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Estimation of phenotypic divergence in a collection of muskmelon germplasm, including Indian and exotic populations

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Abstract

Seventy melon genotypes, comprising of landraces, inbred lines and cultivars collected from diverse geographical locations, were evaluated for 17 morphological traits for determining the patterns of genetic diversity and relationship among genotypes. The genetic diversity based on quantitative and qualitative characters of genotypes was assessed by D^2 analysis. The Euclidean's distance-based clustering divided the germplasm in to nine clusters. Though the analysis of 17 descriptors distinctly separated *momordica* and wild accessions from *cantalupensis*, *reticulatus*, *inodorus* accessions, but it was not able to differentiate *cantalupensis*, *reticulatus*, *inodorus* group accessions. The inter-cluster distance varied from 759.6 between clusters 1 and 2 to 5194.5 between clusters 4 and 8. The maximum intra-cluster distance matrix was observed in cluster 9 (728.5), while cluster 6 exhibited the minimum intra-cluster variation (344.0). Maximum number of genotypes was grouped in cluster 1 and 7 i.e. 15 in each group. The genotype "Canary Yellow-2" present in cluster 4 did not grouped in any cluster. Overall, the clustering patterns exhibited great diversity in the germplasm for certain traits (fruit morphology and quality characteristics) which can be effectively utilized for broadening the genetic base of muskmelon germplasm and for developing specialty melon cultivars/ hybrids with unique traits such as high beta carotene, sweetness and extended shelf life.

Keywords: Cluster analysis, *Cucumis melo*, genetic diversity, morphological characterization

Introduction

Muskmelon is a commercial vegetable crop in India, and its cultivation history dates to 2000 BC (Malik et al. 2014). Annually, 961.87 thousand MT melons are produced from an area of 46.59 thousand ha (Anonymous 2017). Muskmelon is extensively cultivated in hot and dry areas of Uttar Pradesh, Punjab, Rajasthan, Madhya Pradesh, Bihar and Karnataka states. In Punjab, muskmelon is cultivated on an area of 5.23 thousand ha with 93.12 thousand tons of production (Anonymous 2017). Thus, muskmelon is an important commercial crop in the region which calls for precise characterization of locally adapted germplasm for the development high yielding cultivars/ hybrids. *Cucumis melo* L. is a highly polymorphic species for growth habits, flowering behavior and fruit characteristics such as shape, size, color, taste, texture and biochemical constituents (Burger et al. 2006). This intraspecific diversity provides a great opportunity for creating novel genotypes with improved quality traits through introgression and recombination. Thus, natural or deliberate out-breeding has decreased the distinction among cultivar groups of muskmelon. For example, unique genotypes have been created through introgression of orange flesh in honey dew melons, acidity in sweet melons and long shelf-life in cantaloupes (Burger et al. 2006). A reasonable collection of melon germplasm which includes local Indian landraces, exotic accessions and inbred lines generated in the breeding program available at the Department of Vegetable Science, Punjab Agricultural University, Ludhiana. Thus, understanding of the variability in germplasm is critical for creating new melon hybrids/ cultivars with unique combinations of quality components.

The estimates of genetic distance are a pre-requisite for categorization of germplasm into heterotic groups and selection of parental combinations with a desired level genetic diversity for heterosis. Grouping can be based on phenotypic traits, geographical origin, biochemical traits, and molecular marker data. Morphological characteristics are essential to assess the genetic resources for initial diversity studies and to identify traditional cultivars (Konopka and Hanson 1985). Genetic distances among genotypes can be estimated by various

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methods. Cluster analysis can be used to categorize the previously ungrouped materials. The main objective of this study was to investigate the genetic relatedness among melon genotypes collected from various cultivar groups and geographic locations on the basis of several growth and fruit traits and level of biochemical constituents and relationship among these traits.

Materials and Methods

The germplasm collection consisting of 70 muskmelon accessions was used in this study, which consisted of landraces, inbred lines and cultivars collected from diverse geographical locations. It had 63 accessions from India and 7 genotypes from USA, Israel and other countries. Ten accessions corresponding to melon horticultural groups, *cantalupensis*, *reticulatus*, *inodorus* and Indian accessions belonging to *momordica* and two unknown type, wild melon or 'Ra Chibar' were also included in the study as reference genotypes. For easy graphical presentation, each accession was renamed as M1 to M70 (Table 1). These melon accessions were maintained at muskmelon germplasm repository, Department of Vegetable Science, PAU, Ludhiana. This set of germplasm was planted at the Vegetable Research Farm, Punjab Agricultural University (PAU), Ludhiana (30° 54' N and 70° 45' E), India during spring-summer season, 2016. Seeds were sown in a mixture of farm yard manure and clay soil (1:1) and four-week-old seedlings were transplanted in open field. Accessions were arranged in a randomized complete block design with three replications. Eight plants of each accession in each replication were planted at 3 m row to row spacing and 0.45 m plant to plant spacing. Crop was furrow irrigated and fertilized with standard agronomic practices. Four plants from each replication were randomly selected for data recording. The data was recorded on seventeen quantitative and qualitative traits viz. (Table 3)

Four mature fruits from each accession within each replication were harvested at full slip stage for biochemical analysis. Total soluble solids (TSS; ÚBrix), â-carotene content (mg/ 100g), ascorbic acid content (mg/ 100g) and titrable acidity (%) were estimated as described in (Dhillon et al. 2007). The pH of fresh juice extracted from fully ripened fruits was estimated by using a pH meter. Firmness (lb/ inch^2) of cut-fruits was determined using a hand-held penetrometer (Model FT-327, USA). Cluster analysis of 70 accessions based on 17 characters was performed using Windostat (Version 9.2, Indostat Services, Hyderabad, India).

Results and Discussion

In the current study, a large genetic variability was observed for â-carotene content, titrable acidity, number of fruits per vine, fruit weight, yield, and firmness. Similarly, Fergany et al (2011), Malik et al. (2014), reported a significant variability for morphological traits in melon germplasm in India. On the basis of 17 morphological descriptors Euclidean's inter and intra-cluster distance matrices of 70 melon genotypes are presented in Figure 1, where each circle is represented by a cluster number. Numerical within the circle represent intra-cluster distance, whereas figures on the connecting lines denote the inter-cluster distances. The inter-cluster distance varied from 759.6 between clusters

Table 1: Details of accessions used for genetic divergence analysis

S. No.	Genotypes	Geographical region
1.	AFC -1 (M1), AFC -2 (M2), Canary Yellow-1 (M3), Dissected Leaf (M6), Green Flesh (M8), Hara Madhu (M9), Honey Dew (M11), MM Sel.-103 (M15), Kajri-1 (M16), Kajri-2 (M17), Khasta Kharbooza (M19), MC-2012-6 (M20), MC-2013-2 (M21), MC-2014-2 (M22), MM-105 (M23), MM-105-1 (M24), MM-2012-4 (M25), MM-2014-11(M26), MM-2014-13(M27), MM-2014-2(M28), MM-2014-2-1(M29), MM-2014-3 (M30), MS-3(M38), PAUS-11 (M40), PAUS-12 (M41), PAUS-13 (M42), PAUS-14(M43), PAUS-15 (M44), PAUS-16 (M45), PAUS-17 (M46), PAUS-17-1 (M47), PAUS-18 (M48), PAUS-19 (M49), PAUS-26 (M50), PAUS-27 (M51), PAUS-27-1 (M52), PAUS-28 (M53), PAUS-30 (M54), PAUS-31 (M55), PAUS-4 (M56), PAUS-8 (M57), PAUS-9 (M58), PAUS-9-1 (M59), Punjab Sunehri (M60), SM-2013-18 (M62), SM-2013-2 (M63), SM-2013-5 (M64), SM-2013-9 (M65), WM 2013-2 (M69), Canary Yellow-2 (M70)	Ludhiana, Punjab
2.	SM-2014-7 (M66), SM-2015-2 (M67), WM-11 (M68)	Bathinda, Punjab
3.	Durgapura Madhu (M5)	Durgapura, Rajasthan
4.	IC-267375 (M12), IC-274034 (M13)	NBPGR, India
5.	IIVM-3 (M14), MM Var-3(M36), MM var-4(M37)	AICRP (VC), India
6.	Kajri Doctor (M18)	Doctor Seeds Pvt. Ltd., India
7.	MM-3864 (M31)	Gorakhpur, UP
8.	MM-3965 (M32)	US Nagar, Uttarakhand
9.	MM-4226 (M33), MM-4279(M34), MM-4305(M35)	Lucknow, UP
10.	Riogold (M61)	USA
11.	Natal (M39), Carabean Gold (M4)	RijkZwan, Netherlands
12.	Durasol (M7)	Harris seeds, USA
13.	Hemed (M10)	ARO, Volcani Centre, Israel

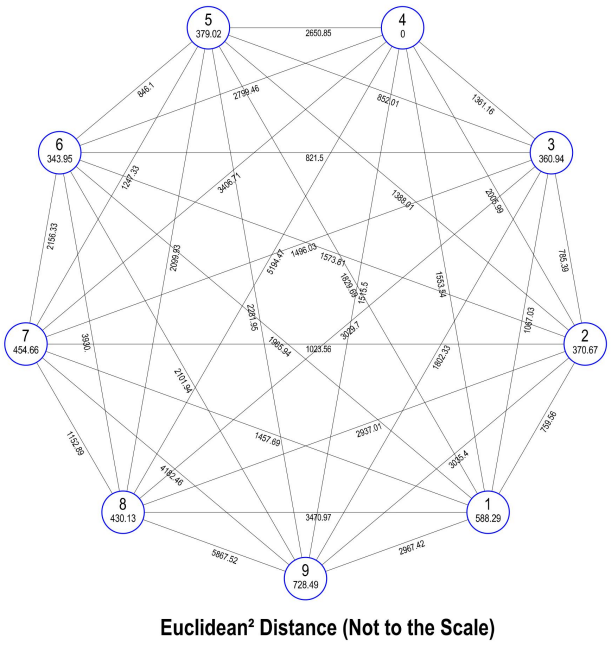

Figure 1: Euclidean distance (not to the scale) showing cluster analysis of the melon genotypes on basis of their phenotypic characters.

1 and 2 to 5194.5 between clusters 4 and 8. This indicated that high level of genetic divergence between any two accessions of melon. The maximum intra-cluster distance matrix was observed in cluster 9 (728.5) followed by clusters followed by cluster 1 (588.3) and 7 (454.6). Cluster 6 exhibited the minimum intra-cluster variation (344.0). Maximum number of genotypes was grouped in cluster 1 and 7 i.e. 15 in each group. However, individual genotype "Canary Yellow-2" present in cluster 4 which did not group in any cluster. Clustering pattern of 70 melon genotypes based on the Euclidean's analysis is given in Table 2 and Figure 2.

The distribution of accessions into clusters by diversity analysis is independent of the horticulture group and geographic origin of the accessions with exception of cluster 9, in which all accessions belonging to *momordica* group were clustered with two accessions of wild melon group. Mean values of clusters for 17 morphological traits are presented in Table 3. For all morphological traits, almost all clusters were distinct from each other. Cluster I exhibited maximum values for rind thickness (4.8 mm), yield per plant (3.5 kg), firmness (9.2lb/ inch^2). While, cluster III showed maximum values for days to 1st fruit harvest (96.8), days to last fruit harvest (107.8), Cluster IV had highest mean values for fruit weight (1475.0 g), seed cavity area (66.3 cm^2), and flesh thickness (3.3 cm). Similarly, highest mean values for vine length (146.7 cm), fruits/ vine (5.4), days to 1st female flower appearance (66.3), and pH (6.7) were observed in cluster VI. For ascorbic acid content, the highest mean value (30.6 mg/ 100 g) was observed in cluster VII. However, cluster VIII had maximum mean values TSS content (8.9 Úbrix), â-carotene (mg/ 100 g) (2.9). Maximum value for fruit shape index (1.4) and titrable acidity (%) (0.5) was observed in cluster IX.

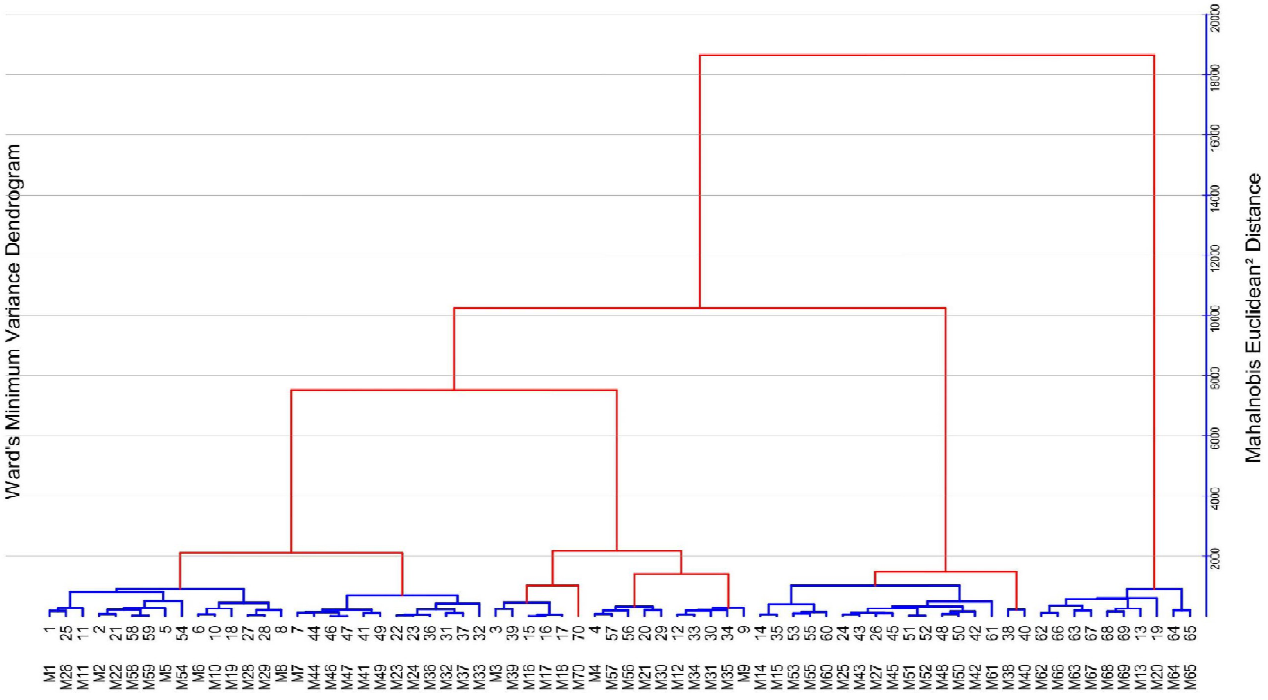

Figure 2: Dendrogram depicting classification of 70 muskmelon genotypes based on 17 morphological descriptors. Genotype codes are given in Table 1.

Table 2: Clustering pattern of 70 melon genotypes on the basis of the Euclidean's analysis

Cluster	No. of genotype(s)	Name of genotype(s)
Cluster I	15	Afghanistan Collection-1, MM-2014-11, Honey Dew, Afghanistan Collection-2, MC-2014-2, Durgapura Madhu, PAUS-9, PAUS-9-1, PAUS-30, Dissected Leaf, Hemed, Khasta Kharbooza, MM-2014-2, MM-2014-2-1, Green Flesh
Cluster II	12	Durasol, PAUS-15, PAUS-17, PAUS-17-1, PAUS-12, PAUS-19, MM-105, MM-105-1, MM Var-3, MM-3965, MM-4226, MM var-4
Cluster III	5	Canary Yellow-1, Natal , Kajri-1, Kajri-2, Kajri Doctor
Cluster IV	1	Canary Yellow-2
Cluster V	5	Carabean Gold , MC-2013-2, MM-2014-3, PAUS-4, PAUS-8
Cluster VI	5	Hara Madhu, IC-267375 , MM-3864, MM-4279, MM-4305
Cluster VII	15	IIVM-3, MM Selection-103, PAUS-28, PAUS-31, Punjab Sunheri, PAUS-14, MM-2012-4, MM-2014-13, PAUS-16, PAUS-27, PAUS-27-1, PAUS-18, PAUS-26, PAUS-13, Riogold
Cluster VIII	2	MS-3 , PAUS-11
Cluster IX	10	SM-2013-18, SM-2013-2, SM-2013-5, SM-2013-9, SM-2014-7, SM-2015-2, WM-11, WM-2013-2, IC-274034, MC-2012-6

Cluster IV had lowest values for fruit/ vine (1.3), days to 1st female flower appearance (58.5), â-carotene (0.035 mg/ 100 g), ascorbic acid content (18.8 mg/ 100 g), pH (4.3). Minimum vine length (88.3 cm), seed cavity area (32.6 cm^2) were observed in cluster V. Cluster VI had least value for fruit shape index (0.9), titrable acidity (0.1) and firmness (4.1 lb/ $inch^2$). Similarly, cluster VIII exhibited minimum value yield/ plant (1.6 kg). Cluster IX had least mean values for fruit weight (351.4 g), flesh thickness (1.3 cm), rind thickness (1.2 mm), TSS content (2.6 Úbrix), days to 1st fruit harvest (93.0), and days to last fruit harvest (102.8). Further, this study will be useful in selection of diverse parent form different clusters. Cultivar Punjab Sunehri, derived from a cross between Hara Madhu × Edisto, had highest ascorbic acid content (46.3 mg/ 100 g), Green flesh and MM-2014-2-1, local collections, had hishest fresh thickness (~3.4 cm), an exotic line, MS-3 had highest â-Carotene content (3.3 mg/ 100 g), Canay yellow accessions exhibited higher values for flesh firmness, a trait associated with longer shelf-life and MM-4226 had maximum fruits per vine (8.5). These genotypes can be utilized for developing improved breeding lines in the muskmelon breeding program at Punjab Agricultural University, India.

In the genetic distance clustering (Table 2), Momordica and wild melon accessions were separated from *Cantalupensis*, *Reticulatus, Inodorus* accessions. These findings are consistent with the intra specific classification proposed by Jeffrey (2001). However, the diversity analysis of 17 measured descriptors was not able to separate *Cantalupensis* and *Reticulatus* accessions which is consistent with intra specific classification proposed by Munger and Robinson (1991) but contradicts with the classifications proposed by Whitaker and Davis (1976) and Pitrat et al. (2000). Further, Malik et al. (2014) indicated that inadvertent out-crossing and intentional inter-crossing of Indian muskmelon germplasm with exotic melon introductions has resulted in introgression of *reticulatus* genes in Indian germplasm. Similarly, honeydew and canary yellow melons belonging to *inodorus* group are also being utilized in the breeding program for broadening genetic base and introgression unique traits which might have resulted in intermixing *inodorus* accessions with *cantalupensis* and *reticulatus* accessions.

Snapmelon accessions exhibits a mealy flesh, lack of sugar content and shows splitting at fruit maturity. However, *Momordica* accessions possessed high levels

Table 3: Cluster mean and contribution of various traits in melon.

Cluster	Vine Length (cm)	Fruit Weight	Fruit Shape Index	Seed Cavity Area	Flesh Thick ness	Rind Thick ness	TSS	Fruits/ Vine	Days to 1st female flower	Days to 1st fruit harvest	Days to Last Fruit harvest	Yield per plant (kg)	ß-carotene	Ascorbic Acid	Titrable Acidity	pH	Firmn ess
1 Cluster	115.2	1327.8	1.3	65.7	2.7	4.8	6.9	2.6	64.2	95.3	104.7	3.5	0.3	22.6	0.2	6.3	9.2
2 Cluster	104.0	707.3	1.0	34.8	2.4	3.3	8.5	3.5	64.9	94.0	104.3	2.3	0.3	29.6	0.2	6.4	8.9
3 Cluster	126.7	737.7	0.9	35.8	2.3	3.2	8.5	3.5	65.5	96.8	107.8	2.6	0.2	23.4	0.2	5.4	7.0
4 Cluster	113.3	1475.0	1.4	66.3	3.3	3.6	8.3	1.3	58.5	95.5	103.0	1.8	0.0	18.8	0.5	4.3	8.5
5 Cluster	88.3	731.3	1.0	32.6	2.4	4.3	7.5	2.9	64.4	95.7	106.5	2.1	1.2	23.5	0.2	6.3	4.8
6 Cluster	146.7	605.3	0.9	35.1	2.0	2.5	8.4	5.4	66.3	95.3	107.1	3.2	0.3	27.0	0.1	6.7	4.1
7 Cluster	111.1	793.0	1.1	38.3	2.6	4.5	8.8	3.2	64.4	96.7	106.4	2.5	1.5	30.6	0.2	6.4	8.5
8 Cluster	115.3	921.9	1.0	37.0	3.0	4.7	8.9	1.7	65.5	94.5	107.0	1.6	2.9	29.8	0.2	5.3	8.3
9 Cluster	104.6	351.4	1.4	40.7	1.3	1.2	2.6	6.0	64.5	93.0	102.8	1.9	0.1	26.8	0.5	4.5	4.4
Contribution (%) towards divergence	2.4	1.8	2.1	0.04	1.7	.08	0.25	1.4	0.08	0.04	0.04	0.8	26.5	2.5	12.2	8.5	39.6

of titrable acidity. Further, Dhillon et al. (2007) in diversity analysis reported that snapmelon accessions are potential source of resistance to fungal disease downy mildew, 3 viruses (CMV, Zucchini yellow mosaic virus, Papaya ring spot virus) and melon aphid. Therefore, genotypes belonging to *momordica* group can be utilized as a resistance source against diseases and insects to develop cultivars with unique flavor. The germplasm used in this study present wide range of variability for morphological characters. Investigation of genetic variability and association among genotypes is of great importance, since it facilitates selection of diverse and desired parents with unique traits for cultivars/ F_1 hybrids. This study depicted a clear picture of 70 melon genotypes based upon seventeen morphological traits which would help in selecting parents for cultivar/ hybrid develop with unique traits and high productivity.

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