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Morphological studies on interspecific F_1 hybrids in genus *Citrullus*

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Abstract

Interspecific crosses in genus *Citrullus* involving two accessions of *C. colocynthis* i. e. cv. GP 3 and cv. GP 177 (diploid, 2n = 22), wild perennial and a cultivar of *C. vulgaris* var watermelon cv. RW 177-3 (diploid, 2n = 22), a cultivated annual yielded 8 hybrid fruits. Interspecific hybrids showed intermediary values between the parents for most of the attributes studied, though reciprocal differences were there. Hybrid plants were earlier in opening of female flower than respective female parent. F_1 hybrids of the crosses involving *C. colocynthis* as female parent showed flower shedding and delayed maturity of fruits, consequently, resulting in high rate of seed abortion. Pulp colour, pulp taste and seed coat colour were intermediate in crosses involving *C. vulgaris* var. watermelon as seed parent. The character expression of F_1s surpassing with the parent either in positive or negative direction was comparable to transgression effect. Resemblance to either of the parent and intermediate character expression were attributed to dominance and additive gene action, respectively.

Key words: *C. colocynthis*, *C. vulgaris*, reciprocal crosses, pollen fertility

Introduction

Family Cucurbitaceae is a difficult one for the study of specific and generic segregation [1]. Excess of parental zygotes indicates operation of powerful restriction to recombination in interspecific crosses [2]. So far, the distant hybridization in this family was mainly concerned with introgression of genes for disease/drought resistance and improving the fruit size and quality of flesh for various purposes. The genetic improvement of the cucurbits could not be done in the direction of improved seed characteristics i.e. test weight, quality of seed and its product (cake and oil). Distant hybridization between compatible species helps in broadening narrow genetic base, improving productivity and transfer of desirable genes [3].

Tumba, *C. colocynthis* Schrad. an unexploited perennial creeper growing wild in hot Indian arid zone has attained new heights in recent past because of its multifarious uses. Tumba is an oil rich plant [4,5]. Major drawback of this plant is its thick testa, which not only adversely affects germination but also increases fiber content of the cake. The variation in oil content is also limited between 20-30 per cent. Indigenous collection of tumba exhibited good amount of variability of attributes other than test weight and testa content. On the other hand *C. vulgaris* a cultivated annual with large fruits, bold seeds and relatively thinner testa is grown in this region. The present investigation was therefore undertaken to explore the possibilities of obtaining hybrids between these two species and to study their behaviour.

Materials and methods

Plant materials: The parental material used in the present study, consisted of two accessions of *C. colocynthis* (L.) Schrad. wild perennial and one cultivar of *C. vulgaris* (L.) Schrad. cultivated annual. The seed of *C. colocynthis* cv. GP 3 and cv. GP 177 (bold seeded) was obtained from Agricultural Research Station, Mandor, Jodhpur (Rajasthan), and the seed of *C. vulgaris* var watermelon cv. RW 177-3 from A.R.S., Durgapura, Jaipur. The hybridization was carried out during *zaid* (summer) 1997 at A.R.S. Durgapura, Jaipur. A total of 8 fruits were obtained from the crosses attempted between these two species involving three parents. The crossed seed and seed of parents were sown in two rows of 4m length and 2.5m apart. Plant to plant distance was maintained 80 cm. Based on morphological attributes, true hybrids were selected for further studies. Data on morphological and other metric traits of parents and their F_1 hybrid plants were recorded in *zaid* 1998.

Pollen fertility analysis: Anthers from freshly opened flowers were dusted on the micro-slides and released in aceto-carmine (1%), mixed with a little of glycerol and mounted using a cover-slip. Pollen was

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observed under a microscope using 10x objective. Deeply stained and well-developed pollen grains were counted as fertile. Ten random fields were observed for each parent/F_1 and ratio of stained pollen to total pollen counted was expressed as per cent fertility. Required photographs of morphological features of leaf, flower, fruit, cut-fruit and seeds were taken from this experiment (Fig. 1).

Results and discussion

Morphology of parents: Parents involved in present study had differences in their morphological attributes. Leaves of *C. colocynthis* (GP 177 and GP 3) were simple, deltoid in outline with three deep lobes, middle lobe being the largest. Each lobe was pinnatifid and obtuse at apex. Leaf margin was crisped. Lower surface in young leaves was densely hirsute. Leaves of *C. vulgaris* var watermelon cv. RW 177-3 were triangular, 3-segmented, however, segments not distinguished. Lateral segments were rounded to obtuse at apex (Fig. 1A). Leaves of parents varied in area and compactness of lobes. Parental genotypes differed in floral morphology. The corolla lobe-size of female flowers ranged from 1.4 cm x 0.9 cm (cv. RW 177-3) to 2.0 cm × 1.7 cm (GP 177). Corolla of *C. colocynthis* (GP 177 and GP 3) was green on outside, yellowish green inside, ovate, 5-nerved from base, acute at apex, shortly hairy outside and glabrous inside. Corolla of RW 177-3 was deep yellow, villous on outside and glabrous inside. Besides habit, pulp colour, pulp taste and seed coat colour, parental genotypes of two species differed in metric attributes *viz.*, fruit circumference, number of fruits per plant, fresh fruit weight, seed weight per fruit, number of seeds per fruit, 1000-seed weight, per cent seed testa and per cent oil content. Per cent pollen fertility of all the three parental genotypes involved in crosses, was more than 90 per cent (Table 1).

Morphology of hybrids: In general, the hybrid plants were vigorous and intermediate as compared to parents. The hybrid vigour was manifested as enhancement in leaf area and early initiation of female flower and profuse vegetative growth in comparison to better parent (Table 1). The leaves of the hybrid plants were triangular in outline scabrous on lower surface, pinnatifid, tri-lobed with crisped margin. Central segment was ovate with acute apex. Lateral segments were bilobed rounded to obtuse at apex (Fig. 1A). The flowers were intermediate in size, however, corolla lobe of male flowers was slightly longer while it was shorter in female/hermaphrodite flowers in cross GP 3 × RW 177-3 (Fig. 1B) and in the cross GP 177 × RW 177-3 male flowers were larger than female flowers. The hybrid exhibited intermediary values between the parents for fruit circumference, number of fruits per plant (GP 177 × RW 177-3), fresh fruit weight, 1000 seed weight (GP 3 × RW 177-3), number of fruits per plant (GP 3 × RW 177-3) and per cent seed testa. While for number of fruits per plant (GP 3 × RW 177-3) seed weight per fruit, number of seeds per fruit, per cent oil in seeds and pollen fertility for hybrid was inferior to either of the parent (Table 1). Pulp colour and pulp taste were similar to female parent, whereas seed coat colour was intermediate (Fig. 1E).

Morphology of reciprocal hybrids: In general the hybrid plants were intermediate between parents in leaf,

Table 1. Mean values for qualitative and quantitative attributes of parents and F_1 hybrids of *C. colocynthis* and *C. vulgaris* var. watermelon (*Zaid* 1998)

Characters	GP 177	GP 177 × RW 177-3	RW 177-3 × GP 177	RW 177-3	RW 177-3 × GP 3	GP 3 × RW 177-3	GP 3
Leaf area (cm^2)	63.4	109.6	168.6	101.2	110.0	115.4	69.3
Days to opening of ♀ flower	60.1	48.4	47.5	52.3	46.9	48.4	58.3
Flower colour	GY	Y	Y	DY	Y	Y	GY
Corolla lobe (♂) L × W (cm × cm)	1.7 × 1.5	2.0 × 1.0	1.8 × 1.2	1.6 × 0.9	1.6 × 1.4	1.5 × 1.3	1.6 × 1.3
Corolla lobe (♀) L × W (cm × cm)	2.0 × 1.7	1. 8 × 0.8	2.0 × 1.3	1.4 × 0.9	1.5 × 1.5	1.6 × 1.3	1.4 × 1.5
Pulp colour	White	White	YW	Red	YW	White	White
Pulp taste	Bitter	Bitter	SB	Sweet	SB	Bitter	Bitter
Fruit circumference (cm)	24.2	32.3	49.9	58.9	40.8	26.7	24.8
Number of fruits per plant	5.8	3.2	4.6	2.6	3.2	2.0	7.3
Fresh fruit weight (g)	347.6	612.5	2037.5	3500.0	1416.5	1181.2	324.6
Seed weight per fruit	14.7	1.35	23.2	19.0	21.4	2.07	12.4
Number of seeds per fruit	425.5	81.2	815.2	648.4	779.8	98.0	503.0
Seed colour	DB	B	B	LB	DB	B	DB
1000 seed weight (g)	33.7	26.4	28.5	26.5	26.3	23.6	22.6
Per cent seed testa	58.4	52.3	48.3	46.7	51.4	54.2	61.3
Per cent oil in seeds	23.2	18.6	27.6	26.8	26.6	19.7	22.5
Per cent pollen fertility	97.7	26.6	84.1	96.3	69.2	24.5	97.8

L-Length, W-Width, GY-Greenish yellow, Y-Yellow, DY-Deep yellow, YW-Yellowish white SB-Slightly bitter, DB-Deep brown, LB-Light brown, B-Brown

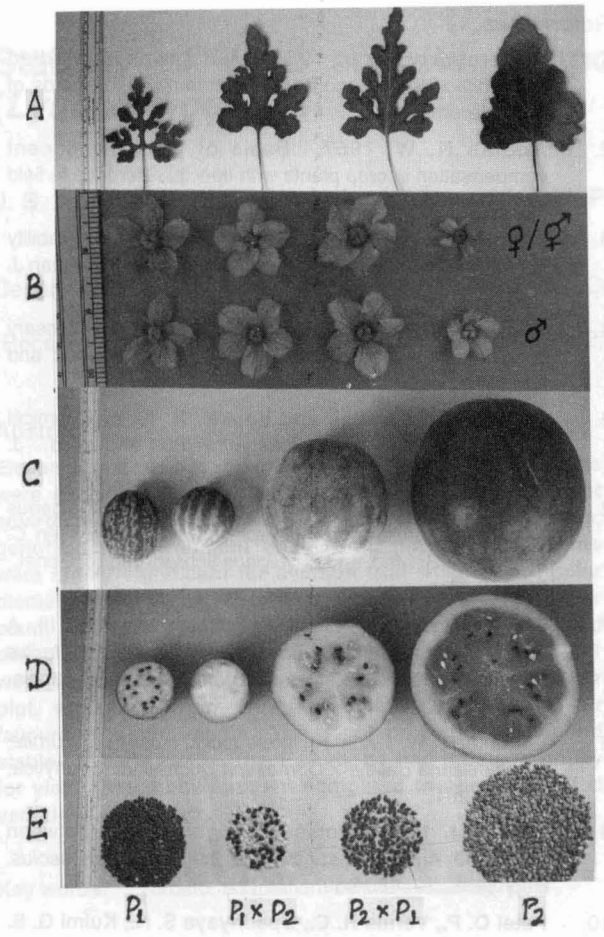

Fig. 1. Morphological features of leaf (A) flower, (B) fruit, (C) cut-fruit, (D) and seed (E) of *Citrillus colocynthis* cv. GP 3 (P_1), *Citrullus vulgaris* cv. RW 177-3 (P_2) and their F_1 hybrids ($P_1 \times P_2$) ($P_2 \times P_1$)

flower and other morphological attributes. The leaves of hybrid plants were triangular pyramid shaped (RW 177-3 × GP 3), pinnatisect, trilobed and hastate. Central segment was ovate with acute apex and lateral segments were bilobed with obtuse apices (Fig. 1A). Size of corolla lobes of both male and female flowers were similar to male parent (RW 177-3 × GP 3) with relatively low width appearing intermediate to both the parents in size (RW 177-3 × GP 177) and colour of the petals (Fig. 1B). The hybrid showed intermediary values between parents for five quantitative attributes *viz.*, fruit circumference, number of fruits per plant, fresh fruit weight, 1000 seed weight and per cent seed testa (Table 1). Whereas the hybrid showed superiority for the characters like leaf area, days to opening of female flower, seed weight per fruit and number of seeds per fruit over both the parents. On the contrary for pollen fertility the hybrid was inferior to either of the parent. Pulp colour (Fig. 1D), pulp testa and seed coat colour were intermediate (RW 177-3 × GP 177).

Reciprocal differences were recorded in crosses between *C. colocynthis* and *C. vulgaris* for the characters like leaf area, fruit circumference, fresh fruit weight, seed weight per fruit, number of seeds per fruit, per cent oil content and pollen fertility (Table 1).

The observations emanating from the current study of interspecific hybridization in genus *Citrullus* clearly indicated that hybrids (F_1) could be obtained from the crosses made between the genotypes of *C. colocynthis* and *C. vulgaris*. Morphologically F_1 hybrids of the cross combinations of *C. colocynthis* and *C. vulgaris* were intermediate between these two parental species. The main features of F_1 plant were vigorous vine growth, with lobed leaves but not deeply cleft as that of *C. colocynthis*. Corolla was more like *C. colocynthis* in size and colour. Similar observations on morphology of F_1 hybrids were made in "ta-tumba", said to be a natural hybrid between these two species [6,7] and in species cross *C. lundelliana* × *C. maxima* [8]. The behaviour of hybrids of crosses *C. colocynthis* × *C. vulgaris*, until the onset of flowering, was similar to that of reciprocals. The flowers of reciprocal F_1 hybrids were normal, whereas, those of hybrids *C. colocynthis* × *C. vulgaris* exhibited deformed anthers and flower shedding which resulted in reduced seed set and delayed maturity of fruits. Smartt [9] and Patel *et al.* [10] also reported reciprocal differences in F_1 hybrids of interspecific crosses in pulses.

Crosses involving *C. colocynthis* as female parent exhibited higher rate of seed abortion than their respective reciprocals. Joshi and Solanki [11] in interspecific crosses in genus *Citrullus*, observed less number of seeds per fruit in F_1 hybrids. Reciprocal differences in F_1 hybrids were observed for average number of days to pistillate flowering, number of fruits per plant, fresh fruit weight, 1000-seed weight and yield per plant. Similar observations were also made in F_1 hybrids and reciprocals in *Cucurbita* species [12]. Comparatively low seed abortion rate in reciprocal crosses (*C. vulgaris* × *C. colocynthis*) indicated cytoplasmic control of fertility [9]. The genome and plasmon must both have a common origin to ensure normal fertility. Mean value of per cent seed testa was low in the hybrids involving *C. colocynthis* as seed parent. Results of the present study further revealed that cultivars having higher per cent of seed testa exhibited low per cent of oil content. Comparatively low value of oil content in the seeds of hybrids (GP 3/GP 177 × RW 177-3) was due to incomplete development of endosperm. Thayer [13] also reported such type of relationship while studying inheritance of cotyledonary character in *Cucurbita pepo*. Seed coat colour in F_1 hybrids was intermediate or akin to seed colour of female parent. Seed coat colour was governed

by single gene difference with incomplete dominance in *Melothria medraspatana* [14] while Poole and Porter [15] stated that this trait was governed by the interaction of at least three genes in genus *Citrullus*.

F_1 hybrids involving *C. colocynthis* (parent with bitter principle) as pistillate parent produced fruits with bitter pulp, whereas, reciprocal hybrids revealed varied degree of bitterness. Contardi [16] concluded that bitterness in fruits of the *Cucurbitaceae* was controlled by single dominant gene. The results of crosses in *Cucumis sativus* and *Lagenaria siceraria* indicated that genetic mechanism for the formation of elaterase activity, existed independent of gene for bitterness [17]. From the results obtained in present study it is clear that the single dominant gene hypothesis would not account for the observed F_1s. In addition to a dominant overall gene for bitterness, genes were also operating to suppress bitterness in the fruit pulp and possibly another concerned with elaterase activity. Further, possibility of cytoplasmic factor could not be ruled out to explain the inheritance of this attribute.

The low pollen fertility in F_1 hybrids in general could be due to genetic imbalance and or cytoplasmic nuclear interaction. Such sterility promoted out-crossing and selection of chromosomally balanced genotypes by sieving out unbalanced gametes. Hence, it might be possible to select some useful gene combinations that have arisen from introgression or chromosomal exchange.

The expression of the characters in hybrids surpassing both the parents either in positive or negative direction may be comparable to transgressive effect; the intermediate character expression may be attributed to partial dominance or additive gene action. Whereas, resemblance to either of the parent may be due to dominant gene action.

Possibility of obtaining a useful product: Since both the species are economically important for the arid regions of Rajasthan. Hybridization between these species provides opportunity of obtaining high recombinations and thereby genetic introgression of desirable traits [18]. This leads to the possibility of synthesizing a new plant-type having higher test weight, higher number of seeds per unit of pulp volume with thinner testa and increased oil content. This will certainly depend on (i) how specific characters combine in the hybrid (ii) the fertility level of F_1 hybrids and stability of hybrid derivatives and (iii) the ease of manipulation of characters in hybrid derivatives. Further, by adopting suitable breeding strategies desirable segregants from the advance generations could be isolated.

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