



## Morpho-biochemical characterization and D<sup>2</sup> analysis of watermelon (*Citrullus lanatus*) landraces from India and exotic germplasm

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### ABSTRACT

Eighty eight watermelon (*Citrullus* spp) genotypes representing Indian landraces and exotic accessions of diverse geography were characterized for 17 plant, fruit, seed and quality traits during 2015 and 2016. The germplasm evaluated showed significant differences for all the agro-morphological and fruit quality characters. Cluster analysis using Mahalanobis (D<sup>2</sup>) assigned genotypes into 10 distinct clusters. Clusters I and II containing landraces collected from Uttar Pradesh and Rajasthan province of India showed least inter cluster distance depicting them to be morphologically close to each other. Other clusters were a mixture of accessions grouped together irrespective of their origin. Cluster V and Cluster III were most divergent. Genotypes of Cluster III were best for earliness in flowering and fruit maturity, Cluster V members best for TSS, lycopene and total carotenoids, whereas Cluster VIII constituted genotypes having largest fruit size and yield/plant. Indigenous landraces excelled in earliness, fruit number, dry matter and lowest 100 seed weight. Exotic germplasm was best for yield and fruit quality parameters. Breeding strategies involving indigenous landraces would serve to broaden the genetic base, crop improvement as well as their conservation.

**Key words:** *Citrullus lanatus*, Cluster analysis, Diversity, Fruit quality, Landraces, Yield

Watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai; family: Cucurbitaceae], is primarily consumed as fresh dessert fruit for its juicy red flesh. Fruits of bitter cultivars are fed to livestock (Goda 2007) and seeds of bitter cultivars are consumed as raw, boiled, fermented, roasted in Africa and India (Mahla and Choudhary 2013). Watermelon is a rich source of citrulline and anti-carcinogenic functions like lycopene and  $\beta$  carotene (Rimando and Perkins-Veazie 2007). Cultivated watermelon is an annual species grown in tropical and subtropical climates worldwide. In India, watermelon occupied an area of about 83500 ha in the year 2014-15 with a production of about 2049 000 t (www.nhb.org). Watermelon production areas in India are spread over three major agro-climatic zones, viz. Zone V (Upper Gangetic plain region), Zone X (Southern Plateau and Hills region) and Zone VII (Eastern Plateau and Hills region). These zones collectively produce 70 % of watermelon over 60 % of total watermelon area in India (www.nhb.org). With the increase in disposable household incomes, more Indians are including fruits and vegetables in their diet.

Genus *Citrullus* consists of three species which are distributed in different parts of African continent, Hindustani centre and south east asia (Bisognin 2002). All *Citrullus* species originated in the Kalahari region of Africa (Maggs-Colling and Christiansen 2003), with wild species scattered in India and China. Zeven and de Wet (1982) suggested Hindustani region comprising India and SE Asia as centre for domestication. It was introduced into India in 8<sup>th</sup> century (Jeffery 1975).

During domestication selection was biased on producing bigger fruits and overlooked other desirable traits like disease resistance and abiotic stresses thereby narrowing genetic base of this crop. Genetic base of the crop was further narrowed due to crossing between genetically alike species resulting in lowering of allelic variation and gradual loss of heterogeneity (Levi *et al.* 2001a and 2001b).

Agro-morphological characterisation of respective domestic watermelon genetic resources in Namibia (Maggs-Kolling and Christiansen 2003), Sudan (Goda 2007), Tunisia (Elbakkay *et al.* 2009), Turkey (Solmaz and Sari 2009), Hungary and Turkey (Szamosi *et al.* 2009), Kenya (Gichimu *et al.* 2009), Mozambique (Munisse *et al.* 2011), Zimbabwe (Mujaju and Nybom 2011), Morocco (Hakimi and Al Madidi 2015) exposed the variation available in the local watermelon germplasm. In India, Mahla and Choudhary (2013) evaluated only the seed type watermelon accessions. All these studies stressed on the conservation of indigenous watermelon resources and advocated their

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utilization in watermelon breeding. Despite India being a secondary centre of diversity for watermelon (Whitaker and Davis 1962), and the Indo-Gangetic region of India rich in watermelon genetic diversity (Wehner 2008), the available genetic resources have still been under exploited. The reason could be ascribed to the scanty information available on the genetic diversity and genetic relationship among watermelon genetic resources endemic to India. Landraces are proven to be more adapted to local conditions and carriers of traits related to both biotic and abiotic stresses, but they considerably lack in fruit quality and yield. The need is to incorporate exotic germplasm resources high on quality and yield to augment and broad base domestic breeding programme. Therefore, the current investigation was conducted to characterise the phenotypic diversity in the hitherto unexplored watermelon germplasm from India and its performance comparison with global watermelon sources. The information generated thus, would enable to identify the newer genetic resources for utilization in broadening the genetic base of cultivated watermelon and would offer future options to enhance productivity, quality and resistance to diseases.

#### MATERIALS AND METHODS

The experiment was carried out at Vegetable Research Farm and Biochemistry Laboratory, Department of Vegetable Science, Punjab Agricultural University, Ludhiana (30° 54' N, 75° 48' E, 248 above main sea level) during the spring summer season of 2015 and 2016. The planting material comprised 88 accessions belonging to different *Citrullus* species (Table 1). Seed sowing was done in polythene bags of size 6 × 4 inches filled with a mixture of soil and FYM in the ratio 1:1. During the 1<sup>st</sup> year sowing was done on 12 February and 2<sup>nd</sup> year on 15 February. Four week old seedlings were transplanted in the field. The accessions were evaluated in a randomized block design replicated thrice. Each entry consisted of 10 plants in each replication and planted at a spacing of 3.5 m (Row to row) × 0.5 m (Plant to plant). Recommended agronomic and plant protection practices (Anonymous 2014) were followed for raising the crop. Five randomly selected plants from each replication were chosen for data collection and mean for each observation was used for statistical analysis. Seventeen morphological traits pertaining to seed, plant and fruit (IPGRI 1991) were measured at seedling, vegetative growth stage and full fruit maturity stage with the help of a hand held ruler or measuring tape. Fully mature fruits were selected for biochemical analysis. Total soluble solids were measured using a hand held refractometer. Lycopene and total carotenoids content was measured as prescribed by (Srivastava and Kumar 2006), ascorbic acid content (Bajaj and Kaur 1981). Dry matter was calculated by the formula:

$$DM (\%) = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

For this, 100 g of fruit flesh was put in a petridish and placed in drying chamber at 65 °C for 5 days. After five

Table 1 Origin of genotypes

Genotypes	Origin
<i>Citrullus lanatus</i> var. <i>lanatus</i>	
WM-6, WM-10, WM-11, WM-14, WM-20, WM-21, WM-22, WM-26, WM-28, WM-29, WM-30, WM-35, WM-36, WM-38, WM-41, WM-43, WM-46, WM-50, Jaipur, Bikaner, Barmer, Jaisalmer, Thar Manik, Arka Manik, Arka Jyoti	India
IC-374795, IC-611626, IC-611623, IC-324819, IC-374820, IC-599377, IC-374802, IC-315328, IC-13773, IC-374803, IC-611626, IC-325808, EC-829846, IC-315321, IC-315324,	India
<i>Citrullus colocynthis</i>	
EC-829813, EC-829815, EC-829816	Iran
EC-829818, EC-829819	Cyprus
<i>Citrullus lanatus</i>	
EC-829820, EC-829822, EC-829823, EC-829824, EC-829825, EC-829826, EC-829827, EC-829828, EC-829829, EC-829832, EC-829833, EC-829836, EC-829838, EC-829840	USA
EC-829851, EC-829852, Asahi Yamato	Japan
EC-829858, EC-829870, EC-829871	China
EC-829872, EC-829873, EC-829874, EC-829875	South Korea
EC-829860, EC-829861, EC-829862	Zimbabwe
EC-829865, EC-829866	Pakistan
EC-829846, EC-829848	Nigeria
EC-829849, EC-829850	Iran
EC-829863, EC-829864	USSR
EC-829867, EC-829868	Spain
EC-829844	Turkey
EC-829853	Belize
EC-829854	Greece
EC-829841	Turkmenistan
EC-829878	Canada
<i>Citrullus amarus</i>	
EC-829810	Unknown

days the petridish was taken out and dry matter calculated.

The data obtained for seventeen agro-morphological and quality characters were subjected to analysis of variance (ANOVA) and multivariate analysis to generate dendrogram and clusters using WINDOWSTAT 9.2 (www.indostat.com) based on D<sup>2</sup> analysis suggested by Mahalanobis (1936). Pooled data was used for performance analysis.

#### RESULTS AND DISCUSSION

##### *Genetic variability*

Analysis of variance (ANOVA not given) revealed significant differences among the genotypes evaluated for all phenotypic traits. Pooled (2 years data), mean value and range for each quantitative character are presented in Table 2. Mean performance of individual genotype is not

Table 2 Mean, range and CV of different characters

Character	Mean	Lowest Range	Highest range	LSD (P=0.05)	CV
Vine length (cm)	283.16	116.50	455.00	24.84	7.73
Internode length (cm)	9.48	4.62	15.70	0.60	5.54
100 seed weight (g)	6.92	1.97	17.96	0.24	9.21
Days to 50% flowering	64.84	52.00	90.00	1.63	8.21
Days to first fruit maturity	96.13	82.00	122.00	1.78	7.63
Fruit length (cm)	17.49	6.20	28.88	1.28	6.45
Fruit width (cm)	15.99	6.30	23.00	1.17	6.46
Pericarp thickness (cm)	1.27	0.37	2.33	0.12	8.30
Average fruit weight (kg)	2.84	0.14	7.15	0.31	9.71
Fruit number/vine	2.12	1.18	2.90	0.15	6.30
Yield/plant (kg)	5.51	0.14	8.48	0.48	7.67
Total yield (q/acre)	127.05	8.68	193.73	10.92	7.57
TSS (°Brix)	6.98	1.40	10.85	0.51	6.38
Lycopene (µg/g)	39.63	1.89	90.38	2.23	12.67
Total carotenoids (µg/g)	49.78	2.24	106.12	3.39	10.79
Ascorbic acid content (mg)	5.10	3.07	8.59	0.28	4.84
Dry matter (%)	3.42	1.77	6.44	0.21	5.29

shown. Only important plant and fruit traits are included in discussion. Days to 50% flowering and first fruit maturity displayed significant variability. EC-829878 (52.00 days) was the earliest to reach 50% flowering and significantly at par with WM-38 (52.33) and Arka Manik (52.50). Days to first fruit maturity followed the pattern of earliness in flowering. EC-829878 and WM-20 took least number of days (82.00) to reach fruit maturity. Earliness through selection performed by farmers' maintained local cultivars has also been observed by Mujaju and Nybom (2011). Lateness in flowering and fruiting was profound in *colocynthis* accessions. Maximum number of days for flowering and fruit maturity were taken by EC-829813 (92) and EC-829813 (121). Among plant traits, longest vines were produced by IC-611625 (455.00 cm), and shortest vines were observed in EC-829816 (116.50 cm). Szamosi *et al.* (2009) also recorded significant differences in flowering and fruit maturity in Hungarian and Turkish watermelon genotypes. Internode length was shortest in case of EC-829816 (4.61 cm) which was significantly at par with EC-829815 (5.11 cm), whereas it was recorded maximum in EC-829840 (15.70 cm). Short internodes did not show any correlation with earliness, although internode length was shortest in *C. colocynthis* accessions, they were all late in achieving flowering and first fruit maturity. Fruit yield/vine and average fruit weight was found to be highest in EC-829840 (8.48 kg and 7.14 kg,

respectively), although it produced least number of fruits/vine (1.18). WM-36 was the most prolific fruit bearer (2.9), statistically at par with WM-11 (2.82). Maximum fruit length was recorded in EC-829870 (28.88 cm), whereas fruit width was highest in genotype EC-829853 (23.00 cm), significantly at par with EC-829840 (22.83 cm), EC-829863 (22.41 cm). Pericarp (rind) thickness was highest in IC-599377 (2.33 cm). Four *Citrullus colocynthis* accessions (EC-829813, EC-829815, EC-829816 and EC-829818) irrespective of their origin were lowest and at par in fruit yield/plant and its contributing characters as well as fruit quality traits such as TSS content, lycopene and total carotenoids. Distinctness of *Colocynthis* accessions from cultivated watermelon in terms of plant and fruit characteristics are in conformity with those of Yaniv *et al.* (1999). 100 seed weight was highest for EC-829841 (17.96 g) and lowest for IC-611630 (1.97 g). Szamosi *et al.* (2009) found that Hungarian accessions had smaller seed size and seed weight as compared to those obtained from Turkey. Among quality traits, TSS content was highest in EC-829858 (10.85 °Brix), statistically at par with EC-829875 (10.41). All *Colocynthis* accessions were poor in TSS content, lowest being EC-829816 (1.4 °Brix). Lycopene content is associated with bright red flesh which goes on decreasing with diminishing levels of red colour; is genotype dependent and lowest in case of white fleshed genotypes (Perkins- Veazie *et al.* 2006). EC-829858 (90.38 µg/g) possessed highest lycopene content, followed by EC-829871 (86.15 µg/g) of fruit flesh. Total carotenoids content corresponded to the lycopene content and was maximum in EC-829858 (101.62 µg/g). It was minimum in EC-829818 (2.24 µg/g). Ascorbic acid content showed significant genetic variation (Leskovar *et al.* 2004). It was highest in IC-611626 (8.59 ml/100 ml fruit juice) and was lowest in EC-829816 and EC-829818 (3.07 ml). Total dry matter was maximum in WM-46, a landrace collected from Uttar Pradesh, India and was minimum in case of EC-829868 (1.95%). Land races and wild types are low on fruit quality parameters than commercial cultivars has also been reinforced by Goda (2007) and Gichimu *et al.* (2009).

#### Clustering based on D<sup>2</sup> analysis

Knowledge of extent of genetic diversity in a given set of genotypes is of immense value in designing efficient breeding programme. The D<sup>2</sup> analysis (Mahalanobis 1936) and further elaborated by Rao (1952) to measure quantitative traits is a bench mark geneticist use to estimate extent of genetic diversity. The non-hierarchical cluster analysis based on seventeen seed, plant, fruit and quality traits, assigned 88 accessions into 10 clusters. Dendrogram illustrating the clustering patterns is shown in the Fig 1. Inter and intra (in bold) cluster distances have been presented in (Table 3). The inter cluster distance exhibited a range from 795.60 to 7314.71. Maximum inter cluster distance was found between Cluster X and Cluster III (7314.71) indicating high levels of dissimilarity for morphological traits. Lower inter cluster distances describe phenotypic performance similarity between the individuals constituting that particular cluster.

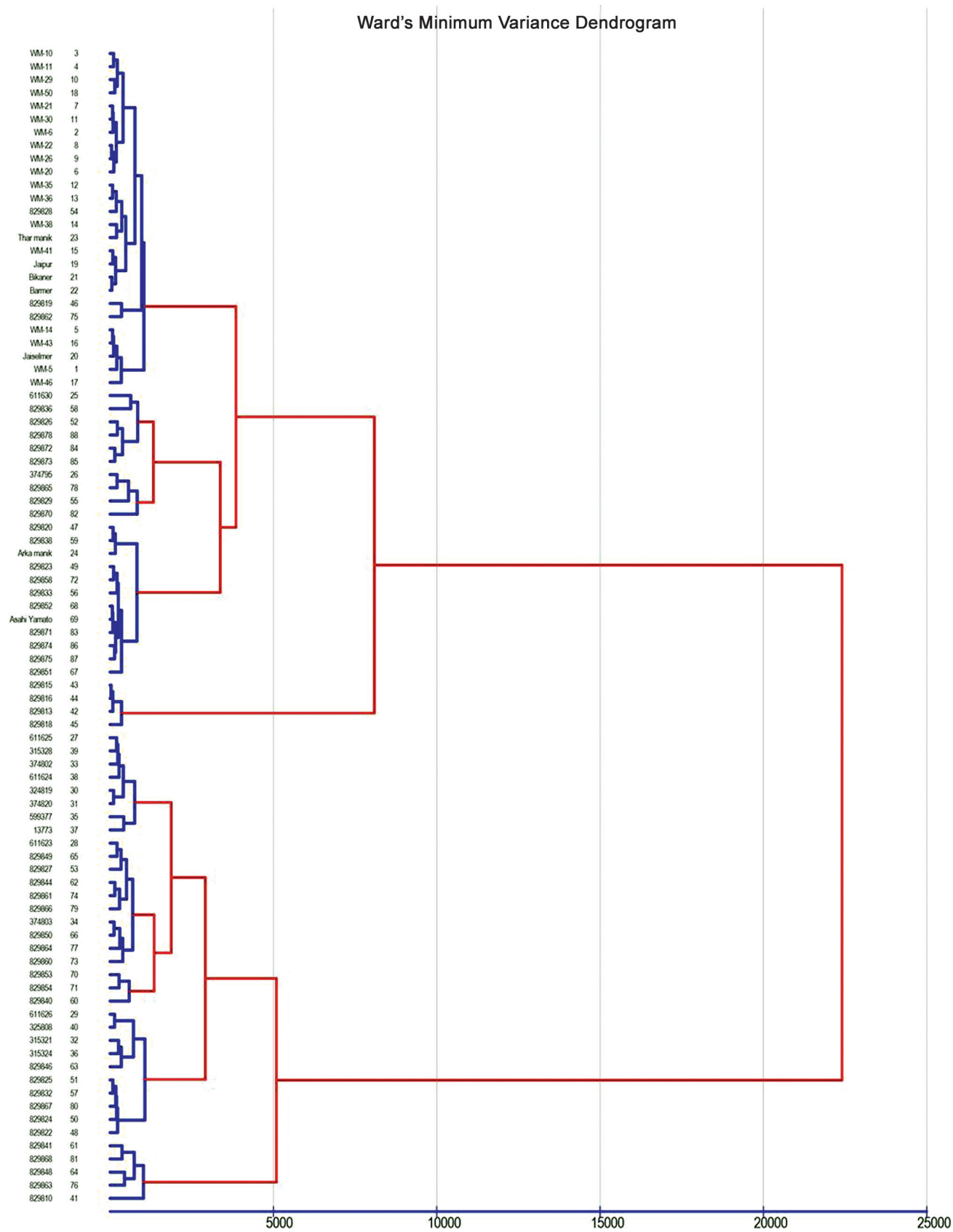


Fig 1 Dendrogram depicting clustering pattern for 88 watermelon genotypes.

Table 3 Euclidean<sup>2</sup>: Inter and intra (in bold) cluster distances

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster 9	Cluster 10
Cluster 1	<b>370.58</b>	795.60	881.10	2116.25	808.80	1169.22	1621.33	1290.00	2286.40	4593.05
Cluster 2		<b>317.29</b>	790.26	2721.74	1285.67	1472.90	2177.41	1724.39	2713.78	5167.15
Cluster 3			<b>451.42</b>	3421.31	1576.02	2250.99	3186.66	2568.32	4314.73	7314.71
Cluster 4				<b>334.08</b>	1759.00	3002.21	2210.61	2443.15	3027.04	5819.05
Cluster 5					<b>377.14</b>	1519.33	1188.61	892.92	1971.16	4236.90
Cluster 6						<b>549.15</b>	886.57	1083.47	1309.11	2496.81
Cluster 7							<b>463.510</b>	819.70	850.73	2086.27
Cluster 8								<b>536.60</b>	984.57	2299.59
Cluster 9									<b>638.93</b>	1204.62
Cluster 10										686.95

Mean cluster performance for each trait is presented in Tables 4 and 5. Based on desirable horticultural performance, Cluster III comprising three genotypes exhibited earliness in days taken in flowering and first fruit maturity, had lowest 100 seed weight (2.07 g). Cluster V contained 18 genotypes of diverse geographical background and possessed highest levels of both lycopene and total carotenoids content (60.29 and 72.41 µg/g, respectively). Yoo *et al* (2012) obtained distinct lycopene and carotenoid profiles for cultivars having differences in flesh colour. Cluster VIII consisted of 11 genotypes showing superior performance for yield and its associated traits. All cultivars from China, Japan and South Korea evaluated in this study were grouped in this cluster, indicating similarity in performance and accentuating narrow genetic base in hypothesis in modern cultivars. However, no relation was observed between genetic variability and geographic origin (Krishna Prasad 2004). Also, commercial cultivars possessing higher fruit weight, fruit length and width over local farmer grown cultivars has been reported by Hakimi and El Madidi (2015).

Cluster IV contained all but one *Citrullus colocynthis* accession, marked by poor horticultural performance. Mujaju

*et al.* (2010) in an analysis of cow melons and cultivated watermelons placed them in separate groups and observed high differentiation among them. One accession EC-829819 of *C. colocynthis* was placed in cluster V. This accession besides having red flesh had significantly higher fruit weight, yield and fruit quality than other *Colocynthis* accessions. Indian land races collected from UP and Rajasthan (Clusters I and II) falling in the Indo-Gangetic plain region showed least inter cluster distance implying morphological similarity. Taking into account both inter cluster distances as well as cluster means for each trait, desirable genotypes from respective clusters can be chosen as parents for targeted trait improvement. For instance, Cluster III was marked by genotypes showing earliness in both days to flowering and first fruit maturity (55.00 and 88.22), whereas cluster V was best for TSS (9.23 °Brix), lycopene (60.29 µg/g), total carotenoids content (72.41 µg/g) and ascorbic acid content (5.69 mg). Members of cluster VIII can be exploited for obtaining larger fruits as well as high yield. Crossing between members of clusters having maximum inter cluster distance can generate relatively higher heterosis than having less inter cluster distance (Krishna Prasad *et al.* 2004). Case in point is though highest cluster distance was observed between clusters III and X, but the cluster X was diverse from others with respect to highest 100 seed weight and lowest fruit number/vine. These are undesirable from agronomic and consumer point of view.

The study focus was on variability and performance evaluation of unimproved watermelon landraces from India with global watermelon accessions. Morphological and clustering analysis demonstrated significant underlying genetic diversity in the Indian landraces. Although less adapted to market acceptability they are rich source of variation and can be candidates for higher fruit number, dry matter content, earliness and low 100 seed weight. Exotic commercial genotypes performed better on fruit quality parameters and yield consistently during the study period. Desirable traits in watermelon pertaining to growers and consumers are flavour, texture, fruit shape and size. Combining the strong and desirable attributes of indigenous and introduced germplasm would give the fillip to future

Table 4 Cluster means for plant, seed and flowering characters

Cluster	Vine length (cm)	Internode length (cm)	100 seed weight (g)	Days to 50% flowering	Days to first fruit maturity
Cluster 1	249.153	8.365	5.170	58.481	89.963
Cluster 2	252.517	7.902	5.447	60.533	92.433
Cluster 3	287.611	8.289	2.072	55.000	88.222
Cluster 4	143.083	5.358	3.866	87.750	117.583
Cluster 5	250.944	9.609	4.574	60.657	90.194
Cluster 6	371.333	12.262	8.900	68.000	102.583
Cluster 7	370.245	11.210	8.556	71.157	103.637
Cluster 8	283.182	10.215	8.117	63.303	94.197
Cluster 9	309.000	9.146	11.491	71.167	102.806
Cluster 10	303.083	10.663	15.777	68.500	99.958
Mean	283.155	9.481	6.919	64.843	96.134

Table 5 Cluster means for fruit, yield and quality traits

Cluster	Fruit length (cm)	Fruit width (cm)	Pericarp thickness (cm)	Average fruit weight (kg)	Fruit number/vine	Yield/plant (kg)	Total yield (kg)	TSS (°Brix)	Lycopene (µg/g)	Total carotenoids (µg/g)	Ascorbic acid content (mg)	Dry matter (%)
Cluster 1	15.89	15.08	1.14	2.19	2.29	4.84	110.51	7.44	35.40	46.81	4.75	3.25
Cluster 2	17.70	17.19	1.27	2.83	2.02	5.47	125.07	6.89	50.07	63.36	4.30	5.53
Cluster 3	18.56	17.10	1.36	3.17	2.04	6.48	148.18	8.99	56.12	66.51	5.46	3.79
Cluster 4	6.92	7.13	0.38	0.17	2.60	0.44	10.16	1.98	2.21	2.89	3.32	3.83
Cluster 5	17.87	16.62	1.19	2.90	2.13	6.13	139.84	9.23	60.29	72.41	5.69	3.92
Cluster 6	18.93	18.21	1.24	3.37	2.05	6.88	157.40	4.68	37.70	48.13	6.00	2.08
Cluster 7	17.58	15.59	1.38	2.57	2.14	5.19	118.70	5.41	2.82	3.64	5.05	2.80
Cluster 8	22.40	17.88	1.48	4.11	1.83	6.91	157.91	7.97	4.61	5.81	5.27	3.36
Cluster 9	16.88	16.61	1.58	2.93	2.05	5.93	135.61	5.56	2.85	3.79	4.95	3.27
Cluster 10	18.70	18.17	1.57	4.05	1.66	6.48	148.12	6.79	2.59	3.38	5.86	2.15
Mean	17.48	15.98	1.27	2.82	2.08	5.47	125.15	6.98	20.03	19.86	5.09	3.41

watermelon improvement strategies. High yield and consumer preferred modern cultivars are replacing the local landraces leading to their genetic erosion and threatening the existing diversity. Therefore, it is vital to utilize the diversity in the germplasm to broaden the genetic base of watermelon by creating new recombinants and conservation of indigenous germplasm as well.

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