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Genetic Variability and Phylogenetic Relationship of Pakistani Snapmelon (*Cucumis melo* var. Momordica) by Using Microsatellite Markers

Ghulam Rasool[1], Muhammad J. Jaskani[1], Amjad Ullakh[1] & Rashid Ahmad[2]

[1] Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan

[2] Department of crop physiology, University of Agriculture Faisalabad, Pakistan

Correspondence: Muhammad J. Jaskani, Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan. E-mail: jjaskani@uaf.edu.pk

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Abstract

Among the major cucurbit vegetables, *Cucumis melo* has one of the highest polymorphic fruit types and botanical varieties. The aim of the present study was to evaluate the genetic diversity among different genotypes of Snapmelon (*Cucumis melo* var. Momordica) collected from all the four provinces of Pakistan. In this study, 18 microsatellite markers were tested on Snapmelon germplasm which yielded valuable information about the genetic relationships among 40 Snapmelon accessions. The mean PIC value of the markers ranged from 0.3706 to 0.8247. For establishing data matrix, an auto radiogram was visually scored for the presence (1) or absence (0) of polymorphic bands. Assessments of genetic relationship among the genotypes were done by cluster analysis, using POPgen software. The genetic analysis through principle coordinate analysis (PCA) and dendrogram showed that the wild accessions were distinguished from all domesticated accessions collected from various regions of the country. Genetic differentiation among the populations using molecular data indicated the importance of the study area for species conservation, genetic erosion estimation, and exploitation in breeding programs.

Keywords: Snapmelon, genetic relationships, microsatellite, PCA, PIC, data matrix

1. Introduction

The genus *Cucumis* is famous in the world with 30 wild and cultivated species, which are mostly found in Africa, tropical America and Southeast Asia. This genus was first originated in Africa but some authors also mentioned the Middle East and Asia as centers of wild type melons (Nakata et al., 2005). Snapmelon is cultivated in various parts of the globe including India and Pakistan. However, the recent literature about Snapmelon indicates that origin of the genus *Cucumis* may be in Asia (Schaefer et al., 2009; Fergany et al., 2010). The center of diversity of melon is believed from the Mediterranean basin (Turkey) to Central Asia (Iran, Uzbekistan) and from India to East Asia (Fergany et al., 2011). Snapmelon exhibits remarkable variation in fruit traits like size, shape, color, taste, texture, and biochemical composition. Snapmelon (*Cucumis melo* L. var. *momordica* (Roxb.) is native to India. It was N.P.S. Dhillon and Ranjana who contributed equally to this intensively grown crop in the 19th century in northern India (Duthie, 1905) where it is commonly known as 'Phut' which means to split

In Pakistan, the Snapmelon is also cultivated under the name of 'Phut' and the group name is referred as Momordica of the family Cucurbitaceae whose fruits crack at maturity. It is a neglected crop in Pakistan and mostly, it is cultivated as a mixed crop in cotton or many other crops. The plants of this crop are monoecious. The fruits of Snapmelon are yellow, orange or light in colour, oval or elliptic in shape with smooth skin. Fruit flesh may be white, creamy, light yellow or light orange, mealy and without sugar or aroma. The variation detected among melons originating in Southern India, can be compared to a reference group of melon accessions from miscellaneous origins (Eastern Asia, Central Asia, Western Asia, Africa, Europe, and other geographic regions) (Monforte et al., 2003, 2005).

It has been reported that germplasm of Snapmelon is a good source of disease and insect resistance (Pitrat et al., 2000). To explore the potentiality of the germplasm of Snapmelon it is believed that the identifying suitable accessions (wild and domesticated) with superior horticultural characteristics and adaptation ability for given

locations and conditions, is useful and necessary for overcoming drawbacks, and providing genetically uniform, cost-effective planting material (Perry, 1987). Genetic variation assortment is important factor for the sustainable agricultural production (Zhou et al., 2002). In spite of tremendous genetic potential of Snapmelon in Pakistan, a comprehensive analysis of genetic variation, plant characteristics, physical and biochemical features of fruits in this taxon, has yet not been conducted.

Moreover, there is a dire need of application of powerful and modern biotechnological techniques like tissue culture, molecular biology, genetic engineering and molecular DNA markers to understand structure and function of different biological and metabolic pathways for assessing diversity, conservation and improvement of characteristics of Snapmelon. Morphological and molecular markers are used to find relationship among accessions of melons (Stepansky et al., 1999; Staub et al., 2004). Morphological markers are not reliable sources to study diversity because they cannot provide accurate and clear information about the accessions. In fact, morphological traits are affected by various environmental factors and by pleiotropic gene action (Tanksley, 1993; Andersen & Lubberstedt, 2003). Molecular markers are more suitable and informative for diversity study as they can be applied for germplasm characterization, genetic diagnostics, characterization of transformants, studies of genome organization, and phylogenetic analysis (Gupta et al., 1998; Gostimsky et al., 2005).

The genetic diversity in Snapmelon extends to both vegetative and reproductive characteristics (Rai et al., 2012). To raise the usefulness of Snapmelon, germplasm for melon conservationists, breeders and growers, the morphological, biochemical and molecular characterization of Pakistani Snapmelon is necessary. In order to characterize melon accessions in systematic way, specific morphological and genetic markers have been developed such as Restriction Fragment Length Polymorphism (RFLP) (SSRs) Risteruccci et al., 2005) and Random Amplified Polymorphism DNA (RAPD) (Feria-Romero et al., 2009; Chen et al., 2007) for the estimation of genetic diversity (Valdes-Infante et al., 2010). Up to now, only dominant polymerase chain reaction (PCR)-based marker technologies such as Random Amplified Polymorphic DNA (RAPD) (Prakash et al., 2002) were applied to study the Snapmelon genetic diversity. Amplify Fragment Length Polymorphism (AFLP) (Hernández-Delgado et al., 2007) analysis is a very useful molecular marker technique, of genome-wide coverage, that allows detection of a high number of polymorphisms. The SSR or micro satellite co- dominant technique, which has proven its advantages and suitability in a large range of applications in genetics, was developed in order to improve the availability of best performing molecular tools for genetic studies and further marker assisted breeding in melon and its close related species. Hence, characterization of genotype at the genetic level supplemented with phenotypic characters could be an important step towards efficient conservation, maintenance and utilization of existing genetic diversity (Mehmood et al., 2014). So this study was planned to explore polymorphism in available Snapmelon genetic resources of Pakistan on molecular level.

2. Materials and Methods

The present study was carried out in the Plant Genomic and Fingerprinting lab., Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan.

2.1 Plant Materials

Forty accessions of Snapmelon were collected from 34 different geographical locations of Pakistan covering all the four provinces (Table 1). For DNA extraction and genetic diversity analysis young and healthy leaves were collected and preserved in ulta-low freezer at -80 °C.

Table 1. Details of Snapmelon accessions collected from different localities

S.No.	Accession code	Location	Accession Specification
1	SG 1585	Sargodhaa	Domesticated
2	HP 1586	Hasalpur	Domesticated
3	MT 1587	Multan	Domesticated
4	RYK 1588	Rahim Yar Khan	Domesticated
5	BW 1589	Burewala	Domesticated
6	TTS 1590	Toba Tek Singh	Domesticated
7	KP 1591A	Khair Pur 1	Domesticated
8	KPW 1591B	Khair Pur 2	Wild
9	MPK 1592	Mir Pur Khas	Domesticated
10	JW 1593A	Jaranwala 1	Domesticated
11	JWW 1593B	Jaranwala 2	WILD
12	DP 1595	Depal Pur	Domesticated
13	PP 1596	Pakpattan	Domesticated
14	NK 1597A	Naakana 1	Domesticated
15	NKW 1597B	Naakana 2	WILD
16	ps 1598	Peshawar	Domesticated
17	KS 1599	Kasur	Domesticated
18	HfA 1600	Hafizabad	Domesticated
19	LL 1601	Lora Lai	Domesticated
20	HdA 1602A	Hydrabad	Domesticated
21	HdA W1602B	Hydrabad	WILD
22	GJ 1603A	Gojra	Domesticated
23	GJW 1603B	Gojra	WILD
24	VH 1604	Vehari	Domesticated
25	MW 1605	Mianwali	Domesticated
26	SW 1606	Swabi	Domesticated
27	KM 1607	Kamalia	Domesticated
28	FA 1608	Faisalabad	Domesticated
29	ChW 1609	Chechawatni	Domesticated
30	TL 1610	Tandla	Domesticated
31	NS 1611	Nawabshah	Domesticated
32	GT 1612	Ghotki	Domesticated
33	AW 1613	Arifwala	Domesticated
34	ShW 1614	Sahiwal	Domesticated
35	SG 1615	Sanghar	Domesticated
36	BD 1616	Badeen	Domesticated
37	SM 1617	Sumandri	Domesticated
38	SK 1618A	Sakhar 1	Domesticated
39	SKW 1618B	Sakhar 2	WILD
40	KL 1619	Kulachi	Domesticated

2.2 DNA Extraction

Total genomic DNA was extracted from 0.5 g of young leaves of each accession by the cetyl trimethyl ammonium romide (CTAB) method of J. J. Doyle and J. L. Doyle (1990) with slight modifications (Padilla-Ramirez et al., 2002). The samples were quantified using spectrophotometry after which the purity was estimated. For the polymerase chain reaction (PCR) procedure, 18 SSR markers were used (Table 2).

2.3 PCR Amplification Conditions

A PeqLab 96 well primus thermal cycler was used in this study. The PCR amplification program used was as follows: 4 min at 94 °C, followed by 30 cycles of 45 seconds at 94 °C, 45 seconds at 55 °C, and 45 seconds at 72 °C, followed by final extension of 45 seconds at 72 °C (Rodrigues et al., 2007).

2.4 Gel Electrophoresis

After PCR reactions, a mixture from SSR amplification was processed for 1.5% agarose gel and stained with ethidium bromide solution.

2.5 Data Scoring and Analysis

For establishing data matrix, an autoradiogram was visually scored for the presence (1) or absence (0) of polymorphic bands. Assessments of genetic relationship among the genotypes was done by cluster analysis; using POPgen (version 1.44). Data was subjected to Principle Component Analysis (PCA) in order to identify genetic variation. Data analysis was performed using SAS statistical software (Version 9.3). For each primer, the polymorph ism information content (PIC) value was taken as the mean of the calculated PIC of all loci. The PIC for SSR (co-dominant markers) was calculated as, $PIC = 1 - \Sigma pi^2$.

3. Results

3.1 Genetic Diversity among Snapmelon Accessions of Pakistan by 18 SSR Markers

Quantification of genetic variation was made through microsatellite analysis on Snapmelon germplasm collected from various forty locations including all the four provinces of Pakistan. Eighteen microsatellite markers were used to evaluate the diversity and all the markers proved effective by producing scorable bands and displaying polymorphism within genotypes (Table 2).

PICs ranged from 0.3706 to 0.8247 with an average of 0.6945 per locus. The primer pair TJ2, CMATN89 and CMCTN86 had the highest PIC value (0.8247), indicating that these were the most informative locus. Among the twelve (12) microsatellites (SSR) primer pairs tested in the Pakistan Snapmelon, all SSR primer pairs successfully analyzed genetic variation and produced multiple fragments in the 40 accession of Pakistani Snapmelon. In total, 21 alleles were discriminated with an average of 1.75 alleles per locus. The number of alleles per locus ranged from 0.5427 for the locus CMCTN86 to 0.9065 for locus CMAT141 followed by TJ31 (0.8963) and CMAT35 (0.8374). The allele frequency varied from 0.5427 for CMCTN86 to 0.9065 for CMAT141. A total of 24 genotypes were scored with an average of 1.6 genotypes per locus, a maximum of 3 genotypes for the locus CMAGN75 and minimum of 1.5 genotypes were recorded in TJ10.

The polymorphic information content parameter showed that CMCTN86 is the most polymorphic locus (0.3722) and that CMAT141 is the least polymorphic one (0.1271). A value of 0.251 was recorded as a mean of the PIC for all detected loci. The results indicated the efficiency of these SSR markers to detect molecular polymorphism. Thus, these markers can be used to perform genetic diversity studies on Pakistani Snapmelon.

These results indicate that the SSR marker system used in this study can also reveal genomic DNA diversity in Snapmelon (*Cucumis melo* var. *momordica*) (Table 2).

Table 2. List of Markers used for genetic analysis of Snapmelon accessions

Markers	Forward	Reverse	Annealing Tem (°C)	PIC values
CMGA128	ATGAAGAAGG-GATATTCAAAG	ACTCCATTGT-TGCTAACCTTT	59	0.7119
CMTAA166	GGAACAGACA-CCTCTTCTGAG	TCCGTCTACA-AGCGTGACTGT	59	0.7861
CMCCA145	GAGGGAAGGC-AGAAACCAAAG	GCTACTTTTG-TGGTGGTGG	59	0.7544
CMCTN86	GTGACAGTTA-TCAAGGATGC	AAGGGAATG-CATGTGGAC	59	0.8247
CMAGN75	TGGGTTTTCT-TCTACTACTG	TGCTTTTACT-CTCATTCAAC	59	0.8152
CMAT35	GTGGGTCAT-CATTATTGTTA	GCTTTTAGC-CTATTAAGTTGC	59	0.6050
CMTA134B	GCTCCTCCT-TAACTCTATAC	GCATTATTAC-CCATGTACGAG	59	0.7119
CMTC160b	GTCTCTCTC-CCTTATCTTCCA	GATGGTGCC-TTAGTTGTTCCG	59	0.6533
CMTCN41	CCCCAAGA-TTCGTATTAATC	TGGTAGTAGA-GATGATATAC	59	0.7397
CSCCT571	CCTTTCTGC-TGTTTCTTCTTC	GAAGGAAGG-AGTGAGGGGAAG	59	0.7119
TJ27	AAGCGGAAC-AAGCTCATCTC	CAAAAGCATC-AATTGCTTGAA	59	0.7786
TJ2	GAGGAATCC-GAGACCACAAC	GCCAAGTGTG-TGTTGGAAAA	59	0.8247
CMCT38	ACGAGGAAA-ACGCAGAATCA	TGAACGTGGA-CGACAGTCTT	59	0.3706
CMTC168	ATCATTGGATG-TGGGATTCTC	ACAGATGGAT-GAAACCTTAGG	59	0.6675
TJ10	ACGAGGAAA-ACGCAAAATCA	TGAACGTGGA-CGACATTTTT	59	0.6414
TJ31	GAGGCCTCC-TCAGCTCTACA	AGCCCATTAG-CACAAGCTGA	59	0.5698
CMAT141	AAGCACACC-ACCACCCGTAA	GTGAATGGTA-TGTTATCCTTG	59	0.5112
CMATN89	CACTACCTTA-AAACAGAATTG	GGACAATTTA-GGGAGGATC	59	0.8247

3.2 Binary Matrix of 40 Snapmelon Cultivars

The binary matrix from the 40 Snapmelon DNA fingerprints produced from the 18 primer pairs was analyzed to reveal genetic similarities (Figure 1). The resulted dendrogram divided the studied accessions into three classes of various genotypes (Table 3). The class one made the largest group among the three and included accessions VH (Vehari), HP (Hasalpur), MT (Multan), KpP (Khair Pur), TL (Tandla), ShW (Sahiwal), FA (Faisalbad), AW (Arifwala), PP (Pakpattan), SM (Samundri), ChW (Chechawatni), KM (Kamalia), Gj (Gojra), Jw (Jaranwala), Mw (Mianwali), Tts (Toba Tek Singh), BM (Burewala), DP (Depalpur), RYK (Rahim Yar Khan),GT (Ghotki), SW(Swabi), NS (Nawab Shah), HfA (Hafizabad), PS (Peshawar), NK (Nankana), BD (Badeen), SG (Sargodha), KS (Kasur), SNG (Sangharr) and LL (Loralai). The class one was further divided into several sub classes. Similarly, the principal coordinate analysis divided the Pakistani Snapmelon accessions into four classes. The group two had thirteen accessions and was the largest of all but the smallest class was consisted of six accessions only. The Punjab (Burewala, Sahiwal and Kasur) and the Sindh accessions (Sakhar and Ghotki) were grouped together in the principal coordinate analysis (Figure 2).

Table 3. Grouping of Snapmelon accessions on the basis of genetic variation

Classes	Genotypes
C1	VH (VEHARI), HP (HASALPUR), MT (MULTAN), KP (KHAIR PUR), TL (TANDLA), ShW (SAHIWAL), FA (FAISALBAD), AW (ARIFWALA), PP (PAKPATTAN), SM (SAMUNDRI), ChW (CHECHAWATNI), KM (KAMALIA), GJ (GOJRA), JW (JARANWALA), MW (MIANWALI), TTS (TOBA TEK SINGH), BW (BUREWALA), DP (DEPALPUR), RYK (RAHIM YAR KHAN), GT (GHOTKI), SW(SWABI), NS (NAWAB SHAH), HA (HAFIZABAD), PS (PESHAWAR), NK (NANKANA), BD (BADEEN), SG (SARGODHA), KS (KASUR), SNG (SANGHARR), LL (LORALAI)
C2	HADW (HYDRABAD WILD), HAD (HYDRABAD), MPK (MIRPUR KHAS), KL (KULACHI), GTW (GHOTKI WILD), SKH (SAKKHAR), KPW (KHAIR PUR WILD)
C3	SKW (SAKKHAR WILD), JWW (JARANWALA WILD), GJW (GOJRA WILD)

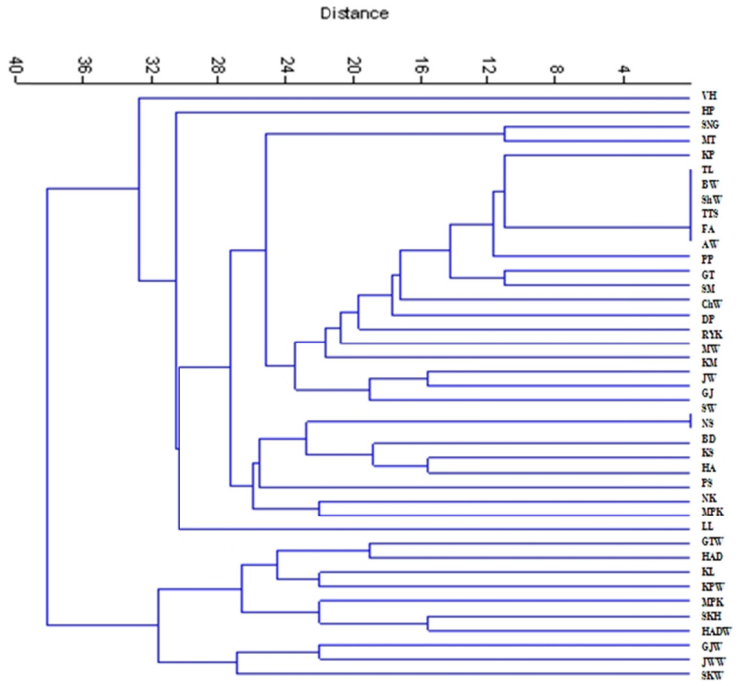

Figure 1. Dendrogram explaining diversity among 40 of Snapmelon accessions

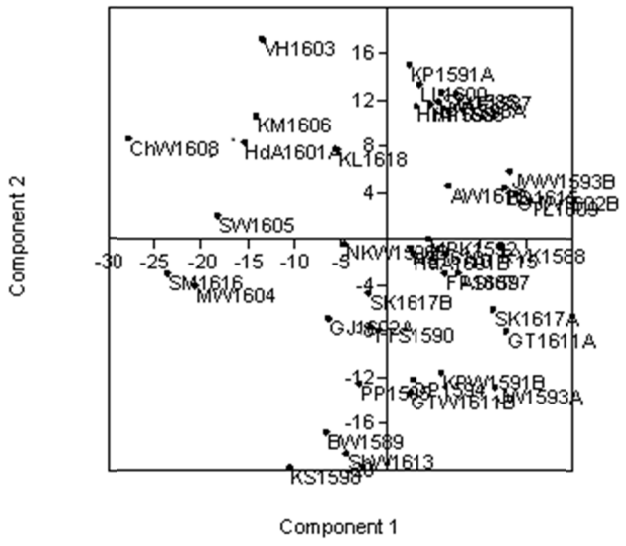

Figure 2. Principal Coordinate Analysis of 40 Snapmelon accessions of Pakistan

4. Discussion

The phenotypic markers and taxonomic studies cannot fulfill the requirements for the germplasm identification in some area. The molecular markers have been becoming more popular to unravel the genetic diversity. The morphological diversity identification is the combined product of the morphological and molecular marker investigations. The phenotypic characteristics are affected by the edaphic factors and results in epigenetic changes. Genetic diversity provides a certain degree of strength against complete destruction for plant population in natural disasters. Extremely low level of genetic diversity may lead to complete elimination of some species and result in loss of biological diversity (Subudhi et al., 2007). There is no definite correspondence between geographic origin and genetic diversity of Snapmelon, indicating that parental selection should be made on the basis of systematic assessment of genetic distance in a specific population rather than on geographic difference.

According to Dias et al. (2003) divergence between any two parents expresses the allelic difference between them. The results indicated the clustering of forty Snapmelon accessions into three main groups. The accessions within these three groups were genetically more similar than the members of other groups. The group one which was the largest group among the three was consisted of the accessions from Vehari, Hasalpur, Multan, Khair Pur, Tandla, Sahiwal, Faisalbad, Arifwala, Pakpattan, Samundri, Chechawatni, Kamalia, Gojra, Jaranwala, Mianwali, Toba Tek Singh, Burewala, Depalpur, Rahim Yar Khan And Ghotki regions. The group two was the second most populated including the accessions from Swabi, Nawab Shah, Hafizabad, Peshawar, Nankana, Badeen, Sargodha, Kasur, Sangharr tehsils and the wild accessions Hydrabad region. Similarly, the domesticated accessions collected from Loralai, Hyderabad, Kulachi, Mirpur Khas, Sakkhar while the wild accessions from Ghotki, Khair Pur, Sakkhar, Jaranwala and Gojra area were the part of third group which was the smallest of all the three classes. The group one was mainly comprised of the accessions from Punjab while the majority in group three were from Sindh province which indicated that some relationship was there among the germplasm of these accessions or they had exchanged the germplasm. The results were interesting as the Snapmelon accessions belong to different geographical locations grouped together and the accessions from neighboring regions expressed more distance in the dendogram. As, the Snapmelon accessions from Swabi and Nawab shah and from that of Sangharr and Multan paired together despite of being from very distant locations. The results showed that these accessions were traveled to different locations from some common origin which is not known. In a similar fashion, the accessions of Hasalpur, Vehari, Loralai and Pakpattan showed no pairing with any other accession and placed on separate independent positions. But, in some cases the geographical proximity was justified. As, the accessions collected from Tandla, Burewala, Sahiwal, Toba tek singh, Faisalabad and Arifwala made a single group which proved that these Snapmelon accessions shared some common ancestors. Furthermore, the class two included HADW (Hydrabad Wild), HAD (Hydrabad), MPK (Mirpur Khas), KL (Kulachi), GTW (Ghotki Wild), SK (Sakkhar) and KPW (Khair Pur Wild) Snapmelon accessions. Also, this comparatively smaller class again showed sub groups in the dendogram. Again the results were not uniform in nature according to the geographical locations of the germplasm. In this class, the wild accessions of Ghotki paired with domesticated accession of Hyderabad. Similarly, the domesticated accessions of Kulachi and Sakhar made pair with the wild accessions of Khair pur and Hyderabad, respectively. While the accession collected from Mir pur khas made no pairing with any other accession under study. In the same fashion, the wild accessions from Gojra, Jaranwala and Sakhar made a single group inspite of the fact that Sakhar had no geographical proximity. In the same way, the principal component analysis divided the forty Snapmelon accessions into four main groups. The first two groups were comprised of mixed type of accessions collected from various regions while the third and fourth group was dominantly composed of the accessions from Punjab and Sindh provinces, respectively. Thus, the clustering of accessions in mixed form of groups indicates the frequent exchange of genetic material among these regions. Moreover, this study provided the estimation and understanding of the genetic diversity patterns present in the areas of Snapmelon cultivation and is also helpful for the conservation and breeding of distinctive accessions. The accessions having wide geographical distribution had more genetic diversity than the species of narrow range distribution (Casiva et al., 2002). Transfer of Snapmelon accessions from one location to the other location may cause mislabeling and mixing of the genetic material present in that area. People are not cultivating this as a commercial crop and are also not using any specific commercial variety. There is no planned cultivation present in the area from where the Snapmelon germplasm was collected. The results of cluster analysis and principal component analysis were not similar due to mixing of some accessions while other accessions remained unexchanged. Different types of genetic markers have been employed to assess the genetic diversity in melon viz. isozymes (Akashi et al., 2002; Mc-Creight et al., 2004), restriction fragment length polymorphisms RFLPs (Neuhausen, 1992), and amplified fragment length polymorphism AFLPs (Garcia-Mas et al., 2000), random amplified polymorphic DNA RAPDs (Staub et al., 2004) and simple sequence repeats SSRs (Monforte et al., 2003). All of these have been equally effective in establishing genetic relationships between melon genotypes. The variation detected among Indian Snapmelons was compared to the reference accessions of melon from diverse origin (S.E. Asia, S. Asia, W. Asia, Europe) (Monforte et al., 2003, 2005) using a set of SSR markers (Gonzalo et al., 2005). These analyses provided the insight into the horticultural worth of Pakistani Snapmelons which is imperative for the organization and conservation of Snapmelon genetic resources and its further utilization. This high polymorphism is in agreement with previous work on melon (Lopez-Sese et al., 2003).

Using 18 SSR markers, we performed PC analysis on Pakistani Snapmelons. The importance of the genetic variability in the Snapmelon collection can be better appreciated on comparing it with the genetic variability observed in other cucurbit genotypes. Though a large sample of Snapmelon population should have been used, these results indicated that the current Snapmelon collection contains a degree of unique genetic variability

which needs to be preserved for future use. Also it supports the previous observations of Akashi et al. (2002) and Monforte et al. (2003) suggesting that the process of introduction and domestication of melons into more regions could have led to the erosion of the variability.

The SSR markers exhibited high levels of polymorphism when they were used to analyze the Snapmelon accessions in Pakistan. The main disadvantage of the SSR markers is that the search strategy of repetitive sequences and the design of the markers employed are usually developed for specific species. In consequence, the laborious and costly procedure that their development implies, limit the number of species that may be assayed (Dayanandan et al., 1997). However, inclusion of additional accessions from other locations of Pakistan or even from other parts of the world can confirm these findings. It was interesting to note in cluster analysis or principal component analysis that the accessions which were closer to each other despite the fact that they belong to very distant locations, clearly indicated that these accessions may be derived from a common population or from few populations. Several researchers have used SSRs for assessing genetic diversity and population structure in plant species as, Collevatti et al. (2001) used SSRs to assess population structure in fragmented population of the various plant species.

Thus, this work shows that Pakistani Snapmelon accessions display a considerable diversity coupled with good horticultural characteristics and there appears to be regional and sub regional differentiation at the molecular level. As Pakistan is a vast geographical land divided into various agro-climatic units, hence this concept of regions and sub-regions can be adopted for future Snapmelon explorations in Pakistan for ensuring the retention of existing variability. Threats to biodiversity are increasing in Pakistan due to population pressure on cultivatable land. Sweet melon evolution under domestication has resulted in better productivity and fruit quality. However, this process narrowed the genetic basis. Natural variation among the relatives of sweet melon (var. momordica for increased high acidity, high tolerance to biotic and abiotic stresses, large number of fruit per vine, drought tolerance and disease resistance) provides an opportunity to enrich the gene pool of sweet melon with novel alleles that eventually could improve productivity, quality and adaptation and reduce the risk of genetic vulnerability.

5. Conclusion

In conclusion, a selected set of 18 SSRs has clearly differentiated the germplasm originating from different regions. The findings of this study demonstrated that a wide genetic diversity is present in Snapmelon. The same set of markers were currently analyzed on multi species and genera of cucurbits with direct sequencing to elucidate some inter generic genetic relationship within the family. The diversity present in accessions collected from Pakistan emphasized that there is a great potential for genetic improvement of Snapmelon to enhance its multipurpose uses, therefore, strategies should be adopted to make maximum use of its diversity. As part of the germplasm collection and breeding program at Institute of Horticultural Sciences, University of Agriculture, Faisalabad (Pakistan), the results of this study will help to improve the conservation and management of germplasm in Pakistan and also facilitate international cooperation on exploiting new plant materials for improving new cultivars in cucurbits.

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