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Genetic diversity in natural watermelon (*Citrullus lanatus)* populations of Rajasthan

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ABSTRACT

The present investigation was carried out to estimate the genetic diversity at molecular level using RAPD primers across 23 genotypes of watermelon [*Citrullus lanatus* (Thunb.) Mastum and Nakai]. RAPD analysis was conducted using 14 random primers of which eight primers produced 41 bands, 39 being polymorphic, generating 95% polymorphism. Jaccard similarity index based on RAPD revealed 49.6% average similarity with a range of 28% to 86%. The SAHN clustering based on UPGMA algorithms has put genotypes in two groups, one with 5 genotypes and another with 9 genotypes. The 9 genotype were left separately these being most diverse. High within and between group diversity resulted in poor grouping. Moreover clustering did not show any regional pattern. However, some population specific bands suggested regional influence on diversity.

Key words: Crop improvement, Ecotypes, Genetic diversity, Natural population, RAPD, Watermelon

Mateera, an ecotype of watermelon [*Citrullus lanatus* (Thunb) Matsun and Nakai] exists in dry arid region of Rajasthan. With the onset of monsoon the plants emerges itself and bears fruits. The local people use the unripe fruit as vegetable and ripped one as desserts. These primitive varieties are the repository of the heat, drought and insect pest tolerant genes. People collected the watermelon land races/ primitive varieties from Rajasthan area and made improvement over it. The today's watermelon has undergone several important cycles as well as some authentic changes during the past 20 years. The first change came when the high yielding improved open pollinated (OP) varieties were introduced; these varieties became dangerous to the primitive varieties and the second when open pollinated varieties gave way to hybrid varieties. Eventhough OP varieties were inbred varieties maintained by isolation, some variability in fruit size and shape did appear from time to time. As cultural practices such as plastic mulch, closer spacing, drip irrigation and more precise fertilization maximized the environment for watermelon production, it became clear that the hybrids were yielding more than inbreeds. The grower could afford to pay more for seed, and seed companies could afford to increase the research efforts. This all development has worked as a devastating factor for the germplasm diversity.

With these developments, it is being seen as an important alternative crop for diversification in Bikaner region. Before, the improved genotypes take over the local populations germplasm needs to be collected for preservation. In this context, determination of diversity in populations of various regions is important. Moreover, diversity present in the germplasm would also determine possibilities of further improvement. Thus, determination of diversity in the germplasm makes an important primary study area for any crop introduction and improvement programme.

In recent years, DNA based molecular markers are preferred for the estimation of diversity. A large number of DNA based molecular markers have been developed of which hybridization based RFLP marker technology was first and widely used in research. But, being costlier, labor intensive and time consuming, a more simple technique amenable to automation, PCR based (Mullis *et al.* 1987) RAPD (Williams *et al.* 1990) was developed that has gained much importance due to its simplicity and efficiency, selective ease to perform and non-requirement of sequence information. The RAPD technique uses single primers with random sequences to amplify discrete DNA fragments, which makes it appropriate to reveal polymorphisms among individuals (Williams *et al.* 1990). It has been used with success on several wild species of forest or horticultural crops (Besnard *et al.* 2001, Gao *et al.* 2001). Chowdhury *et al.* (2002) studied that RAPD markers can detect varietal differences in soybean though they are morphologically more or less alike. Thus, the present investigation aimed at determining diversity available at molecular level, among

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different genotypes of *Mateera* in region of Rajasthan.

MATERIALS AND METHODS

The present investigations were carried out at the Plant Biotechnology Centre, Rajasthan Agricultural University, Bikaner. The experimental material consisted of 23 genotypes of watermelon representing wild stands from Nagour, Churu, Bassi, Pali, Jaisalmer, and released varieties namely, AWH 19, Arka Manik, Asai Yamato. These genotypes were grown in experimental plot of Plant Biotechnology Center, Rajasthan Agricultural University, Bikaner, Rajasthan.

Three grams of tender twigs along with leaves were homogenized in liquid nitrogen (liq. N_2) after though washing. The homogenized material was handled as per the method described by Doyle and Doyle (1990). RNA was removed by treating the sample with DNase free RNase. Protein including RNase was removed by treating with chloroform: Isoamyl alcohol (24:1). The integrity of DNA was judged through gel analysis and the quantitation of DNA was done by observing it at 260 nm and 280 nm wavelengths by using a UV- VIS spectrophotometer (made by UNICAM). The quantitated DNA was diluted to final concentration of 25 ng/µl in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) for RAPD amplification. Random amplification of polymorphic DNA was done by using 14 primers of OPF and OPG series obtained from 'Operon Technologies'. PCR reactions were performed in final volume of 25 µl containing 10X assay Buffer, 1.0 unit of Taq DNA polymerase, 200 µM each of dNTPs, 10 pmols/reaction of random primers (Operon Technologies) and 50 ng of template DNA. The PCR was performed in 'Biometra Thermocycler'. Following the amplification, the PCR products were loaded on 1.2% Agarose gel (Himedia, molecular grade), which was prepared in 1X TBE buffer containing 0.5 µg/ml of the ethidium bromide. The amplified products were electrophoresed for 3 - 3.5 hr at 100 V with cooling. After separation the gel was viewed under UV trans-illuminator and photographed by digital camera. RAPD bands were designated on the basis of their molecular sizes (length of polynucleotide amplified). λ DNA EcoRI/Hind III double digest was loaded with each primer products to estimate the molecular size. The distance run by amplified fragments from the well was translated to molecular sizes with reference to molecular weight marker. The presence of each band was scored as '1' and its absence as '0'. Faintly visible bands were not scored but a major band corresponding to faint band was considered for scoring. In order to confirm the presence of bands and determine reproducibility of all the primers were replicated twice and if necessary thrice. The scores (0 or 1) for each band obtained from photograph were entered in the form of a rectangular data matrix (qualitative data matrix). The pair-wise association coefficients were calculated from qualitative data matrix using Jaccard's similarity coefficient. Cluster analysis for the genetic distance was then carried out using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships of the clones using computer program NTSYS pc version 2.02 (Rohlf 1998). To compare the efficiency of the markers in varietal identification, the discriminating power (D) of each primer was estimated. A single numerical index of discrimination (D) based on the probability that two unrelated strains sampled from the test population will be placed into different typing groups was calculated based on Simpson's index of diversity (Simpson 1949) and described by Hunter and Gaston (1988).

RESULTS AND DISCUSSION

Random amplified polymorphic DNA (RAPD) analysis was performed with 14 decamer primers. Out of fourteen primers used, 6 did not amplify any fragment. While other 8 primers generated amplicons ranging from 4 (OPF 18, OPF 16) to 7 (OPG 4). The reproducibility of the bands generated by these 14 primers was confirmed by replicating the amplification twice and if needed thrice. Only the bands showing reproducible amplification were considered for scoring and for further analysis. The number of polymorphic bands ranged from 4 to 7 with range of polymorphisms 80% (OPG-5,OPF 19) to 100% (OPF 18,OPF 20, OPF16,OPG 4, OPG 3 and OPG 6) (Table 1). The total number of

Table 1 List of single arbitrary primers showing total and polymorphic amplicons generated along with PIC and discriminatory power of each pattern for 23 genotypes of *Citrullus lanatus*

Primers	Sequences (5'→3')	Total No. of bands (a)	Total No. of polymophic bands (b)	Polymorphism (b/a × 100)	PIC	Discrimination index (D)
OPF-18	TTCCCGGGTT	4	4	100	0.349	0.786
OPG-5	CTGAGACGGA	5	4	80	0.319	0.759
OPF-20	GGTCTAGAGG	5	5	100	0.733	0.707
OPF-19	CCTCTAGACC	5	4	80	0.589	0.600
OPF-16	GGAGTACTGG	4	4	100	0.289	0.562
OPG-4	AGCGTGTCTG	7	7	100	0.422	0.822
OPG-3	GAGCCCTCCA	5	5	100	0.342	0.691
OPG-6	CTGAGACGGA	6	6	100	0.289	0.443
Total		41	39	95 (Aver.)		

bands generated by eight amplifying primers was 41 with an average amplification of 5.125 bands per primer. The average polymorphism generated by these bands was 95%. The size of the amplicons generated varied from 100 bp (OPG 3) to 3000 bp (OPG-5).

The banding pattern generated and polymorphism reflected in these patterns was used to identify the accessions taken for present study. All the 8 amplifying arbitrary primers produced polymorphic bands. Though, none of the single primers produced unique patterns for all the twenty three genotypes, they could distinguish all the genotypes collectively. A combination of more than two primers can be selected to distinguish all the accessions. The ability of a primer to distinguish between unrelated strains can be determined by the number of types (pattern types) defined by the primer and the relative frequencies of their types. We have calculated a single numerical index of discrimination (D) based on the probability that two unrelated genotypes amplified from the test population will be placed into different typing groups. The value of D is one when all the patterns are unique and 0 when patterns generated for all the varieties contain monomorphic bands. The value of D ranged from 0.443 (OPG 6) to 0.822 (OPG 4) for single primer based RAPD patterns. However, both of these primers had 100% polymorphism level (Table 1).Whereas PIC (Polymorphic information content) ranges from 0.255 (OPF-20) to 0.439 (OPF-16). The PIC and Discriminatory values had a poor correlation coefficient being 0.174.

Genetic similarity estimates based on RAPD banding patterns were calculated using method of Jaccard's coefficient analysis (Table 2). The similarity coefficient matrix generated was subjected to algorithm "Unweighted Pair Group Method for Arithmetic Average (UPGMA)" to generate clusters using NTSYS 2.02 pc program (Rohlf 1998). The dendrogram showing relationship among various genotypes was constructed using these clusters (Figure 1). The Jaccard's pairwise similarity coefficient values ranged from 0.28% (p-3, N-2 and C-2, J-2) to 0.86% (C-1 and B-4) with an average of 0.496, for single primer based RAPD patterns (Table 1). The clusters constructed through NTSYS (2.02 pc) presented in the form of dendrogram has been shown in Fig 1. The dendrogram has generated two major groups (group A and B) at 46% between group similarities whereas C-2, P-2, P-3, B-3, J-3, N-1 AWH 19, Asai Yamato has been left out of the groups. Group A consists of J-1, C-1, C-4, B-1, B-4 and N-3, total 5 genotypes having 67.2% within group similarity and group B consists J-2, J-4, N-2, N-4, P-1, P-4, C-3, and Arka Manik; total 8 genotypes with 53.9% similarity.

Though, great deal of diversity average 50.4% was shown by the material, there was no regional influence observed and the cluster analysis showed that both the cluster A and B included genotype from various places. Similar trend was evident for the outliers. Three of the varieties taken represented different types, Asai Yamato developed in Japan and Arka Manik developed at Banglore represent summer

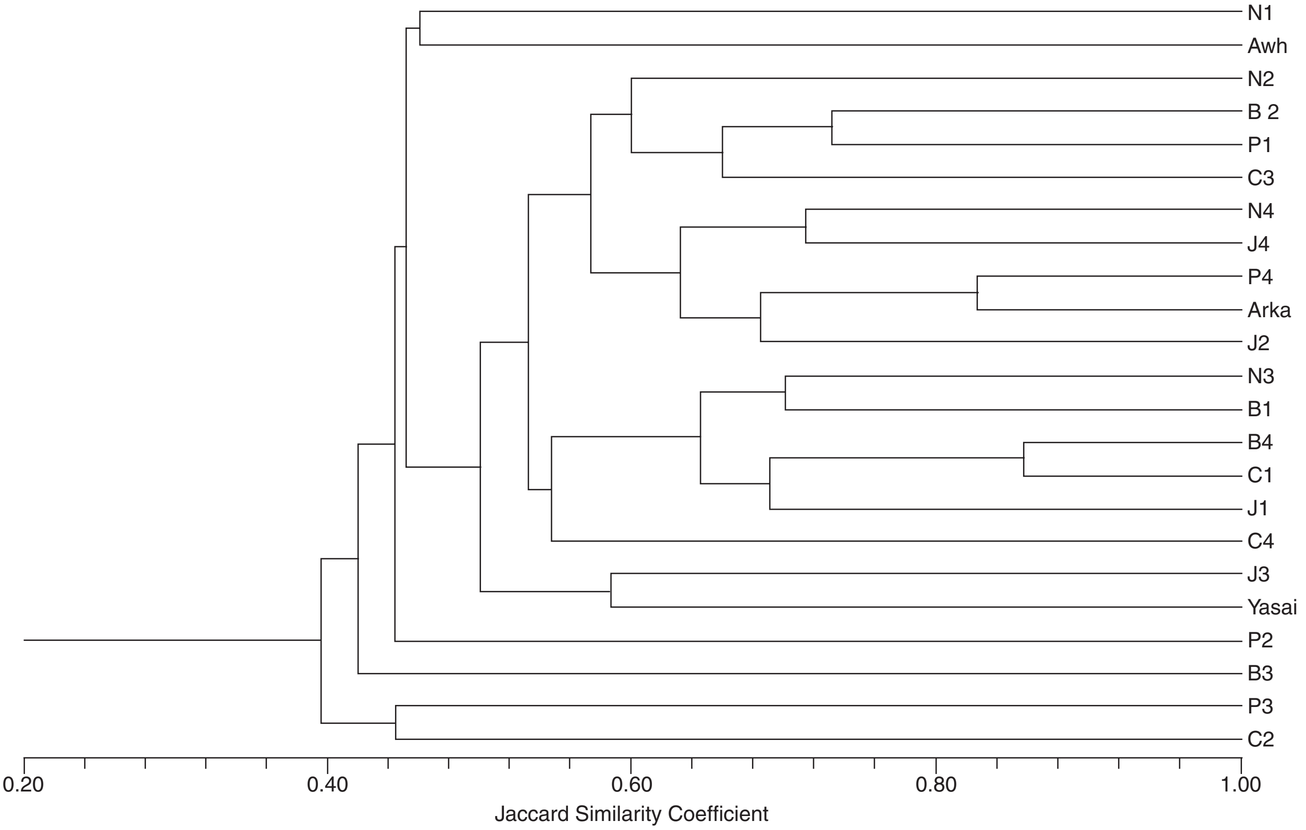

Fig 1 Dendrogram showing relationship among twenty three watermelon genotypes generated by UPGMA analysis based on single primers.

type (Tarbooj) and AWH 19 is a monsoon type. There was no difference seen in clustering on the basis of such types. The two Tarbooj types show more similarity with the local collection most similar being Arka Manik (57.9%) followed by Asai Yamato (50.3%) with the rest genotype, where as the mateera type AWH 19 showed less similarity with rest of the genotypes (43.0%). However comparisons within three varieties differentiate AWH 19 at higher dissimilar coefficient *i.e.* 53% from Asai Yamato, and 48% from Arka Manik. The dissimilarity between Arka Manik and Asai Yamato is 37%.The result obtained are in accordance with the findings of Dane and Liu (2007). Dhillon *et al.* (2007) also studied the comparative analysis of genetic variability among Indian snapmelon using RAPD primers.

The absence of regional effect on genetic similarity was also evident from comparatively lesser similarity coefficient for within region genotypes compared to between region genotypes least similarity was observed for Pali collection, whereas Jaisalmer collection shows highest similar (52.6%). The three varieties collectively were more similar to Jaisalmer collection (57.0%), However, there were a few population specific bands generated, for example 600bp band of OPF-16 was present in one of the Pali sample (p 2) and in variety Asai Yamato. Similarily, 2000bp band of OPG-4 was present in Pali (p3) sample and Asai Yamato and OPG-6,900bp band in Churu (c-3) sample.

The discriminatory power of RAPD patterns was normally found to be higher for most of the primers showing higher polymorphism and PIC values, however this was not the case always. Amplicon patterns for all the primers produced D value of 0.822 or more. Thus, RAPD patterns generated may efficiently be used in identifying accessions. Similar results have been obtained (Chin and Smith1993, Khalil 1998, Barkar *et al.* 1999, Chen and Liu 2001, Xu *et al.20*04, Dane and Liu 2007). In addition, the genotype and population specific bands generated for Pali (p2, p3) Asai Yamato and Churu (c3) could directly be used identify such populations and accessions.

The Jaccard similarity coefficient generated on the basis of RAPD profiling showed a average similarity of 0.496 (49.6%) among all the 23 genotypes of watermelon. The level of diversity with in a population is important for the adaptability of the species. In case of *C. lanatus* both within and between populations diversity is very high and makes it adaptive in wider range of climatic and other edaphic conditions. This is evident from their suitability to grow in various seasons and diverse regions covering various continents of world. The SAHN clustering based on UPGMA algorithism has put the genotypes in two groups, one with six genotypes and another with eight genotypes. The eight genotypes were left separately indicating these being most diverse. The high within group diversity comparable to the level of between group diversity has resulted in poor grouping indicating that populations are quite diverse. The inclusion of genotypes from various regions indicated absence of regional influence on adaptability of genotypes or free migration of seed between regions overcoming the effect of selection. The population specific bands however, indicated presence of differential selection pressure. Pali representing more diverse geo-climatic regions compared to other regions, viz. Nagore, Churu, Bassi and Jaisalmer had more population specific bands, thus, suggesting climatic influence over selection. The findings are in line with the findings of Lee *et al,*(1996); Levi *et al.* (2001) and Levi and Thomas (2007).

It is concluded from the present investigation *C. lanatus* collected from various regions of Rajasthan harbors a great deal of diversity. The poor clustering and distribution of genotypes among groups from various regions suggested no or very less influence of regions in shaping the diversity. Presence of population specific bands however, indicated some regional effect on survival.

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