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Heterotic effects in triploid watermelon hybrids

Flávio de França Souza[1*], Manoel Abilio de Queiroz[2], and Rita de Cássia Souza Dias[3]

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ABSTRACT - *Twelve experimental triploid hybrids were evaluated to estimate heterotic effects for some plant and fruit traits in triploid watermelon (*Citrullus lanatus*). Tiffany, a triploid hybrid was used as standard cultivar. The experiment in a randomized block design was carried out in three replications at the Experimental Station of Embrapa Semi-Árido in Petrolina, state of Pernambuco. The following traits were evaluated: earliness, prolificacy, main stem length, fruit weight, soluble solid content, rind thickness, flesh color, and hollow heart disorder. All evaluated traits presented significant variability among the hybrids. Mid-parent heterosis effects were found as well as the standard cultivar ranging from negative to positive values attaining up to 300% prolificacy in some cases. The tetraploid lines should be improved for several traits in order to produce earlier triploid hybrids with more compact plants and fruits without the hollow heart disorder.*

Key words: *Citrullus lanatus*, seedless watermelon, polyploidy.

INTRODUCTION

Triploid watermelon plants, which produce seedless fruits, are hybrids and therefore prone to show heterotic effects. These effects can be of value to achieve superior genotypes that include more desirable features, e. g. phenotypic stability under stress conditions, higher fruit yield, among others. When the first seedless watermelon hybrids appeared, the need of establishing more heterotic combinations became evident, especially such that with a lower number of white immature seeds per fruit (Kihara 1951).

Heterosis is a manifestation of the hybrid vigor expressed by the difference between generation F_1 and the mean value of the parents. It can also be measured in relation to the better parent (heterobeltiosis) or to a standard cultivar (Paterniani 1973). Therefore, heterosis is essentially a phenotypic effect and is defined as the performance of the descendents (Mayo 1980).

Heterotic effects have been studied in some polyploidy species such as alfalfa and potato, mainly for yield-related traits. Positive heterosis was observed in most of these cases (Cubillos and Plaisted 1976, Ray et al. 1998).

[1] Embrapa Rondônia, BR 364, km 5,5, C. P. 406, 78.900-970, Porto Velho, RO, Brasil. E-mail: falviofs@cpafro.embrapa.br
[2] DTCS-UNEB, C. P. 171, 48.900-000, Juazeiro, BA, Brasil
[3] Embrapa Semi-Árido, BR 428, km 152, Zona Rural, C. P. 23, 56.302-970, Petrolina, PE, Brasil

Regarding the triploid watermelons hybrids, practically no research work on heterotic effects has been done even with diploid genotypes, despite the importance and usefulness of heterosis in watermelon plant breeding. Nath and Dutta (1970) mentioned that some hybrid combinations showed over 50% heterosis for yield and fruit quality. Kale and Seshadri (1988) detected heterosis for yield and fruit quality-related traits in some crosses of Indian with exotic cultivars. Ferreira et al. (2002) evaluated 21 experimental watermelon hybrids and detected positive heterotic effects in relation to the parent's mean for the number of fruits per plant, fruit weight, width of the flesh, transversal diameter of the fruit, number of seeds per fruit, and the weight of a hundred seeds.

This study had the objective to determine the heterotic effects related to the fruit yield and quality in 12 hybrids and their parents (three tetraploid and four diploid lines of commercial cultivars).

MATERIAL AND METHODS

The experimental triploid hybrids were obtained by crosses among three tetraploid lines (LT7-48.1, LT9-24.14 and LTCC-24) and four diploid lines from selfing commercial cultivars (Crimson Sweet, Perola, New Hampshire Midget, and Charleston Gray).

The triploid seeds were produced through hand pollination. The male and female flowers were protected before anthesis using a device made of a plastic cup stapled to a stick which kept the device in the ground and prevented pollen contamination by bees. Normally, two male flowers are protected to pollinate a female flower. After anthesis the male flowers were removed from the plant and rubbed onto the tetraploid stigmas. The female flower was tagged and protected again for at least one day (Figure 1). Mature fruits of all treatments were harvested and the seeds were extracted and left in the shade to dry.

Seeds were sown in a tray filled with a humus – vermiculite mixture and the seedlings transplanted to the field after 15 days.

The hybrids and parents were grown at the Experimental Station of Embrapa Semi-Árido in Petrolina-PE, from April to August 1999, on a Latossol. The experiment was carried out in a randomized block design with three replications. The plots had seven plants in rows spaced 3.0 m apart and 1.0 m between plants. The triploid hybrid Tiffany was used as standard cultivar. To guarantee fruit production in the triploid plants, an experimental diploid hybrid was used as pollinator. One such plant was planted at one and another at the other end of each plot.

The plants were watered by furrow irrigation and the crop management was done according to the specific technical recommendations for watermelon crop. Fruits were harvested 82 days after planting.

The treatments were evaluated for the following traits: number of days to the emergence of the first female flower (NDF); length of the main stem (LMS); number of fruits per plant (NFP); fruit weight (FW) in kilograms; total soluble solid content (TSS), rind thickness (RT), flesh color (FC) and occurrence of hollow heart (OHH). The last two traits were evaluated using a scale of grades. The flesh color was graded from one to four (1 = deep red; 2 = light red; 3 = pink; 4 = white). The following scale was established for the occurrence of hollow heart: 1 = absence of hollow heart; 2 = up to 10% hollow heart of the flesh; 3 = over 10% hollow heart of the flesh. The plant traits of the five middle plants in the row were evaluated and the fruit traits were determined by a random sample of five fruits per plot.

The variance analyses were performed according to the ramdomized complete block model. The means were compared by the test of Scott and Knott (1974) at 5% probability (Cruz 1997).

Heterosis in relation to the parent's mean (Ramalho et al. 1990) and the standard cultivar was estimated according to the following formulae:

$$H_{mp} = F_1 - (P_i + P_j)/2 \text{ and } H_{cp} = F_1 - CP$$

where

H_{mp} : is the heterosis in relation to the parent's mean;
F_1 : hybrid mean;
P_i and P_j : mean performances of each parent;
H_{cp} : heterosis in relation to the standard cultivar; and
CP : mean performance of the standard cultivar.

The values were transformed to percentage for the parent mean and the standard cultivar.

RESULTS AND DISCUSSION

Significant differences were observed among the treatments for plant and fruit traits (Tables 1 and 2). The

Figure 1. Hand pollination of watermelon showing: (a) protected male flower, (b) female flower on the day of anthesis, (c) two male flowers and one female flower protected in a watermelon plant, (d) male flower removed from the plant and rubbed onto the stigma, (e) after the pollination the female flower is tagged and protected for one more day. Arrow indicates fruit set two days after pollination

parents showed great variability for all traits. The tetraploid and diploid lines presented a range of variation for several traits, mainly for earliness, prolificacy, fruit size, and flesh color.

For earliness, hybrid 3 x 5 was the latest to flower and was comparable to the earliness of the tetraploid lines, but much later than the standard cultivar. There were no significant differences among the other hybrid combinations and almost all these hybrids showed negative heterosis in relation to the parent mean or small positive heterosis in relation to the standard cultivar, i.e., they were earlier than the tetraploid lines but were later than the standard cultivar indicating that the tetraploid lines should be improved for earliness. This trait presented non-additive effects (Souza et al. 2002) indicating that there is a need to select a pair of lines which can achieve the desirable earliness in the triploid crosses.

Hybrid 3 x 4 was the most vigorous while the hybrids 1 x 4, 1 x 5, 2 x 5 and 2 x 7 produced more compact plants, but all hybrids showed positive heterosis for this trait in relation to the parent mean and the standard cultivar (Table 1). Since compact plants are interesting for farmers and no hybrid performed better than the standard cultivar, this trait should also be improved in the tetraploid lines. One of the alternatives could be a crossing with tetraploid lines of population Charleston Tetra Number 3 which has a shorter stem. However, it introduces susceptibility to powdery mildew and therefore requires the selection of recombinants which are resistant to the disease but maintain the shorter stem (Queiróz et al. 2001).

The triploid hybrids 3 x 6 and 3 x 7 showed a high prolificacy, which differentiated them from the others, reaching a heterosis over 130% (3 x 6) in relation to the mid-parents and 300% (3 x 7) in relation to the standard cultivar. The remaining hybrids had a positive heterosis in relation to the parent mean, most of them above 50%. The same tendency was however not observed regarding the standard cultivar, which had negative heterosis in some cases. If prolific plants with small fruits are to be selected the diploid parent New Hampshire Midget and the tetraploid line LTCC-24 should be considered due to their high specific as well as general combining ability for prolificacy, and since the number of fruits per plant increased in their crosses (Souza et al. 2002) although they will have to be selected for other plant and fruit traits in order to produce commercial hybrids.

Positive heterotic effects for fruit weight were verified in relation to the mid-parents and the standard cultivar for hybrids 1 x 4 and 1 x 7, which shows that the tetraploid line LT7-48.1 had a significant positive heterotic effect on fruit weight. On the other hand, the lines New Hampshire Midget and LTCC-24 had prolific hybrids of smaller fruit size (Table 1). Prolificacy is negatively correlated with the fruit size, indicating that the higher the number of fruits per plant, the smaller they are (Ferreira et al. 2003). The fruit size defines the type of market for a cultivar. In this case, hybrids with small fruits and a high percentage of negative heterosis in relation to the standard cultivar could be sold as “ice box” type: 8 to 12 pounds per melon, while the hybrids from the LT7-48.1 line could be delivered to markets which prefer large fruits. However, the hybrids with small fruits (3 x 4 and 3 x 7) (Tables 1 and 2) presented poor flesh color and sugar content The tetraploid lines from LTCC-24 therefore need to be improved for these traits, although the triploid hybrid 2 x 6 of good flesh color and sugar content, if cultivated in small plant spacing, could produce acceptable fruits for small-fruit markets.

As the watermelon fruit yield depends strongly on the number of fruits and mean weight of fruit per plant (Kale and Seshadri 1988), more prolific hybrids could provide yield increases, which is desirable for most breeding programs. Prolific plants also offer a greater flexibility to tailor different fruit sizes according to market preferences since they can be adjusted to different plant spacings.

All hybrids, except the ones derived from line LTCC-24, had a satisfactory sweet taste of flesh (over 10.0 °Brix) particularly 1 x 5 and 1 x 7. The positive heterotic effects for sugar content exceeded the parent mean by 3.0 to 24 % but were very low when the standard cultivar was considered. The lines LT9-24.14 and LTCC-24, however, presented negative heterosis over the standard cultivar for sugar content in most hybrids (Table 2). New Hampshire Midget had low values for the soluble solid content *per se*, but could be of help when improving the sugar content of hybrids. Ferreira et al. (2002) also pointed out positive heterotic effects for solid soluble contents regarding this line. At that time the authors worked with diploid hybrids of this parental. According to the scale of grades to evaluate the color of the fruit flesh, negative heterosis is the desirable result. The hybrids of LT7-48.1 and LT9-24.14, with the exception of combination 2 x 7, had deep red flesh color. The hybrids from LTCC-24 with Perola and New Hampshire Midget had a light red and pink flesh color (Table 2). Hybrids from the New Hampshire Midget line presented negative heterosis in relation to the mid-parents. This showed a superiority regarding the parents, which had a low performance *per se*. Hybrids 1 x 6, 2 x 7 and all hybrid combinations with LTCC-24 had positive heterosis in relation to the standard cultivar, which shows that their fruits do not meet the market standards.

Some watermelon fruits presented superficial to deep cracks in the flesh. This disorder is designated as hollow heart (Mohr 1986) and disqualifies these fruits for the market. All triploid hybrids had fruits with hollow heart incidence at different degrees (Table 2). Tetraploid line LT9-24.14 is prone to hollow heart, a trait that has appeared in its crosses with all diploid lines (Table 2). Although LTCC-24 did not present hollow heart *per se*, its hybrids had deeply cracked flesh, even when the diploid lines did not have this problem. Regarding the fruit quality of triploid hybrids, the hollow heart of fruits is a defect that should be a prior concern in watermelon breeding programs. According to Queiróz et al. (2001) there are different sets of tetraploid lines in the watermelon breeding program at Embrapa Semi-Árido, as well as diploid lines with a different fruit structure from commercial cultivars. Therefore, a range of triploid combinations can be raised by crossing different tetraploid lines with diploid lines from commercial cultivars in order to select desirable triploid combinations. However, when selecting for triploid crosses without cracks in the flesh the breeder has to take into consideration that this trait has presented non-additive gene action (Souza et al. 2002) and the specific combining ability of each pair of lines should therefore be a major concern.

The thickness of the rind is a relevant trait in view of transportation and post-harvest fruit conservation. Very thick rinds (over 2.0 cm) are however not appreciated by customers, who prefer thinner ones.

The hybrids 1 x 4 and 1 x 7 had the thickest rind (Table 2). Among the parental lines, only LT7-48.1, Crimson Sweet and Charleston Gray presented a thick rind and transferred this trait to their hybrids (Table 2). All LTCC-24 hybrids presented thin rind showing good combining ability for this trait (Souza et al. 2002). However, a tough rind is not determined only by the rind thickness, since the rind of some fruits tends to break easily because they have the recessive allele *e* (explosive) (Mohr 1986). Therefore, in the triploid crosses there is a need to select contrasting pairs of lines for this trait in order to achieve hybrid combinations with tough rinds. According to Mohr (1986), a tough rind can be selected by cutting a thin strip of rind (0.3 cm thick and 7.5 cm long) and bend it into a circle. If it forms a complete circle it is considered very tough, but if it breaks easily in the attempt to shape a circle it is tender due to the explosive allele.

Table 1. Mean values for the number of days to the first female flower shooting (NDF), length of the main stem (LMS), number of fruits per plant (NF) and fruit weight (FW). Heterosis in relation to the mid-parents (H_{mp}) and the standard cultivar (H_{cp}) in three tetraploid lines, four diploid lines and twelve watermelon hybrids

	Mean				**Relative heterosis** (%)							
	NDF	**LMS**	**NF/P**	**FW**	**NDF**		**LMS**		**NF/P**		**FW**	
	(days)	(m)	(unit)	kg	Hmp	Hcp	Hmp	Hcp	Hmp	Hcp	Hmp	Hcp
Tiffany (cp)[1]	41.7 d	2.7 d	2.6 e	6.29 d								
LT7-48.1 (1)	49.7 b	3.6 d	0.9 e	7.90 c								
LT9-24.14 (2)	51.0 b	4.1 d	1.7 e	7.19 d								
LTCC-24 (3)	53.7 a	5.7 b	6.2 b	2.92 e								
Crimson Sweet (4)	45.3 c	4.8 c	1.7 e	9.72 b								
Pérola (5)	41.7 d	4.2 d	1.3 e	8.15 c								
New H. Midget (6)	43.7 d	4.2 d	4.4 d	3.48 e								
Charleston Gray (7)	44.0 d	3.9 d	1.5 e	9.17 b								
1x4	47.3 c	4.6 c	2.0 e	11.13 a	-0.42	13.51	9.52	70.37	53.85	-23.08	26.33	76.95
1x5	46.7 c	4.6 c	1.9 e	9.68 b	2.19	11.91	17.95	70.37	72.73	-26.92	20.62	53.90
1x6	46.0 c	6.0 b	4.2 d	6.28 d	-1.50	10.31	53.85	122.22	58.49	61.54	10.37	-0.16
1x7	45.7 c	5.2 b	1.4 e	12.06 a	-2.45	9.51	38.67	92.59	16.67	-46.15	41.30	91.73
2x4	45.0 c	5.6 b	2.7 e	8.16 c	-6.54	7.91	25.84	107.41	58.82	3.85	-3.49	29.73
2x5	46.0 c	4.8 c	2.3 e	7.98 c	-0.76	10.31	15.66	77.78	53.33	-11.54	4.04	26.87
2x6	46.7 c	5.9 b	4.9 c	4.58 e	-1.37	11.91	42.17	118.52	60.66	88.46	-14.15	-27.19
2x7	45.3 c	4.8 c	2.7 e	8.22 c	-4.63	8.71	20.00	77.78	68.75	3.85	0.49	30.68
3x4	46.3 c	6.9 a	7.1 b	3.68 e	-6.46	11.11	31.43	155.56	79.75	173.08	-41.77	-41.49
3x5	48.3 b	5.6 b	5.3 c	3.27 e	1.26	15.91	13.13	107.41	41.33	103.85	-40.92	-48.01
3x6	47.3 c	5.9 b	10.4 a	2.17 e	-2.87	13.51	19.19	118.52	96.23	300.00	-32.19	-65.50
3x7	47.3 c	5.8 b	9.1 a	3.06 e	-3.17	13.51	20.83	114.81	136.36	250.00	-49.38	-51.35

[1]cp = standard cultivar (commercial triploid cultivar)

[2]Means followed by the same letter in the column did not differ by the Scott & Knott test at 5% probability

Table 2. Mean values for the soluble solid content (TSS), flesh color (FC), occurrence of hollow heart (OHH), and rind thickness (RT). Heterosis in relation to the mid-parents (H_{mp}) and to the standard cultivar (H_{cp}) in three tetraploid lines, four diploid lines and twelve watermelon hybrids

	Mean				Relative heterosis (%)							
	TSS	**FC**	**OHH**	**RT**	**TSS**		**FC**		**OHH**		**RT**	
	(°Brix)	(Scale[1])	(Scale[2])	(cm)	Hmp	Hcp	Hmp	Hcp	Hmp	Hcp	Hmp	Hcp
Tiffany (cp)[3]	11.5 b[4]	1.0 d	1.4 b	1.05 c								
LT7-48.1 (1)	11.2 c	1.0 d	1.0 b	1.37 b								
LT9-24.14 (2)	11.2 c	1.0 d	2.2 a	1.07 c								
LTCC-24 (3)	7.5 g	2.9 a	1.0 b	1.20 c								
Crimson Sweet (4)	12.0 a	1.0 d	1.2 b	1.44 b								
Pérola (5)	10.5 d	1.0 d	1.1 b	1.18 c								
New H. Midget (6)	7.3 g	3.0 a	1.0 b	1.15 c								
Charleston Gray (7)	10.7 d	1.0 d	1.3 b	1.32 b								
1x4	11.7 b	1.0 d	2.2 a	1.60 a	0.86	1.74	0.00	0.00	100.00	83.33	13.88	52.38
1x5	11.9 a	1.0 d	1.5 b	1.39 b	9.68	3.48	0.00	0.00	42.86	25.00	9.02	32.38
1x6	11.5 b	1.1 d	1.7 b	1.32 b	24.32	0.00	-45.00	10.00	70.00	41.67	4.76	25.71
1x7	12.4 a	1.0 d	2.1 a	1.61 a	13.24	7.83	0.00	0.00	82.61	75.00	19.70	53.33
2x4	10.9 d	1.0 d	2.6 a	1.40 b	-6.03	-5.22	0.00	0.00	52.94	116.67	11.55	33.33
2x5	11.2 c	1.0 d	2.9 a	1.38 b	3.23	-2.61	0.00	0.00	75.76	141.67	22.67	31.43
2x6	10.5 d	1.0 d	1.8 b	1.07 c	13.51	-8.70	-50.00	0.00	12.50	50.00	-3.60	1.90
2x7	11.5 b	1.3 c	2.4 a	1.35 b	5.02	0.00	30.00	30.00	37.14	100.00	12.97	28.57
3x4	8.5 f	2.1 b	2.1 a	1.25 c	-12.82	-26.09	7.69	110.00	90.91	75.00	-5.30	19.05
3x5	9.5 e	1.4 c	2.4 a	1.12 c	5.56	-17.39	-28.21	40.00	128.57	100.00	-5.88	6.67
3x6	8.1 f	2.4 b	2.1 a	1.12 c	9.46	-29.57	-18.64	140.00	110.00	75.00	-4.68	6.67
3x7	8.3 f	2.2 b	1.8 b	1.14 c	-8.79	-27.83	12.82	120.00	56.52	50.00	-9.52	8.57

[1]Scale of records: 1 – deep red; 2 – light red; 3 – pink; 4 – white;
[2]Scale of grades: 1 – no hollow heart disorder; 2 – slight hollow heart (less than 10% of the flesh); 3 - expressive hollow heart (over 10% of the flesh);
[3]cp = Standard cultivar (commercial triploid cultivar)
[4]Means followed by the same letter in the column did not differ by the Scott & Knott test at 5% probability

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Efeitos heteróticos em híbridos triplóides de melancia

RESUMO - *Este trabalho estimou efeitos heteróticos em caracteres de planta e fruto em melancias triplóides (*Citrullus lanatus*). Doze híbridos experimentais foram avaliados e o híbrido triplóide 'Tiffany' foi utilizado como cultivar padrão. O experimento, em blocos casualizados com três repetições, foi conduzido na Estação Experimental da Embrapa Semi-Árido, em Petrolina-PE. Avaliaram-se os seguintes caracteres: precocidade, prolificidade, comprimento de rama principal, peso de fruto, teor de sólidos solúveis, espessura de casca, cor da polpa e ocorrência de ocamento na polpa. Verificaram-se diferenças significativas para todas as características avaliadas. Foram encontrados efeitos heteróticos em relação à média dos pais e em relação à cultivar padrão, variando desde valores negativos até positivos em vários híbridos, chegando até 300% para prolificidade. As linhas tetraplóides devem ser melhoradas para vários caracteres a fim de produzir híbridos triplóides que sejam mais precoces, de plantas mais compactas e de frutos sem problemas de ocamento da polpa.*

Palavras-chave: *Citrullus lanatus*, melancia sem sementes, poliploidia.

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