

Inheritance of fruit flesh colour in some botanical varieties of muskmelon, *Cucumis melo*

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Abstract

This study was carried out from 2016 to 2017 at Agricultural Research Center, Cairo, Egypt. From former evaluation work on several inbred lines of melon, eight inbred lines were chosen as parents for 4 crosses, viz., RIL D51 × RIL 154 (*C. melo* var. *cantaloupensis*, galia type), RIL Mg₅ × RIL 148 (*C. melo* var. *cantaloupensis*, charentais type), RIL A10 × RIL A5 (*C. melo* var. *anas*) and RIL Si819 × RIL Ab11 (*C. melo* var. *aegyptiaca*) to interpret the genetics of fruit flesh colour. Parental, F₁, F₁′, F₂ and BCs populations of each cross were sown in a randomized complete block design (RCBD) with 4 replicates in the 2017 early summer season in open field using a drip-irrigation system. One pair of genes governed the fruit flesh colour character in all the four crosses. The type of dominance was no dominance of the dark green over orange flesh colour or the reverse in the first hybrid, complete dominance of the reddish orange over dark green flesh colour in the second hybrid, partial dominance of the white over orange flesh colour in the third hybrid and complete dominance of the orange over greenish white flesh colour in the fourth hybrid. Mid and better parent heterosis values were 0.00 and -15.50 % in the first hybrid, 25.00 and 0.00 % in the second hybrid, -57.89 and -77.46 in the third hybrid and 44.90 and 0.00 % in the fourth one, respectively. Hundred percent broad sense heritability (BSH) was recorded in the four hybrids, but narrow sense heritability (NSH) differed from moderate to elevated, being 36.5, 72.15, 28.48 and 26.46 % in the first, second, third and fourth hybrids, respectively. These results proved that melon flesh colour is influenced by genotypic variability. Also, the melon flesh colour inheritance was complex and this may be due to flesh colour gene has multiple alleles (polygenic inheritance).

Key words: *Cucumis melo*, heritability, heterosis, botanical varieties, flesh colour, degree of dominance, number of genes

Introduction

Melon (*Cucumis melo* L.; 2n = 2x = 24) is an important horticultural crop worldwide. It is also one of the economically important species of the Cucurbitaceae family (Pitrat *et al.*, 2000). Few reports indicate that muskmelon originated in Africa while recent research suggest that melon may probably be of Asian origin (Sebastian *et al.*, 2010). It has worldwide existence as wild and cultivated melon types (Pitrat *et al.*, 2000). There are many types or botanical varieties of melon. The higher yield, market-standard volume, better flesh colour and thickness, narrow internal cavity, favorite flesh texture and great soluble solids content have to be available in the new varieties (Nunes *et al.*, 2005). In this respect, the aim of the breeding program define the selected traits of the superior population (Barros *et al.*, 2011).

A study on hereditary behavior of some melon characters becomes necessary due to the wide genetic variability in this crop. In the same direction, internal flesh colour is reported. Several of the former studies on muskmelon (*Cucumis melo* L.) stated that fruit traits such as netting and flesh colour are monogenically inherited (Whitaker and Davis, 1962). Likewise, Hughes (1948) found that orange flesh being dominant and a 3 orange : 1 green flesh segregation within F₂ plants in a cross between ‘Honey Dew’ (green fleshed) and ‘Smith’s Perfect’ (orange fleshed).

White, green, and orange colors of melon fruit flesh are due to a combination of chlorophyll and carotenoid pigments (Burger *et al.*, 2009). β-carotene is the major carotenoid accumulated in orange-flesh varieties of melon (Nuñez-Palenius *et al.*, 2008).

Most of the variation in color density are due to quantitative differences in β-carotene (Burger *et al.*, 2009). A perfect concept of the inheritance pattern of carotenoids accumulation in melon fruit flesh is not available. However, many works found at least four specified varied quantitative trait loci (QTL) governing melon flesh colour in various inheritance backgrounds (Monforte *et al.*, 2004; Paris *et al.*, 2008; Cuevas *et al.*, 2009; Harel-Beja *et al.*, 2010; Diaz *et al.*, 2011). Qualitatively, two main genes, green flesh (gf) and white flesh (wf) (working epistatically), widely govern fruit flesh color. Orange flesh is identified by Gf which is dominant to green flesh (gf). If a melon fruit contains gf gf, it has either green (Wf-) or white flesh (wfwf) (Hughes, 1948; Clayberg, 1992). Up to now, the molecular verification of either gf or wf has not been done, and the accurate interaction among these two genes is still obscure.

According to Ramaswamy *et al.* (1977) genetics of flesh pigments in green x white crosses illustrated that green flesh was controlled by a recessive gene. While, in orange x green, green x orange and orange x white, flesh colour genetics is complex. Maternal effect genetics of the pigment and an interaction among nuclear and extra-nuclear genes was reported. Different genes may be governing chlorophyll and carotenoid pigments through biochemical pathways.

Moreover, Cuevas *et al.* (2010) found that broad and narrow-sense heritability (h²_N) for amount of β-carotene as determined by F₁, F₂, and BC (by individuals) were 0.99 and 0.55, respectively. Likewise, flesh color segregation (F₂ and BC₁P₂) fit a two genes

recessive epistatic model, that react with other secondary genes. So, the genetics of amount of β -carotene is complex. Abo Sedera *et al.* (2016) also stated that broad and narrow-sense heritability for melon flesh colour were 0.99 and 0.51, respectively. Eduardo *et al.* (2007) confirmed that four QTLs controlled flesh colour in melon.

This study was carried out to illustrate the inheritance of fruit flesh colour in four hybrids produced using inbred lines of three botanical varieties of *C. melo*.

Materials and methods

This work was carried out from 2016 to 2017. Seeds of various inheritance populations and seedlings were sown in the polyhouse, but inheritance work was carried out utilizing a drip-irrigation system in the open area through the early summer season at Kaha Vegetable Research Farm (KVRF), Kalubia, Egypt.

Depending on the former field experiments of several inbred lines (IL) of melon through the 2014 and 2015 seasons, 8 inbred lines were chosen to use as parents for 4 hybrids to follow the inheritance of fruit flesh colour in three botanical varieties of melon. In 2016 early summer season, the F_1 hybrid seeds and their reciprocals were obtained. The F_2 , backcross (BC) populations to both parents and additional F_1 seeds were obtained through 2016 late summer season in KVRF, Kalubia.

The six inheritance populations of every hybrid, *i.e.*, parents, F_1 , F_{1r} (reciprocal), BCs, and F_2 , were placed in a randomized complete block design (RCBD) with 4 replicates. Every replicate contained 15, 30 and 40 plants of every of the non-segregating populations (parents and F_1 's), every BC and the F_2 , respectively.

The field experiment was carried out through the 2017 early summer season in the open area utilizing a drip-irrigation system. Seed sowing and transplanting were on February 10 and March 10, 2017, respectively. Plant rows were 1.5 m wide and 60 m long. Plants were placed 50 cm apart along.

Fruit flesh colour was defined in ripening fruits of the hybrids RIL D51 (dark green flesh) $\times$ RIL 154 (orange flesh), *C. melo* var. *cantaloupensis*, galia type, RIL Mg₃ (reddish orange flesh) $\times$ RIL 148 (dark green flesh), *C. melo* var. *cantaloupensis*, charentais type, RIL A10 (white flesh) $\times$ RIL A5 (orange flesh), *C. melo* var. *ananas* and RIL Si819 (orange flesh) $\times$ RIL Ab11 (greenish white), *C. melo* var. *aegyptiaca*. Fruit flesh colour were measured on individual plants of every inheritance population. Two ripe fruits of the same physiological age of every plant in the third picking were picked to define the fruit flesh colour by naked eye. It was determined as the flesh color graded from one to eight according to the scale of Abo Sedera *et al.* (2016) as follows, 1 = white, 2 = yellowish white, 3 = greenish white, 4 = green, 5 = dark green, 6 = greenish orange, 7 = orange, 8 = reddish orange.

Maternal effect was calculated by determining the significance of variations among F_1 averages and their reciprocals, utilizing the t test. Potence ratio, *i.e.*, the relative potency of gene action (P) was utilized to define the tendency of dominance as stated by Smith (1952) formula:

$$P = \frac{\bar{F}_1 - \bar{MP}}{(\bar{P}_2 - \bar{P}_1)^{1/2}} \times 100$$

where, $\bar{F}_1$ = first generation average, $\bar{P}_1$ = average of the smaller parent, $\bar{P}_2$ = average of the larger parent and $\bar{MP}$ = mid parent value = $\frac{1}{2}(\bar{P}_1 + \bar{P}_2)$.

The lack of dominance was supposed when the variation among the parents was significant and $\bar{F}_1 - \bar{MP}$ was not significant. Complete dominance was supposed when potence ratio equaled to or did not vary from ± 1.0 . While, partial dominance was stated when potence ratio was among + 1.0 and - 1.0, but was not equal to zero. Over dominance was supposed when potence ratio surpassed ± 1.0 .

Mid and better parent heterosis were determined by Sinha and Khanna (1975) equation:

$$\text{Mid-parent heterosis} = \frac{\bar{F}_1 - \bar{MP}}{\bar{MP}} \times 100$$

where, $\bar{MP}$: two parents average and $\bar{F}_1$: the first hybrid generation average.

$$\text{Better-parent heterosis} = \frac{\bar{F}_1 - \bar{BP}}{\bar{BP}} \times 100$$

where, $\bar{BP}$: the better- parent average, depending on the favorite fruit flesh colour in the botanical variety.

The least number of genes governing the trait in every hybrid was estimated utilizing Castle-Wright formula (Castle and Wright 1921) as follows:

$$N = \frac{D^2}{8(V_{F_2} - V_{F_1})} \times 100$$

where, N = Gene Numbers governing the trait in every hybrid, D = Parental average differences and V_{F_1} and V_{F_2} = Variances of the F_1 and F_2 populations, respectively.

Broad sense heritability (BSH) was estimated according to Allard (1960) formula:

$$\text{BSH} = \frac{V_G}{V_P} \times 100$$

where, V_G = Genetic variance which was estimated by subtracting the environmental variance (V_E) from the phenotypic variance (V_P) and V_E was calculated as the geometric average of variances of the non-segregating populations, *i.e.*, parents and F_1 s.

Narrow sense heritability (NSH) was estimated according to Falconer (1981) formula:

$$\text{NSH} = \frac{V_A}{V_P} \times 100$$

where, V_A = Additive variance which was estimated by subtracting the variance of BCP₁ and BCP₂ ($V_{B_1} + V_{B_2}$) from two times of the phenotypic variance ($2V_P$).

Results and discussion

Data recorded on fruit flesh colour of parental, F_1 , F_{1r} , F_2 , and backcross populations of the hybrids are presented in Tables 1, 2, 3 and 4.

In the four hybrids, parents of every hybrid clearly varied in fruit flesh colour. In the first hybrid, all the F_1 plants had greenish orange flesh colour and its mean was equal to mid-parents. In contrast, F_2 average was lower than the smaller parent. The flesh colour of F_2 plants were largely spread among both of parents and graded from greenish white to orange. Plants of the backcrosses to both RIL D51 and RIL 154 were largely spread with a great trend to this parent.

In the second hybrid, all the F_1 plants had reddish orange flesh colour and its mean was equal to higher parent. In contrast, F_2 average was lower than the better parent and close to mid-parents. The flesh colour of F_2 plants were largely spread among both of parents and graded from green to reddish orange. Plants of the backcrosses to both RIL Mg₅ and RIL 148 were largely spread with a great trend to this parent.

In the third hybrid, all the F_1 plants had yellowish white flesh colour and its mean was near to the smaller parent, but it's smaller than mid-parents. F_2 mean was equal to mid-parents. The flesh

colour of F_2 plants were largely spread among both of parents. Plants of the backcrosses to both RIL A10 and RIL A5 were largely spread with a great trend to this parent.

In the fourth hybrid, all the F_1 plants had orange flesh colour and its mean was equal to mid-parents. Also, F_2 mean was close to mid-parent. The flesh colour of F_2 plants were largely spread among both of parents. Plants of the backcrosses to both RIL Si819 and RIL Ab11 were largely spread with a relative trend to this parent.

In the four hybrids, no significant variations was showed among the F_1 and its reciprocal in fruit flesh colour, denoting no maternal effect. This result contradicts with the findings of Ramaswamy *et al.* (1977).

The inheritance determinations estimated for fruit flesh colour of four hybrids are recorded in Table 5. In the first hybrid, the P value was zero, denoting lack of dominance of the dark green over orange flesh colour or the opposite. In contrast, the P value of the second and fourth hybrids equaled one, indicating complete

Table 1. Frequency distribution, mean ($\bar{x}$) $\pm$ standard error (SE), and variance of fruit flesh colour of parental, F_1 , F_1r , F_2 , BCP_1 , and BCP_2 populations of the cross RIL D51 $\times$ RIL 154, galia type

Population	Frequency of fruit flesh colour in class ^(a)								Total number of plants	Fruit flesh colour ^(c)	Variance (S^2)
	0.5 (White)	1.6 (Yellowish-White)	2.7 (Greenish-White)	3.8 (Green)	4.9 (Dark-Green)	6.0 (Greenish-Orange) ^(b)	7.1 (Orange)	8.2 (Reddish-Orange)			
RIL D51 (P ₁)					40				40	4.9 ± 0.015	0.00
RIL 154 (P ₂)							40		40	7.1 ± 0.019	0.00
F_1						40			40	6.0 ± 0.013	0.00
F_1r						38			38	6.0 ± 0.012	0.00
F_2			20	34	31	20	15		120	4.68 ± 0.13	1.92
BC_1			12	37	26	17	8		100	4.59 ± 0.12	1.56
BC_2				18	14	26	42		100	5.91 ± 0.13	1.58

^(a) Each class represents a fruit flesh colour range of 1.1 and class values indicated class centers. ^(b) Greenish-Orange means 50 % green flesh and 50 % orange flesh. ^(c) Each value is the mean $\pm$ SE.

Table 2. Frequency distribution, mean ($\bar{x}$) $\pm$ standard error (SE), and variance of fruit flesh colour of parental, F_1 , F_1r , F_2 , BCP_1 , and BCP_2 populations of the cross RIL Mg₅ $\times$ RIL 148, charentais type

Population	Frequency of fruit flesh colour in class ^(a)								Total number of plants	Fruit flesh colour ^(c)	Variance (S^2)
	0.5 (White)	1.6 (Yellowish-White)	2.7 (Greenish-White)	3.8 (Green)	4.9 (Dark-Green)	6.0 (Greenish-Orange) ^(b)	7.1 (Orange)	8.2 (Reddish-Orange)			
RIL Mg ₅ (P ₁)								40	40	8.20 ± 0.00	0.00
RIL 148 (P ₂)					40				40	4.90 ± 0.00	0.00
F_1								40	40	8.20 ± 0.00	0.00
F_1r								40	40	8.20 ± 0.00	0.00
F_2				15	5	20	20	60	120	6.96 ± 0.14	2.37
BC_1					15	5	5	75	100	7.54 ± 0.12	1.52
BC_2				16	55	15	4	10	100	5.31 ± 0.12	1.51

^(a) Each class represents a fruit flesh colour range of 1.1 and class values indicated class centers. ^(b) Greenish-Orange means 50 % green flesh and 50 % orange flesh. ^(c) Each value is the mean $\pm$ SE.

Table 3. Frequency distribution, mean ($\bar{x}$) $\pm$ standard error (SE), and variance of fruit flesh colour of parental, F_1 , F_{1r} , F_2 , BCP_1 , and BCP_2 populations of the cross RIL A10 $\times$ RIL A5, ananas type

Population	Frequency of fruit flesh colour in class ^(a)								Total number of plants	Fruit flesh colour ^(c)	Variance (S^2)
	0.5 (White)	1.6 (Yellowish-White)	2.7 (Greenish-White)	3.8 (Green)	4.9 (Dark-Green)	6.0 (Greenish-Orange)*	7.1 (Orange)	8.2 (Reddish-Orange)			
RIL A10 (P_1)	35								35	0.5 ± 0.00	0
RIL A5 (P_2)							37		37	7.1 ± 0.00	0
F_1		40							40	1.6 ± 0.00	0
F_{1r}		38							38	1.6 ± 0.00	0
F_2	26	32	5			15	42		120	3.88 ± 0.26	8.18
BC_1	35	20	18			10	17		100	2.78 ± 0.25	6.4
BC_2	15	17	5			16	42		95	4.66 ± 0.28	7.63

^(a) Each class represents a fruit flesh colour range of 1.1 and class values indicated class centers. ^(b) Greenish-Orange means 50 % green flesh and 50 % orange flesh. ^(c) Each value is the mean $\pm$ SE.

Table 4. Frequency distribution, mean ($\bar{x}$) $\pm$ standard error (SE), and variance of fruit flesh colour of parental, F_1 , F_{1r} , F_2 , BCP_1 , and BCP_2 populations of the cross RIL Si819 $\times$ RIL Ab11, Egyptian type

Population	Frequency of fruit flesh colour in class ^(a)								Total number of plants	Fruit flesh colour ^(c)	Variance (S^2)
	0.5 (White)	1.6 (Yellowish-White)	2.7 (Greenish-White)	3.8 (Green)	4.9 (Dark-Green)	6.0 (Greenish-Orange)*	7.1 (Orange)	8.2 (Reddish-Orange)			
RIL Si819 (P_1)							40		40	7.1 ± 0.00	0
RIL Ab11 (P_2)			38						38	2.7 ± 0.00	0
F_1							39		39	7.1 ± 0.00	0
F_{1r}							40		40	7.1 ± 0.00	0
F_2	19	10	5	18		30	33	3	118	4.63 ± 0.23	6.35
BC_1	18	5	40	12		10	13		98	3.29 ± 0.21	4.47
BC_2	14	10	13	10		22	26	5	100	4.54 ± 0.26	6.55

^(a) Each class represents a fruit flesh colour range of 1.1 and class values indicated class centers. ^(b) Greenish-Orange means 50 % green flesh and 50 % orange flesh. ^(c) Each value is the mean $\pm$ SE.

Table 5. Inheritance determinations recorded for fruit flesh colour of melon hybrids

Cross	Potence ratio (P)	Mid-parent heterosis (%)	Better-parent heterosis (%)	Minimum number of genes	Broad sense heritability (%)	Narrow sense heritability (%)
RIL D51 $\times$ RIL 154	0.00	0.00	-15.50	0.32	100.00	36.5
RIL Mg ₅ $\times$ RIL 148	1.00	25.00	0.00	0.57	100.00	72.15
RIL A10 $\times$ RIL A5	-0.67	-57.89	-77.46	0.67	100.00	28.48
RIL Si819 $\times$ RIL Ab11	1.00	44.90	0.00	0.38	100.00	26.46

dominance of the reddish orange over dark green flesh colour and orange over greenish white flesh colour, respectively. As for the third hybrid, the negative P value (-0.67) denoted partial dominance of the white over orange flesh colour.

Results recorded on potency ratio in the second and fourth hybrids are in the same direction with those of Hughes (1948) and Clayberg (1992) who reported that orange flesh is controlled by Gf gene and is dominant to green flesh (gf), beside the findings of Ramaswamy *et al.* (1977) who reported that green flesh was governed by a recessive gene. In contrast, data in the first and third crosses are in disagreement with them.

In the first hybrid, the better parent had the dark green flesh colour (the smallest value), as for the second cross, the better parent had the reddish orange flesh colour (the highest value), while, in the third and fourth hybrids the better parent had the orange flesh colour (the greatest value). So, mid and better parent heterosis values were 0.00 and -15.50 % in the first hybrid, 25.00 and 0.00 % in the second hybrid, -57.89 and -77.46 in the third hybrid and 44.90 and 0.00 % in the fourth hybrid, respectively.

The fruit flesh colour was defined to be governed by one pair of genes in the four hybrids as calculated by Castle-Wright formula. Although one pair of genes controlled in this trait, the present

results illustrated that the flesh colour inheritance was complex and this may be due to flesh colour gene has multiple alleles (polygenic inheritance). This result agreed with Ramaswamy *et al.* (1977) who stated that flesh colour inheritance was complex.

Findings recorded on genes number of the four hybrids contradict with results of Monforte *et al.* (2004), Eduardo *et al.* (2007), Paris *et al.* (2008), Cuevas *et al.* (2009), Harel-Beja *et al.* (2010) and Diaz *et al.* (2011) who reported that fruit flesh colour of melon was controlled by four QTLs. On the contrary, Hughes (1948), Clayberg (1992) and Cuevas *et al.* (2010) stated that the fruit flesh colour of melon was governed by two genes.

Hundred percent of broad sense heritability (BSH) in the four hybrids denotes the secondary role of the environment on this trait and the one pair of genes has full control in this trait. While narrow sense heritability (NSH) fluctuated from moderate to high, being 36.5, 72.15, 28.48 and 26.46 % in the first, second, third and fourth hybrids, respectively. The moderate to high NSH determinations denotes the greatest additive effect of genes governing this trait.

These findings agreed with those of Cuevas *et al.* (2010) and Abo Sedera *et al.* (2016) in BSH who stated that broad-sense heritability for melon flesh colour were 99 %, but it is disagreement with them in NSH, however they found that narrow-sense heritability for melon flesh colour was 55 and 51 %, respectively.

In conclusion, the melon flesh colour inheritance is complex and this may be due to flesh colour gene has multiple alleles (polygenic inheritance). Also, the melon flesh colour inheritance varied in the three botanical varieties.

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