Genetic Diversity in Genus Citrullus (C. colocynthis and C. lanatus)

B.R. Choudhary* and S.R. Kumhar

Agricultural Research Station, SK Rajasthan Agricultural University, Mandor 342 304, Jodhpur, India Received: July 2011

Abstract: Genetic diversity was assessed among 39 genotypes of Citrullus, including 17 of Citrullus colocynthis and 22 of C. lanatus using eight characters such as seed yield plant⁻¹ (SYPP), fruit yield plant1 (FYPP), number of fruits plant1 (NFPP), single fruit weight (SFW), fruit diameter (FD), seed weight fruit (SWPF), number of seeds fruit (NSPF) and 100-seed weight (HSW). The high genotypic (GCV) and phenotypic coefficient of variations (PCV) were noticed for HSW, NFPP and NSPF. The heritability in broad sense varied from 51.0 to 97.3%. The traits like HSW, SFW, NFPP and NSPF showed high heritability. Cluster analysis through Ward's minimum variance method grouped the entries into seven clusters. Maximum number of 11 genotypes was included in cluster VII followed by eight each in clusters I and V, five in cluster IV, three in cluster VI and two each in clusters II and III. The group IV, comprized of five C. colocynthis genotypes, had high values for characters like SYPP, NFPP and NSPF while the cluster V, with eight C. lanatus genotypes, was superior for FYPP, SFW, SWPF and HSW. Among the characters studied, NSPF (48.2%) contributed the maximum towards the total divergence followed by SWPF (13.8%), HSW (10.1%) and FYPP (6.9%). The present study indicates greater diversity between genotypes of these two species and thus provides good chance to improve both the species through interspecific hybridization and selection for desired traits.

Key words: Citrullus colocynthis, Citrullus lanatus, genetic diversity.

The genus *Citrullus* Schrad. ex Eckl. & Zeyh. (family Cucurbitaceae) comprises four diploid (n=11) species i.e. (i) Citrullus lanatus (Thunb.) Matsum. & Nakai, includes the cultivated watermelon (C. lanatus var. lanatus) or 'egusi' melon, and the preserving melon (C. lanatus var. citroides), (ii) C. colocynthis (L.) Schrad. ('bitter apple'), (iii) C. ecirrhosus Cogniaux, and (iv) C. rehmii De Winter (Levi et al., 2001a). Colocynth, bitter apple, Indrayan [Citrullus colocynthis (L.) Schrad.], locally kwon as 'tumba', is an important medicinal plant and a source of valuable oil. It is drought tolerant species with perennial deep root system. It is useful in stabilizing sand dunes due to its creeping nature of vine and sand binding ability of roots (Yadav and Singh, 1992). It has been commonly used as catharsis and anti-diabetic agents in traditional Egyptian and Indian Ayurvedic medicines (Yoshikawa et al., 2007; Kumar et al., 2009), and paediatric homoeopathy (Ekins-Daukes et al., 2004). It is growing wild in the hot Indian arid zone and during the drought years, it forms a source of income as well as food for the inhabitants of the desert. Its seeds are extracted, washed with water, dried and

mixed in equal proportion with pearl millet for providing subsistence food while its fruits are used as a feed for cattle, goats and camels (Pareek and Vashishtha, 1980). The seeds have 17-19% oil content and in ancient times its oil was used as candle light (Palevitch and Yaniv, 1991), but these days it is used in soap industry (Pareek and Vashishtha, 1980). Its oil is similar to that of safflower oil with a total of 80-85% unsaturated fatty acids, thus making it a high quality oil (Dane et al., 2007). Other local types of Citrullus found in Indian desert are mateera and kalingada or karinga (C. lanatus (Thunb.) Matsum. & Nakai). These are annual creepers and are drought hardy due to their well developed root system and are well adapted to the soil and climate of the desert. These are different forms of watermelon, which are less sweet and have more seeds fruit-1, and are cultivated in rainy season as an intercrop in western India (Raiger et al., 2009). The seeds of kalingada/mateera have multipurpose values as snack food, oil seed, culinary, etc. and these are also considered to have cooling and diuretic

The nature and magnitude of genetic divergence in a population is essential for

^{*}E-mail: choudharybr@yahoo.com

selection of diverse parents which upon hybridization leads to a wide spectrum of gene recombination for quantitatively inherited traits. For many species, especially underutilized crops, it is still the only approach used by breeders. Among the multivariate analyses, the Ward's minimum variance method (Ward, 1963) is a unique tool to assess the degree of genetic divergence in a biological population and was frequently used in different crops. The studies on genetic variability and diversity of these desert species were reported by Pareek and Vashishtha (1980), Hashizume et al. (1996) and Levi et al. (2001a). The present study was under taken to assess genetic diversity based on agronomic traits of C. colocynthis (tumba) and C. lanatus (kalingada) genotypes which would be useful in improvement.

Materials and Methods

Experimental material had 39 genotypes of Citrullus Schrad. ex Eckl & Zeyh. including 17 of tumba [C. colocynthis (L.) Schrad.] and 22 of kalingada [C. lanatus (Thunb.) Matsum. & Nakai]. Amongst 17 genotypes of tumba, 16 were advance breeding lines and remaining one was released variety RMT 59, while all the 22 genotypes of kalingada were obtained from SD Agricultural University, SK Nagar, Gujarat. All the genotypes were grown in two blocks based on their species and randomized within blocks in three replications during kharif 2009 at research farm of the Agricultural Research Station, Mandor, Jodhpur. Each genotype was sown in a single row of 3 m length. Line-to-line distance was 3 m while plant-to-plant distance within row was 1 m. All cultural operations were done normally. The data were recorded on all the plants of each genotype in each replicate

on seed yield plant⁻¹ (SYPP), fruit yield plant⁻¹ (FYPP), number of fruits plant⁻¹ (NFPP), single fruit weight (SFW), fruit diameter (FD), seed weight fruit⁻¹ (SWPF), number of seeds fruit⁻¹ (NSPF) and 100-seed weight (HSW). The data were subjected to analysis of variance following standard methods. Clustering of genotypes after standardization of data was performed following Ward's minimum variance method (Ward, 1963).

Results and Discussion

In the present study, analysis of variance revealed significant differences among the genotypes for all the characters depicting sufficient variability in the experimental material. Estimates of different variability parameters i.e. genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), heritability in broad sense (h2) and genetic advance as a percentage of mean are given in Table 1. The high GCV and PCV were displayed by characters like HSW, SFW, NFPP and NSPF indicating greater variability for these traits in the present material. There was a considerable role of environmental factors in expression of all the traits as depicted by higher values of PCV than corresponding GCV. The estimates of heritability varied from 51.0 to 97.3%. All the attributes, except SYPP and FYPP, had recorded high heritability. The expected genetic advance as a percentage of mean was the highest for HSW (85.4%) followed by SFW (76.5%), NFPP (67.3) and NSPF (61.2%). High heritability values coupled with high genetic advance (as % of mean) were recorded for HSW, SFW, NFPP and NSPF indicating that these attributes are governed by additive gene action and direct selection for these traits would be

Table 1. Estimate of genetic parameters of seed yield and its component traits in Citrullus spp.

Characters	Mean ± S.Em	GCV (%)	PCV (%)	h² (%)	GA as % of mean
NFPP*	8.6 ± 0.9	36.7	41.2	79.3	67.3
SFW (g)	394.1 ± 40.1	40.5	44.3	83.8	76.5
FD (cm)	9.0 ± 0.4	12.4	14.4	74.2	22.1
SWPF (g)	15.1 ± 1.1	21.7	25.0	74.8	38.6
NSPF	324.1 ± 26.1	32.4	35.4	84.1	61.2
HSW	5.34 ± 0.21	42.0	42.6	97.3	85.4
FYPP (kg)	3.07 ± 0.46	29.3	39.4	55.5	45.0
SYPP (g)	123.5 ± 16.8	24.3	34.1	51.0	35.8

*NFPP- Number of fruits plant¹, SFW- Single fruit weight (g), FD- Fruit diameter (cm), SWPF- Seed weight fruit¹ (g), NSPF- Number of seeds fruit¹, HSW- 100-seed weight (g), FYPP- Fruit yield plant¹ (g) and SYPP- Seed yield plant¹ (g), GCV- Genetic coefficient of variation, PCV- Phenotypic coefficient of variation, h²- Heritability in broad sense, GA- Genetic advance.

more effective for genetic improvement of the crop. These results are similar to those reported by Rakhi and Rajamony (2005) in *Cucumis melo*.

The clustering of genotypes through Ward's minimum variance method first separated the genotypes into two main groups, which were in accordance with the species. This observation is corresponded with the clustering reported by Levi et al. (2001a) in watermelon (C. lanatus and C. colocynthis) accessions. Further, none of the genotype crossed species boundary indicating that inspite common genus Citrullus, both species i.e. C. colocynthis and C. lanatus are maintaining their identical genetic dissimilarity. These two groups were designated as A and B having 17 and 22 genotypes, respectively. All the 17 genotypes of C. colocynthis accommodated into group A, which recorded higher values for SYPP, NFPP and NSPF, while all 22 genotypes of C. lanatus were grouped into B, which had higher means for FYPP, SFW, FD, SWPF and HSW compared to other group. This indicated that C. colocynthis has more number of fruits plant⁻¹ compared to *C. lanatus*. For this reason, Levi et al. (2006) successfully transferred higher female to male ratio from C. colocynthis to C. lanatus through interspecific hybridization between these species. Finally based on Euclidean² cluster analysis, all the genotypes were grouped into seven clusters.

Maximum of 11 genotypes were included in cluster VII followed by eight each in clusters I and V, five in cluster IV, three in cluster VI and two each in clusters II and III. Genotypes of *C. colocynthis* were spread over to four clusters while genotypes of *C. lanatus* were accommodated in three clusters, indicating greater diversity in *C. colocynthis* compared to *C. lanatus*. This showed that *C. lanatus* lost diversity during its domestication and selection for a fewer traits (Navot and Zamir, 1987; Maggs-Kölling *et al.*, 2000) whereas *C. colocynthis* is still unaffected with such genetic manipulation.

The intra-cluster distances ranged from 0.22 (cluster III) to 3.08 (cluster I) and did not transgress the limits of any of the intercluster distances (Table 2). The inter-cluster distances varied from 3.23 (between clusters V and VI) to 42.81 (between clusters III and V). The inter-cluster distance was the maximum between clusters III and V (42.81) followed by

Table 2. Intra and inter-Euclidean² cluster distances among different clusters in Citrullus spp.

	O	22				, ,	
Cluster	I	II	III	IV	V	VI	VII
I	3.08	5.62	9.34	8.10	26.77	24.13	18.13
II		0.63	12.59	11.44	24.88	20.18	13.81
III			0.22	26.42	42.81	35.14	21.17
IV				2.67	30.37	33.25	31.50
V					1.56	3.23	9.35
VI						0.92	4.72
VII							2.35

clusters pair III and VI (35.14), and IV and VI (33.25) indicating enormous diversity among the genotypes evaluated in this study. The crosses between genotypes of these diverse clusters might result into greater variability and produce diverse recombinants (Arunachalam et al., 1984). Levi et al. (2001b) reported low genetic diversity among watermelon cultivars and emphasized the need to expand the genetic base of watermelon. The present study indicated greater diversity between clusters pair having different species and thus provide good chance to improve both the species of Citrullus through interspecific hybridization and selection for desired traits. For this reason, Sain (1999) obtained transgressive segregations from derivatives of crosses between C. lanatus and C. colocynthis.

The mean values of the characters for all the seven clusters are given in Table 3. The group IV, having five C. colocynthis genotypes had the maximum values for traits like SYPP, NFPP and NSPF, while the cluster V, with eight C. lanatus genotypes, was superior for FYPP, SFW, SWPF and HSW. Cluster VI, with three C. lanatus genotypes, was characterized by their larger FD. This showed that different clusters were superior in respect of different characters. Therefore, intercrossing of genotypes of diverse group would be useful for inducing greater variability in the respective trait. Among the characters studied, NSPF contributed the maximum towards the total divergence (48.2%) followed by SWPF (13.8%), HSW (10.1%) and FYPP (6.9%). These characters should be given more importance for effective selection and the choice of parents for hybridization.

The assessment of genetic diversity and clustering of genotypes are helpful in the choice of the parent material for specific breeding objectives, since it is known that exploitation

Clusters	Characters							
	NFPP*	SFW (g)	FD (cm)	SWPF (g)	NSPF	HSW	FYPP (kg)	SYPP (g)
I	10.6	237.3	8.1	11.9	444	2.69	2.545	126.1
II	11.8	207.5	8.0	10.5	247	4.27	2.430	124.3
III	6.0	181.7	6.9	11.5	445	2.60	1.070	68.5
IV	14.7	243.3	7.7	12.5	468	2.68	3.569	185.1
V	7.2	603.1	10.2	19.7	267	7.39	4.367	142.0
VI	6.5	591.5	10.5	16.6	227	7.35	3.862	108.3
VII	5.7	443.5	9.6	16.4	231	7.14	2.547	94.1

Table 3. Cluster means for different characters in Citrullus spp.

*NFPP = Number of fruits plant⁻¹, SFW = Single fruit weight (g), FD = Fruit diameter (cm), SWPF = Seed weight fruit⁻¹ (g), NSPF = Number of seeds fruit⁻¹, HSW = 100-seed weight (g), FYPP = Fruit yield plant⁻¹ (g) and SYPP = Seed yield plant⁻¹ (g).

of heterosis for yield and success of obtaining desirable recombinants is dependent on degree of divergence between the parents chosen. Such analysis quantify the degree of divergence between populations to understand the trend of evolutionary pattern, to assess the relative combination of different components of yield to total divergence and to determine the nature of forces operating at intra and inter cluster levels have been emphasized by various workers in different crops. In the present study, maximum inter-cluster distance was observed between III and V. The parents for hybridization could be selected on the basis of their greater inter-cluster distance for getting desirable recombination. Therefore, it is logical to attempt crosses between the genotypes belonging to cluster III (MGPT 8 and MGPT 18 of tumba) and V (SKGPK 1, SKGPK 15, SKGPK 4, SKGPK 14, SKGPK 18, SKGPK 7, SKGPK 20 and SKGPK 8 of kalingada) to improve various characters in both the species. This offers ample scope for selection of desirable segregants from cross combinations involving genotypes from these diverse groups, rather than utilizing genotypes falling within the same group, since this will be based on narrow genetic differences and is bound to limit the scope for useful genetic advance.

References

Arunachalam, V., Bandyopadhyay, A., Nigam, S.M. and Gibbons, R.W. 1984. Heterosis in relation to genetic divergence and specific combining ability in groundnut (*Arachis hypogea* L.). *Euphytica* 33: 33-39.

Dane, F., Liu, J. and Zhang, C. 2007. Phylogeography of the bitter apple, *Citrullus colocynthis*. *Genetic Resources and Crop Evolution* 54: 327-336.

Ekins-Daukes, S., Helms, P.J., Taylor, M.W., Simpson, C.R. and McLay, J.S. 2004. Paediatric homoeopathy in general practice: Where, when and why? *British Journal of Clinical Pharmacology* 59: 743-749.

Hashizume, T., Shimamoto, I., Harushima, Y., Yui, M., Sato, T., Imai, T. and Hirai, M. 1996. Construction of a linkage map for watermelon (*Citrullus lanatus* (thumb.) Matsum & Nakai) using random amplified polymorphic DNA (RAPD). *Euphytica* 90: 265-273.

Kumar, V., Rathee, P., Kohli, K., Chaudhary, H. and Rathee, S. 2009. Phytochemical and biological potential of *Indrayan*: An overview. *Pharmacognosy Reviews* 3: 182-194.

Levi, A., Thomas, C.E., Keinath, A.P. and Wehner, T.C. 2001a. Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. *Genetic Resources and Crop Evolution* 48: 559-566.

Levi, A., Thomas, C.E., Thies, J.A., Simmons, A.M., Ling, K., Harrison, H.F., Hassell, R. and Keinath, A.P. 2006. Novel watermelon breeding lines containing chloroplast and mitochondrial genomes derived from the desert species *Citrullus colocynthis*. *HortScience* 41: 463-464.

Levi, A., Thomas, C.E., Wehner, T.C. and Zhang, X. 2001b. Low genetic diversity indicates the need to broaden the genetic base of cultivated watermelon. *HortScience* 36: 1096-1101.

Maggs-Kölling, G.L., Madsen, S. and Christiansen, J.L. 2000. A phenetic analysis of morphological variation in *Citrullus lanatus* in Namibia. *Genetic Resources and Crop Evolution* 47: 385-393.

Navot, N. and Zamir, D. 1987. Isozyme and seed protein phylogeny of genus *Citrullus* (Cucurbitaceae). *Plant Systematics and Evolution* 156: 61-67.

Palevitch, D. and Yaniv, Z. 1991. *Medicinal Plants of the Holyland. Tel Aviv*, Tamus Modan Press.

- Pareek, O.P. and Vashishtha, B.B. 1980. Variability in *Citrullus colocynthis* in the Thar desert. *Annals of Arid Zone* 19: 277-281.
- Raiger, H.L., Dua, R.P., Sharma, S.K., Phogat, B.S. and Rathi, R.S. 2009. Stability for seed yield and quality traits in *kalingada* (*Citrullus lanatus*). *Indian Journal of Agricultural Sciences* 79: 745-747.
- Rakhi, R. and Rajamony, L. 2005. Variability, heritability and genetic advance in landraces of culinary melon (*Cucumis melo L.*). *Journal of Tropical Agriculture* 43: 79-82.
- Sain, R.S. 1999. Interspecific hybridization in genus *Citrullus. Ph. D. Thesis*, Rajasthan Agricultural University, Bikaner, India.

- Ward, J.H. 1963. Hierarchical grouping to optimize an objective function. *Journal of American Statistical Association* 58: 236-244.
- Yadav, N.D. and Singh, P.M. 1992. *Tumba and Mateera Cultivation in the Indian Arid Zone*, pp 1-15, CAZRI, Jodhpur, India.
- Yoshikawa, M., Morikawa, T., Kobayashi, H., Nakamura, A., Matsuhira, K., Nakamura, S. and Matsuda, H. 2007. Bioactive saponins and glycosides. XXVII. Structures of new cucurbitane-type triterpene glycosides and antiallergic constituents from *Citrullus colocynthis. Chemical and Pharmaceutical Bulletin* 55: 428-434.