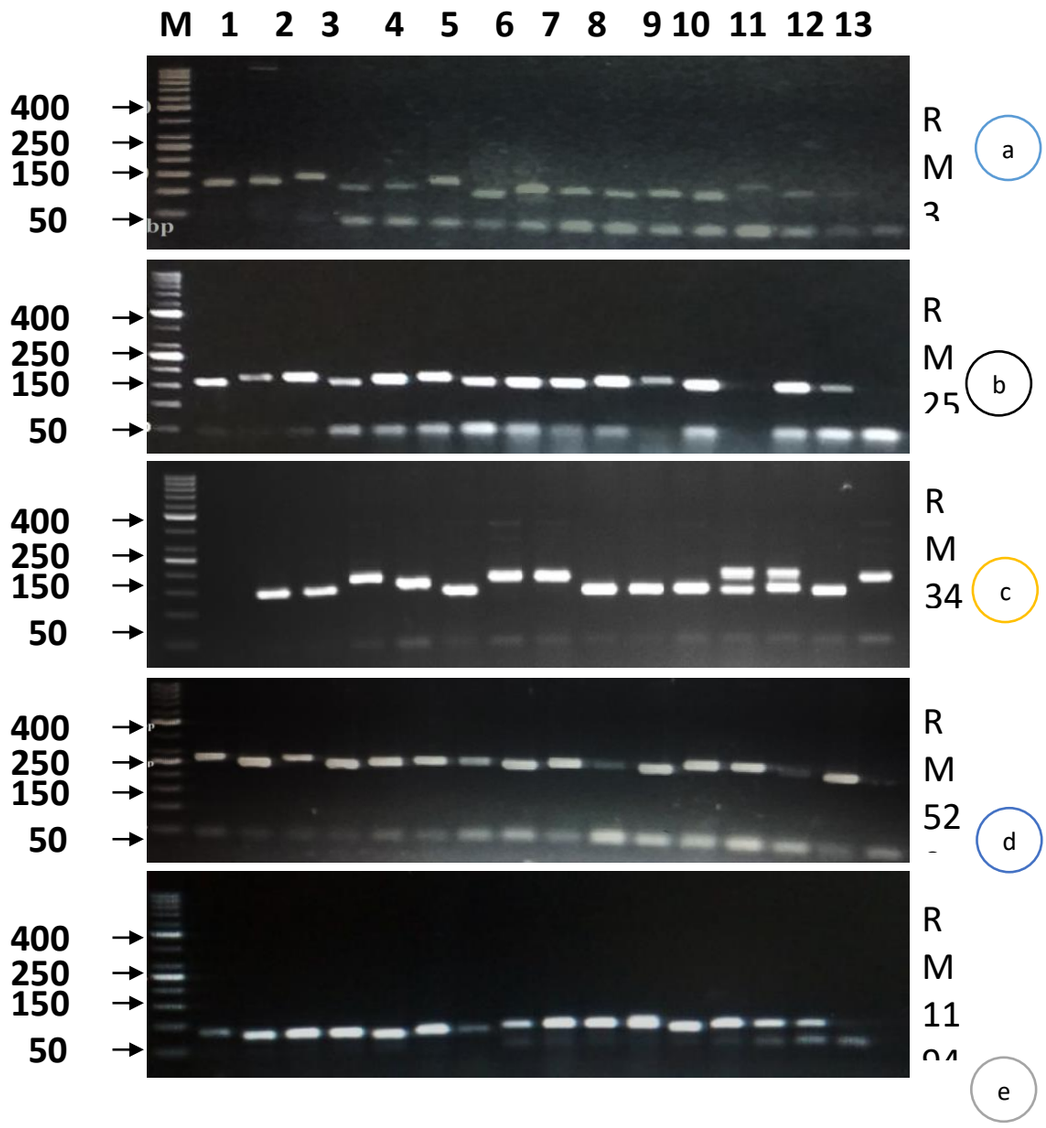

Figure 6. PCR amplification profile of SSR marker (a) RM3 (b) RM259 (c) RM 341 (d) RM 520 (e) RM 11943. M represents 50 Kb ladder; 1. Swati-2014; 2. Originario; 3. Brio; 4. Ibge-2; 5. Onice; 6. Marte; 7. Ibge1; 8. Musa; 9. Mushkan; 10. Irri-Pak; 11. Begami; 12. Calmochi; 13. Ibge-3; 14. FM and 15. Sug-Dasi/ DR-83. PCR product was separated on 2% agarose gel.