CHAPTER 3

MATERIALS AND METHODS

The present investigation was carried out during three successive seasons of the 2011, 2012 and 2013.This study was divided into two separate experiments. The experimental site was the green house and the experimental field of Sabahya (Horticulture Research Station, Alex, Egypt).

3.1 The first experiment (Selfing and selection)

This experiment was conducted to study numerous of genetic parameters which affect the improvement progress of melon breeding program. These genetic parameters included the following parameters: Selection index, genotypic and phenotypic variance, genotypic and phenotypic coefficient of variations, broad-sense heritability and genetic advance.

3.1.1 Experimental details

1- Plant materials

- **Line 1 $_{\text{Orange flesh}}$:** This genotype was selected which was originated as a segregation result in the sixth of inbreeding generation, with orange flesh color of the hybridization between " Charantaise x land race from Matroh governorate with green flesh and not pure" and selfing pollinated was done on it for 6 generations, but not arrives to the homogeneity yet.
- **Line 2 $_{\text{Sandafa}}$:** (selected from Sandafa cultivar (local cultivar, cultivated in Upper Egypt, Beni Suif governorate by selfing and selection for twelve generations) not pure line.
- **Shahd El-Doki (local cultivar)**: pure cultivar originated by vegetables research department, horticulture research institute, as a control variety.

The characteristics of the three previous genotypes are tabulated in table 3.1.

2- The original population (S0) of two lines were planted in green house at the end of August 2011 in foam plates and transplanted after one month on ridges 80 cm wide and 30 cm between parts. Selfing technique was described as follows.

A day before anthesis, the mature male and female flower buds at the same plant that were likely to open on the next day were coating by cotton. Next day, between 6 and 8 am pollen grains from the coated male flower were dusted on to the female flower of the same plant the female flower thus pollinated were labeled and covered by cotton until fruit set.

3- Ten percent from each genotype were selected and its seeds were mixed (mass selection) to get the first selected generation (S1), the same practices was done to get second selected generation (S2) at mid of February 2012. Characters which have a great consideration in selection, netting degree; placenta hardness; flesh thickness; net weight and tss.

4- S0 (original population), S1 (first selected generation), S2 (second selected generation) and Shahd Eldoki as a check cultivar were sown in experimental evaluation in early summer season in first March 2013 and late summer season first June 2013 to test the progress in the traits under studies in an factorial experiment with two factors (genotypes and seasons) in randomized complete block design with three replicates (RCBD) in the experimental field of Sabahya Horticulture station. Each replicate contained 16 rows 4 rows for each genotypes So, S1, S2 and Shahd El-Doki, the rows 5 m long and 130 cm width, the hills were thinned to one plant each, with 35 cm a part after three weeks later.

Table 3. 1 Special features of the original population for two genotypes and the check variety:-

Genotypes	Characterization	Pictures
Line 1 Orange flesh	- Have moderate vegetative growth. - Fruit skin has yellow green color with heavy netted. - The flesh color was dark orange and the placenta hardness was very hard, sweetness was very good with good flavor also.	 
Line 2 Sandafa	- Have strong vegetative growth. - Fruit skin color was canary yellow and light netted. - The flesh color was yellow cream and the placenta hardness was hard, the flesh sweetness was moderate and good flavor.	 
Shahd El-Doki	- Have strong vegetative growth. - Fruit skin color was brown reddish with heavy netting. - Fruit shape was long oval. - Flesh was Orange in color and has good flavor and moderate sweetness.	 

3.2 The second experiment (hybridization)

This experiment was carried out to study general and specific combining abilities and heterosis over mid and better parents

3.2.1 Experimental details

1- Plant materials

- **Line 1 $_{\text{Kos-El-Asal}}$:** Originated as a result of planting Koz El-Asal (local cultivar cultivated in Assut governorate) and applied selfing technique for 20 generations.
- **Line 2$_{\text{Charantais}}$:** Charantaise (European cultivar) pure line.
- **Line 3$_{\text{green flesh}}$:** Improved line selected from F1 hybrid (Charantaise *one of the landraces cultivated in Matroh governorate), F1 plants was individually selfed for six generations, then only one line was selected and selfed for another fourteen generations until arrived to homogeneity.
- **Line 4$_{\text{Matroh}}$:** originated after applying the selfing technique on local landraces from Matroh governorate for twenty generations and arrived to homogeneity.
- **Line 5$_{\text{primal}}$:** originated from the primal F1 hybrid, by selfing program for twenty generations, till the selected individual line arrived to homogeneity.
- **Ananas monanasa (commercial cultivar)** as control variety.

The characteristics of the six previous genotypes are tabulated in table (2).

2- Genotypes (parents) were planted in the green house at the end of August 2011 in foam plats and transplanted after one month on ridges 80 cm wide and 30 cm between plants. A5×5 diallel crosses were made between parents. Crossing technique was described as follows

A day before anthesis fully matured male flower buds of male parents covered by cotton and on the next day this flowers were taken to female emasculated flower and dusted it by pollen grain from this male flower. The pollinated flower were labeled and covered with cotton until complete set.

3- The parents, hybrids, reciprocals and Ananas monanasa (26 genotypes) were sown in separated experiments for evaluation during two successive summer seasons of 2012 and 2013 in first of March. The experimental design used was randomized complete block design with three replicates (RCBD). Each replicate contained 52 rows, 2 rows for each genotypes, rows were 5m long and 130cm wide approximately. The hills were thinned to one plant each 35 cm a part three weeks later.

Table 3. 2. Special features of parents used for crossing and control variety:-

Genotypes	Characterization	Pictures
Line $1_{Koz\text{-}El\_Asal}$	- Have moderate vegetative growth. - Fruit skin color was yellow cupper with moderate netting degree. - Placenta hardness was hard with moderate sweetness and good flavor	
Line $2_{Charantais}$	- Have strong vegetative growth. - Fruit skin color was canary yellow and heavy netted and clear contour in the fruit. - The flesh color was dark orange with small placenta diameter and placenta was very hard, Flesh sweetness was excellent with good flavor but fruit which more ripe have acetone odor	
Line $3_{Green\ flesh}$	- Have strong vegetative growth. - Fruit skin color was green yellow and heavy netted and clear contour in the fruit. - The flesh color was green with moderate placenta diameter and the placenta hardness was very. - The flesh sweetness was excellent with good flavor	

To be conts….

Table 3. 2. Contd….

Genotypes	Characterization	Pictures
Line 4_{Matroh}	- Strong vegetative growth, late flowering. - Fruit skin color was canary yellow with good netting and oval fruit shape. - Yellow orange flesh color and the placenta hardness was hard with very thin placenta. - The flesh sweetness was moderate having very good flavor with soft texture.	
Line 5_{Primal}	- Moderate vegetative growth. - Fruit skin color was yellow with heavy netting degree and clear contour in the fruit. - The flesh color is green with thin hard placenta. - The flesh sweetness was excellent with crunchy texture and moderate flavor.	
Ananas monanasa (control)	- Strong vegetative growth. - Fruit skin color was canary yellow with light netting degree and oval in shape. - Flesh color was yellow cream and moderate hard thin placenta - Sweet flesh and very good flavor with soft texture.	 

All of these lines were provided by "The Project of development of main vegetable crops" Horticulture Research Institute, Agriculture Research Center

3.3 Recorded data

Normal agriculture practices used for sweet melon production were done as normal in the area. Data were recorded on 5 plants per plot as follows:-

3.3.1 Vegetative measurements

- Plant length (cm); measured from soil surface to the terminal buds of the main stem.
- Branches number; Number of branches at the end of fruit picking.

3.3.2 Yield and its components

- Flowering date; Days from planting to the first full flower (Hermaphroditic flower)
- Maturity duration; Days from planting to the first mature fruit.
- Average fruit number/plants.
- Average fruit weight(kg)
- Total yield/plant(kg)

3.3.3 Fruit characteristics

- Net weight%; was calculated according to the following equation:

$$\frac{\text{Fruit weight} - \text{Placenta weight}}{\text{Fruit weight}} \times 100$$

- Flesh thickness%; was determined as a ratio between flesh thickness and fruit diameter
- Placenta hardness; was rating from 1to10, 1 denoted the juicy placenta tissues and 10 is the hard placenta.
- Netting degree: was rating from 1 to 10, 1 denoted the extreme smooth fruit skin and 10 the heavily rough fruit.
- Fruit shape index; was determined by dividing fruit length by fruit diameter according to Winger and ludwing (1974).

3.3.4 T.S.S % , moisture content % and chemical analysis

- Total soluble solids (TSS) %; determined using the Zeiss hand refractometer.
- Fruit moisture content; was determined by weight out 100 gm of fruit flesh then chopped and dried at 70° C for 5 days until constant weight.
- Chemical analysis:
  1- Total carotenoids (mg/100gm fresh weight), was determined as β-carotene using the method which described by Nakdiman and Gabelman (1971), using A Milton Roy, Spectrophotometer 601 at 40 nm, using the following equation:

$$\beta\text{-carotene (mg /100 gm fresh weight)} = \frac{K \times R \times 50 \times 100 \times 100}{\text{Sample weight} \times 100}$$

Where;

K= The extension (0.0754583).

R= Spectrophotometer reading for sample.

2- Vitamin C content (mg/100 ml juice): was determined by titration the 2,6 dichlorophenol,endophenol on fruit juice which described by Jacobs (1951). Using the following equation.

$$\text{VC mg/100 ml juice} = \frac{(B-X)\times 2\times 100}{Y\times 5}$$

Where;

B = Blank

X = The size of the distillation.

Y = Pigment determination

3- Total sugars(gm/100 ml juice) : was determined using the phenol, sulphoric acid method according to the method of Dubios *et al.* (1972), using the following equation.

$$\text{Total sugars mg/100ml juice} = \frac{R\times 150\times 100\times 100}{K\times 1\times 1\times 1000\times 1000}$$

Where;

R = Spectrophotometer reading.

K = The extension (7.13).

3.4 Sensory evaluation (panel Taste)

Healthy fruit were taken from each genotypes (S0, S1, S2 and check cultivar "Shahd El-Doki") and parents, hybrids, reciprocals and control type "Ananas monanasa" to evaluate the exterior properties (fruit skin color, fruit skin texture, fruit odor , fruit firmness and general acceptable) and interior prosperities (flesh color, flesh sweetness, flesh flavor, flesh texture and flesh odor). Sensory evaluation was assessed by the panel taste, using Hedonic Scale as proposed by Ranganna (1977). Taste panel was scaled from 1 to 10 (1,2 poor; 3,4 fair; 5,6 moderate; 7,8 good; 9,10 very good). The experimental design was Randomized complete design with 20 samples, as well as analysis of variance according to Stell and Torrie (1980). Sensory evaluation was done on the average of two seasons.

3.5 Statistical analysis

All the collected data were statistically analyzed according the following:

3.5.1 Selection indices

Classical selection index was performed according to Smith (1936) and as illustrated by Singh and Chaudhary (1985) Smith described the method as follow:

1- The first function

$$H \text{ (Genetic worth)} = a_1G_1 + a_2G_2 + \ldots\ldots\ldots a_nG_n$$

Where,

G_1 $G_2$and G_n = genotypic variation values for analysis of variance and covariance for individual character.

a_1 a_2 and a_n = economic weight for all studied trait.

2 – The second function

I (Phenotypic performance of various characters) = $b_1p_1 + b_2p_2 + \ldots\ldots b_np_n$

Where;

b_1 b_2 and b_n = correlation between H and I, *ie*, r (H, I).

p_1 p_2 and p_n the phenotypic variation values for analysis of variance and covariance.

The maximization of r (H, I) lead to a set of simultaneous equation which upon solving give the desired values of b_n. Considering 5 characters, the simultaneous equation looks like as follows :-

$$b_1 p_{11} + b_2 p_{12} + b_3 p_{13} + b_4 p_{14} + b_5 p_{15} = a_1G_{11} + a_2G_{12} + a_3G_{13} + a_4G_{14} + a_5G_{15}$$
$$b_1 p_{21} + b_2 p_{22} + b_3 p_{23} + b_4 p_{24} + b_5 p_{25} = a_1G_{21} + a_2G_{22} + a_3G_{23} + a_4G_{24} + a_5G_{25}$$
$$b_1 p_{31} + b_2 p_{32} + b_3 p_{33} + b_4 p_{34} + b_5 p_{35} = a_1G_{31} + a_2G_{32} + a_3G_{33} + a_4G_{34} + a_5G_{35}$$
$$b_1 p_{41} + b_2 p_{42} + b_3 p_{43} + b_4 p_{44} + b_5 p_{45} = a_1G_{41} + a_2G_{42} + a_3G_{43} + a_4G_{44} + a_5G_{45}$$
$$b_1 p_{51} + b_2 p_{52} + b_3 p_{53} + b_4 p_{54} + b_5 p_{55} = a_1G_{51} + a_2G_{52} + a_3G_{53} + a_4G_{54} + a_5G_{55}$$

Which in matrix

$$\begin{bmatrix} p_{11} & p_{12} & p_{13} & p_{14} & p_{15} \\ p_{21} & p_{22} & p_{23} & p_{24} & p_{25} \\ p_{31} & p_{32} & p_{33} & p_{34} & p_{35} \\ p_{41} & p_{42} & p_{43} & p_{44} & p_{45} \\ p_{51} & p_{52} & p_{53} & p_{54} & p_{55} \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \\ b_4 \\ b_5 \end{bmatrix} = \begin{bmatrix} G_{11} & G_{12} & G_{13} & G_{14} & G_{15} \\ G_{21} & G_{22} & G_{23} & G_{24} & G_{25} \\ G_{31} & G_{32} & G_{33} & G_{34} & G_{35} \\ G_{41} & G_{42} & G_{43} & G_{44} & G_{45} \\ G_{51} & G_{52} & G_{53} & G_{54} & G_{55} \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \\ a_3 \\ a_4 \\ a_5 \end{bmatrix}$$

The solution of this equation gives the values of b_n by the following manner:

$$B = p^{-1} G.\ a$$

Where;

b = column vector for correlation between H and I

p^{-1} = inverse of phenotypic variance and covariance matrix

G = genotypic variance and covariance matrix and a is the column vector for economic weight.

Then, we can calculation selection index values from this formula.

$$(S.I) = b_n x_{ij}$$

Where;

b_n = the column vector for correlation between r (H, I),

x_{ij} = the matrix which contain the values for several traits for each genotypes.

3.5.2 Analysis of variance (ANOVA)

Analysis of variance for individual character was done on the basis of the mean values as suggested by Snedecor and Cochran (1980). The model of analysis of variance adopted is given below.

*Factorial experiment (First factor = Genotypes, Second factor = Seasons)

S.O.V	DF	MS	EMS
Blocks	(r-1)	MB	
Treatments	(gs-1)	MT	
Genotypes	(g-1	MG(M1)	$\delta^2e + r\delta^2gs + rs\delta^2g$
Seasons	(s-1)	MS(M2)	$\delta^2e + r\delta^2gs + rg\delta^2s$
Genotypes*Seasons	(g-1)(s-1)	M G*S(M3)	$\delta^2e + r\delta^2gs$
Error	(gs-1)(s-1)	ME(M4)	δ^2e
Total	rgs-1		

r = Number of replications, g = Number of genotypes, s = Number of seasons

3.6.3 Estimation of genetic parameters

1- Components of variance

Genotypic and phenotypic variance were computed from ANOVA table based on the expected mean sum of squares as follows:

- Genotypic variance (VG) = (M1-M3) / rs
- Seasons variance (VS) = (M2-M3) / rg
- Interaction variance (VGS) = (M3-M4) / r
- Phenotypic variance (VP) = VS + VG + V(GS) +VE

2- Coefficient of variation

Genotypic and phenotypic of variation were computed according to Burton (1952).

Genotypic coefficient of variance (GCV) = $(\sqrt{VG} / \overline{X}) \times 100$

Phenotypic coefficient of variance (PCV) = $(\sqrt{VP} / \overline{X}) \times 100$

Where;

VG = Genotypic variance.

VP = Phenotypic variance

$\overline{X}$ = General mean of the trait

3- Heritability

Broad sense heritability was estimated as the ratio of genotypic variance to the phenotypic variance and was expressed in percentage (Hanson *et al* 1956).

$$\text{Heritability} = (VG / VP) \times 100$$

Where;

VG = Genotypic variance

VP = Phenotypic variance

4-Genetic advance (GA)

Was computed according to the formula given by Johanson et al (1955).

$$GA = h^2 \times i \times \delta p$$

Where;

h^2 = Broad sense heritability.

i = selection differential 1.76 at 10 selection intensity.

δp = Phenotypic standard deviation.

5-Genetic advance over percent of mean(GAM) for S0, S1 and S2

$$GAM = (GA / \overline{X}) \times 100$$

Where;

GA = Genetic advance

$\overline{X}$ = Mean of population

3.5.4 Coefficient of correlation

Coefficient of correlation between various pairs of characters in 7 entries (six selection generation and Shahd el-Doki cultivar) was worked out to determine the degree of association among the characters as shown by Dospekhove (1984) by the following equation.

$$R = \sum X_1X_2 / \sqrt{(\sum X_1)^2 (\sum X_2)^2}$$

Where;

X1 = Character number 1

X2 = Character number 2

Test of significance of correlation in T test by the following equation:

$$\pm T = r / \sqrt{S^2 r}$$

$$S^2r = [1- (r_{1,2})^2] / n-p$$

Where;

n = Sample number

p = number of variable (traits = 2)

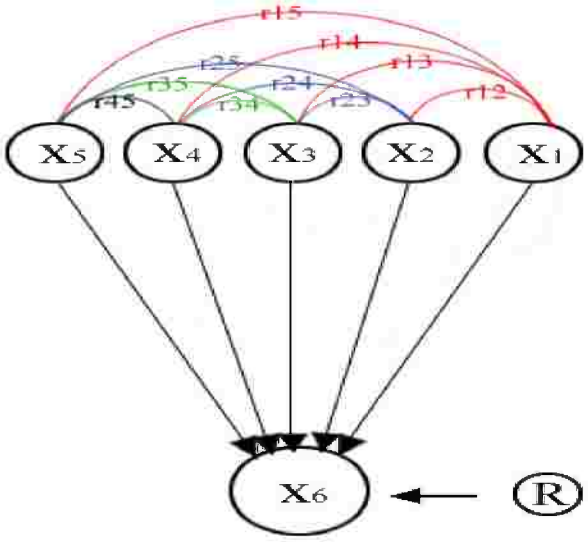
3.5.5 Path coefficient analysis

Path coefficient analysis was calculated as initially proposed by Wright (1921) and illustrated by Dewey and Lu (1959) the direct path coefficient were calculated by solving the formula sets of "p" simultaneous equations by the abbreviated "Doo-Little Technique" as described by Goulden (1959):

$$P_{16} + r_{12} P_{26} + r_{13} P_{36} + r_{14} P_{46} + r_{15} P_{56} = r_{16}$$
$$r_{12} P_{16} + P_{26} + r_{23} P_{36} + r_{24} P_{46} + r_{25} P_{56} = r_{26}$$
$$r_{31} P_{16} + r_{32} P_{26} + P_{36} + r_{34} P_{46} + r_{35} P_{56} = r_{36}$$
$$r_{41} P_{16} + r_{42} P_{26} + r_{43} P_{36} + P_{46} + r_{45} P_{56} = r_{46}$$
$$r_{51} P_{16} + r_{52} P_{26} + r_{53} P_{36} + r_{54} P_{46} + P_{56} = r_{56}$$

Traits which used in this analysis were, 1= Flesh fruit thickness; 2= Moisture content; 3= Netting degree; 4= Placenta hardness; 5= fruit shape index and Net weight (main trait). Also 1= Fruit number; 2= Fruit weight; 3= Plant length; 4= Branches number; 5= Maturity duration and Total yield (main trait).

Plate (3. 1): Path diagram with 5 predictor variables " x_1 " to " x_5" and the response variable x_6. The variable "R" is the reminder portion or residual (1-R) $^{1/2}$.



3.5.6 Heterosis

Heterosis for each cross was calculated according to Bhatt (1971) as follows:

1- Heterosis over mid parents (MP)

The heterosis expressed as percentage increase or decrease in the mean value of hybrids over its parental value:

$$\text{Per cent heterosis over mid parent (MP)} = \frac{\overline{F1} - \overline{MP}}{\overline{MP}} \times 100$$

Where; __

$\overline{F1}$ = Mean of the F1 hybrid

$\overline{MP}$ = Mean of the parents of that particular F1cross

2- Heterosis over better parent (BP)

The heterosis expressed as percentage increase or decrease in the mean of F1 hybrids over its better parent.

$$\text{Per cent heterosis over better parent (BP)} = \frac{\overline{F1} - \overline{BP}}{\overline{BP}} \times 100$$

Where;

$\overline{F1}$ = Mean of the F1 hybrid

$\overline{BP}$ = Mean of the better parent of the particular cross

Significance of the ADH % values was tested using " t " test at error degrees of freedom as shown by Chaudhary *et al.* (1978).

$$\text{Heterosis over mid parent value} = \frac{F1 - MP}{\sqrt{\frac{Me}{r} X \, 3/2}}$$

$$\text{Heterosis over high parent value} = \frac{F1 - HP}{\sqrt{\frac{Me}{r} X 2}}$$

Where;

Me = error variance

r = number of replicates.

3-Potence ratio was calculated by equation as follow which adopted by Peter and Frey (1966):

$$PC = \frac{\overline{F1} - \overline{MP}}{\overline{BP} - \overline{MP}}$$

Where,

$\overline{F1}$ = Mean of the F1 hybrid

$\overline{MP}$ = Mean of the parents of that particular F1 cross

$\overline{BP}$ = Mean of the better parent of that particular cross

3.5.7 Inbreeding depression: was calculated by formula which as follow

$$ID = ((S0 - S2) / S0) \times 100$$

3.5.8 Combining ability

Combining ability analysis for the F1 hybrid was based on the Griffing (1956) using diallel analysis, which was computed on all crosses, reciprocal and their parents (parents + F_1's + reciprocals = full diallel) the analysis described as follow:

Source of variation	DF	SS
GCA	(p-1)	$^1/_{2n} \sum (Yi. + Yi.)^2 - {}^2/_n{}^2 Y^2..$
SCA	(p(p-1)) /2	$^1/_2\sum\sum Yij\,(Yij-Yji) - {}^1/_{2n} \sum(Y.j + Yj.)^2 + {}^1/_n{}^2 Y^2..$
Reciprocals	(p(p-1)) /2	$^1/_2\sum\sum(Yij-Yji)^2$

p = parent number.

1- Estimation of components of variation and their general interpretation .

$$\delta^2 g = \frac{1}{2n}\left[Mg - \frac{Me + n(n-1)Ms}{n^2 - n + 1}\right]$$

$$\delta^2 s = \frac{n^2}{2(n^2-n+1)} \times (Ms - Me')$$

$\delta^2 r = {}^1/_2 (Mr - Me')$

$\delta^2 e = Me'$

$\delta^2 g = {}^1/_2 \delta^2 A$

$\delta^2 s = \delta^2 D$

A/D ratio = $\delta^2 A / \delta^2 D$

2- Estimation of general, specific and reciprocal effects

- General combining ability (gca) effects:
  As $g_1 = {}^1/_{2n} (Y1. + Y.1) - {}^1/_{n2} Y..$
  Similarly, all other values have been calculated

- Specific combining ability (sca) effects:
  As $s_{12} = {}^1/_2 (Y12 + Y21) - {}^1/_{2n} (Y1. + Y.1 + Y2. + Y.2) + {}^1/_{n^2} Y..$
  The sca effects for all other combination have been similarly calculated.

- Reciprocal effects are defined as follows:
  $R12 = {}^1/_2 (Y12 - Y21)$
  Obviously, if Y12 = Y21 there will be no reciprocal effect.

3- Estimation of critical differences (CD) for making comparison between differences effect.

CD for GCA = $\sqrt{}{}^1/_n \delta^2 e \times$ t tabulated at error variance

CD for SCA = $\sqrt{({}^1/_{2n^2} (n^2 - 2p + 2)\delta^2)} \times$ t tabulated

CD for Reciprocals effects = $\sqrt{}{}^1/_{2\delta^2} \times$ t tabulated