CHAPTER III

RESEARCH METHODOLOGY

The experiment was conducted at Suranaree University of Technology Farm, Suranaree University of Technology, Nakhon Ratchasima. The inbred line selection from F_3 seeds of original research (Tira-umphon, 2017). The experiment detail as follow:

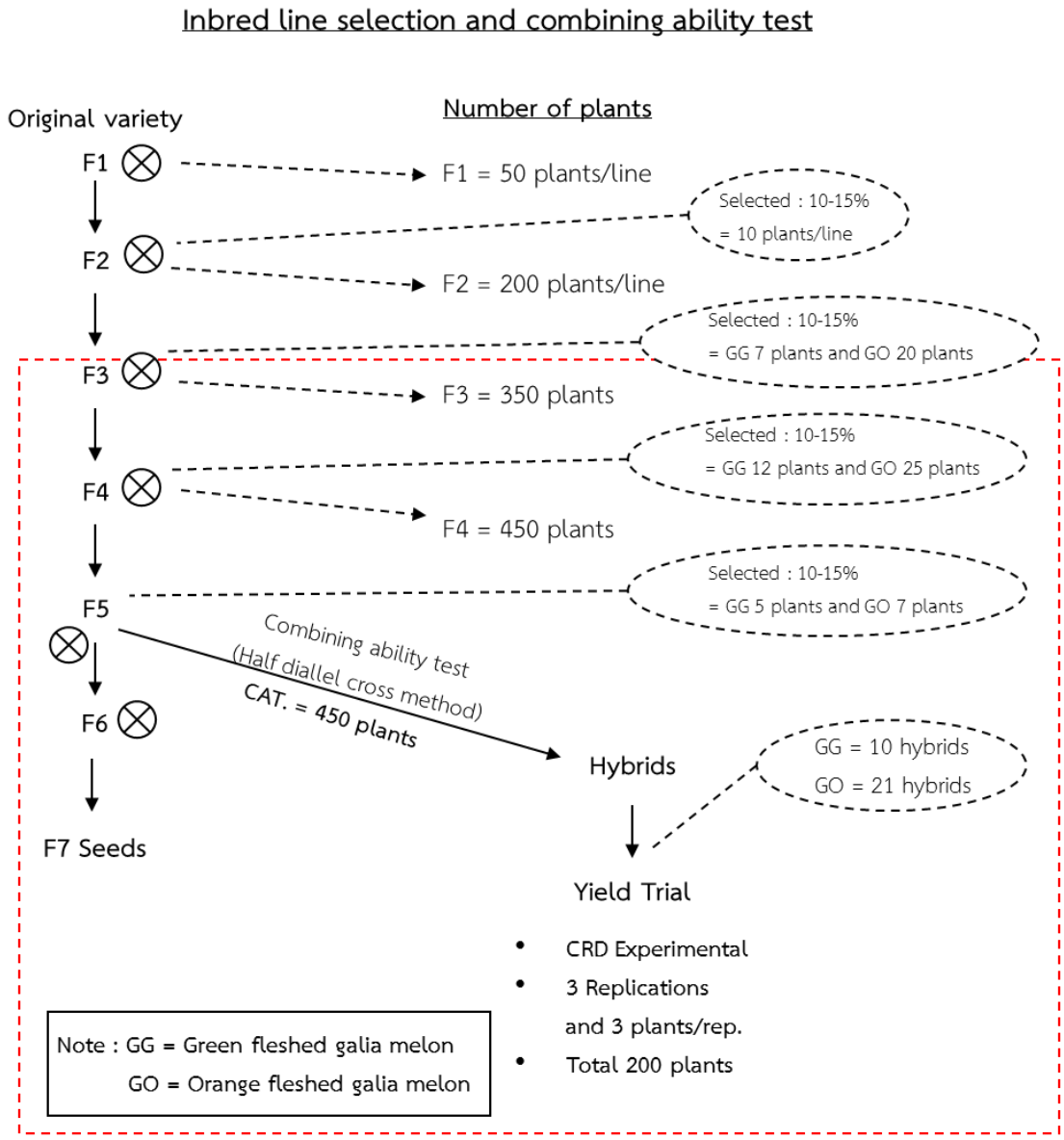

Figure 3.1 The plan of experimental in research.

The original research

F_1 hybrids (Original variety): The original variety from "Rattanasook farm" which was green-fleshed galia melon and orange-fleshed galia melon. The plants were planted in 50 plants per varieties all total 100 plants. Then, self-pollination, and 10-15% of selected were 10 plants per lines which to seeds and continue planting in F_2 plants.

F_2 plants: From the previous selection of 2 lines. The plants were planted in 200 plants per lines all total 400 plants. Then, self-pollination, and 10-15% of selected were 7 plants (lines) in green-fleshed galia melon, and 20 plants (lines) in orange-fleshed galia melon. Which to seeds and continue planting in F_3 plants.

3.1 Thesis experimental

The thesis was experimental all 3 parts as follow:

3.1.1 The inbred line selection from F_3 plants to F_6 plants

F_3 plants: From the previous selection of 2 lines. The plants were planted in 15 plants per line of each lines all total 405 plants. Then, self-pollination, and 10-15% of selected were 12 plants (lines) in green-fleshed galia melon, and 25 plants (lines) in orange-fleshed galia melon. Which to seeds and continue planting in F_4 plants.

F_4 plants: From the previous selection of 2 lines. The plants were planted in 12 plants per line of each lines all total 444 plants. Then, self-pollination, and 10-15% of selected were 5 inbred lines in green-fleshed galia melon, and 7 inbred lines in orange-fleshed galia melon. Which to seeds and continue planting in F_5 plants and combining ability test.

F_5 plants: From the previous selection of 2 lines. The plants were planted in 12 plants per line of each lines all total 144 plants. Then, self-pollinated which to seeds and continue planting in F_6 plants.

F_6 plants: The plants were planted in 9 plants per inbred line with 3 replications and 3 plants per replications, green-fleshed galia melon were planted 45 plants, orange-fleshed galia melon were planted 63 plants, all total 108 plants. Then, self-pollination

which the test plants to find general combining ability value of parent lines and last output were F_7 seeds**.**

3.1.2 The combining ability test

The combining ability test was using half diallel cross method, followed Griffing's Method 2 Model 1 (Griffing, 1956), which seeds of F_5 seeds in selected (Table 3.1). For green-fleshed galia melon gave 10 hybrids, and orange-fleshed galia melon gave 21 hybrids. The experiment plan uses the completely randomized design (CRD). There were 3 replications and 3 plants per replication, all total 279 plants.

Table 3.1 The pedigree of the inbred lines of green-fleshed galia melon (A) and orange-fleshed galia melon (B).

Lines	Pedigree	Code
Green-fleshed galia melon (GG)	GG-01-08-02-05-27	A1
	GG-03-08-02-01-29	A2
	GG-03-08-02-12-13	A3
	GG-03-08-05-08-28	A4
	GG-07-01-09-11-28	A5
Orange-fleshed galia melon (GO)	GO-02-17-11-06-35	B1
	GO-03-23-10-11-35	B2
	GO-03-27-10-08-36	B3
	GO-04-16-06-01-40	B4
	GO-04-18-06-05-37	B6
	GO-04-21-05-06-xx	B7
	GO-05-07-14-01-36	B8

3.1.3 Test planting (yield trial)

The yield trial of hybrids all lines of fruit and yield component characteristics and planted for comparison with parent lines. The hybrids of green-fleshed galia melon, 10 hybrids and 5 parent lines, all total 15 entries (Table 3.2). The hybrids of orange-fleshed galia melon, 21 hybrids and 7 parent lines, all total 28 entries (Table 3.3). The

experiment plan uses the completely randomized design (CRD). There were 3 replications and 3 plants per replication, all total 387 plants.

Table 3.2 The 5 parentals and 10 hybrids of green-fleshed galia melon.

Inbred lines	A1	A2	A3	A4	A5
A1	X	X	X	X	X
A2		X	X	X	X
A3			X	X	X
A4				X	X
A5					X

Table 3.3 The 7 parentals and 21 hybrids of orange-fleshed galia melon.

Inbred lines	B1	B2	B3	B4	B6	B7	B8
B1	X	X	X	X	X	X	X
B2		X	X	X	X	X	X
B3			X	X	X	X	X
B4				X	X	X	X
B6					X	X	X
B7						X	X
B8							X

3.2 Planting methods

The seeding using peat moss in the seed tray. When they are 12 days old of plants move the seedlings into green house and transfer them into 7x14 inch planting bags which have planting materials consisting of SUT planting soil. Place the planting bags in a double zigzag row, making the distance between the plants and between the rows 50x 50 centimeters. Gave chemical fertilizer formulas 16-16-16, 13-13-21 and 0-0-60 and provide water through a drip system twice a day. Make the plants climb up and

pick the 1st to 8th lateral buds out, begin raising from the 9th lateral buds. Before pollination, prepare staminate flowers and pistillate flowers by choosing the flowers 1 day before the flowers bloom, use wire to strap staminate flowers and pistillate flowers close together to prevent crossing from other lines. The 1 week after fruiting, choose 1 fruit per plant, when the fruit grows use a rope to help support the weight of the fruit. Pick treetops out when they have 25 large leaves, trim leaves from the 1st to 4th wood joints. Get rid of pests and diseases according to the epidemic. After that, select good vigor plants according to the need, to inbred line selection up to F_6 plants.

3.3 Harvesting

Harvest the melons, after 45 days of self-pollination, by observing the characteristics of slip of the fruit, fruit appraisal, nets, mesh, volume, and if the melon has a net. For melons that have no polarity of the fruit, by observing if meshes are fully formed around fruits, observing the dark color of the fruit and if the smell of the fruit has increased (Sripongprapai, 2014).

3.4 Data collection

The data recording per plant is as follows:

1. Fruit peel color and fruit pulp color measure by Chromameter CIELAB system, measure peel, every treatment, by measuring the average of 3 points, head of the fruit, middle of the fruit and bottom of the fruit.

2. The net pattern will be scored by the net pattern of the fruits as follows:

    5 = With had net 76-100%

    4 = With had net 51-75%

    3 = With had net 26-50%

    2 = With had net 5-25%

    1 = With don't have net or with had net 0 or < 5%

3. Slip; with slip or without slip.

4. Fruit weigh, record data when harvesting melon, weighing fruits by using scales in kilograms.

5. Fruit width, records data when harvesting products, by vernier caliper in centimeters.

6. Fruit length, records data when harvesting products, by vernier caliper in centimeters.

7. Fruit cavity width, records data when harvesting products, by vernier caliper in centimeters.

8. Fruit cavity length, records data when harvesting products, by vernier caliper in centimeters.

9. Fruit pulp thickness, records data when harvesting products, by vernier caliper in centimeters.

10. Fruit peel thickness, records data when harvesting products, by vernier caliper in centimeters.

11. Sweetness, records data when harvesting products, by squeezing the juice from the melon to measure the Brix value by hand refractometer, measure in percentage units (Cantwell, 2011).

12. Firmness, records data when harvesting products by the fruit firmness meter, units in Newton (N).

13. Percent of pulp, the measure as follow:

$$\% \text{ of pulp} = \frac{2 \times \text{Fruit pulp thickness}}{\text{Fruit width}} \times 100$$

3.5 Selection criteria

3.5.1 Evaluation of peel color and pulp color.

Lines	Fruit pulp color	Type	Selected
Green-fleshed galia melon	White-Green, or White	1	
	Light Green	2	✓
	Dark Green		
Orange-fleshed galia melon	White-Orange, or White	1	
	Light orange	2	✓
	Dark Orange		

***Remark*:** The selection was type 2 was chosen for F_1 hybrid to F_3 hybrid.

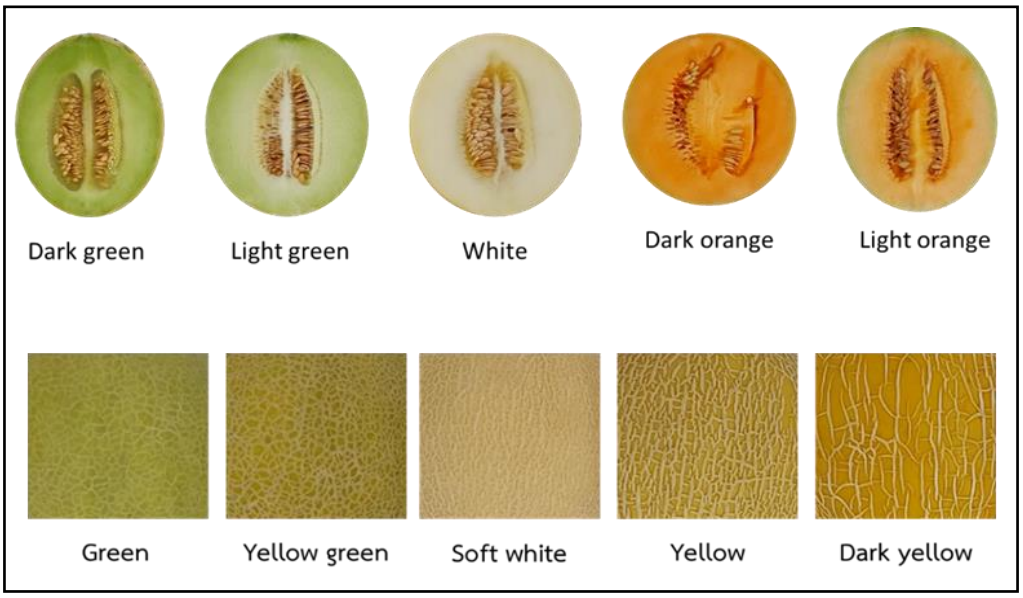

Figure 3.2 The pulp color and peel color of galia melon.

3.5.2 Evaluation of netting density.

Phonotype	Score	Selected
With had net 76-100%	5	✓
With had net 51-75%	4	✓
With had net 26-50%	3	
With had net 5-25%	2	
With don't have net or with had net low than 5-25%	1	

***Remark*:** The netting was at score 2 or more was chosen for F_1 hybrid to F_3 hybrid.

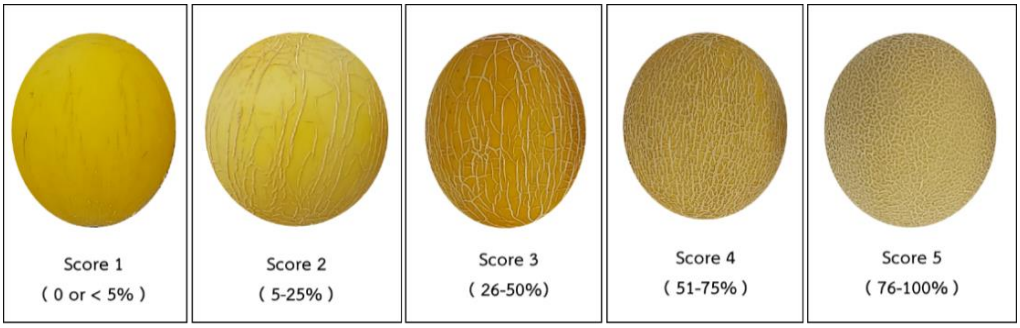

Figure 3.3 Rating level of the nets of galia melon.

3.5.3 Evaluation of slip type.

Phonotype	Type	Selected
With vertical and horizontal for slip	4	✓
With horizontal for slip	3	✓
With vertical for slip	2	✓
Non-slip	1	

Remark: The slip was at Type 2 or more was chosen for F_1 hybrid to F_3 hybrid.

Figure 3.4 The type of slip for galia melon.

5.5.4 Evaluation of fruit weight.

Weight range (kg)	Selected
1.20 – 1.49	✓
0.90 – 1.19	
0.60 – 0.89	
0.30 – 0.59	
0.10 – 0.29	

Remark: Fruit weight was at least 12 kg. was chosen for F_4 hybrid selection.

3.5.5 Evaluation of sweetness.

Sweetness (%Brix)	Selected
12.0 up	✓
10.0 – 11.9	
8.0 – 9.9	
6.0 – 7.9	
4.0 – 5.9	

Remark: The fruit was at least 12%Brix was used for further F_4 hybrid selection.

For the case of the selected does not meet the criteria set forth in F_3 hybrid with the selection criteria from the pulp color, net, and slip. If the selection is not defined will bias to be net primary. For the F_4 hybrid onwards that the case of the selected does not meet the criteria, will bias to be sweetness primary.

3.6 Statistical data analysis

3.6.1. Statistical variability analysis

The variance was analyzed according to the CRD experimental plan, and the mean was compared by Duncan's New Multiple Range Test (DMRT) at the significance level. 0.05 and analyze the relationship between yield composition and yield of melon. using statistical program SPSS for windows version 14.0.

3.6.2 Combining ability analysis

Analysis of variance According to the CRD experimental plan to find differences between the experiments in the way the data is recorded If any differences are found between the experiments Therefore, the performance of the combination of Griffing (1956) Method 2 with mathematical model was analyzed as follows:

$$x_{ij} = \mu + g_i + g_j + s_{ij} + \frac{1}{b}\sum_k e_{ijk}$$

where: $(i = j = 1 \ldots p;\ k = 1 \ldots b)$

where, μ = the population mean.

g_i = the general combining ability effect of the ith parent.

g_j = the general combining ability effect of the jth parent.

s_{ij} = the specific combining ability effect of the cross between i^{th} and j^{th} parents such that $s_{ij} = s_{ji}$ and

e_{ijk} = the environmental effect associated with ij k^{th} observation.

Analysis of variance for method 2 giving expectations of mean squares of model 1 (Griffing, 1956).

source	df	SS	MS	Expectation of Mean Squares
GCA	p-1	SS_g	MS_g	$\sigma^2+(p+2)[\frac{1}{p-1}]\sum_{gi}2$
SCA	p(p-1)/2	SS_s	MS_s	$\sigma^2+\frac{2}{p(p-1)}\sum_i\sum_j sij^2$
Error	m	SS_e	$MS_{e'}$	σ^2

Note: $MS_{e'}$ = Me/rc (where M_e was the error MS of CRD ANOVA, r was the number of iterations and c was the number of plants stored in each iteration).

$SS_g = \frac{1}{p+2}\{\sum(x_{i.}+x_{jj})^2 \simeq \frac{4}{p}x\ldots^2\}$

$SS_s = \sum\sum x_{ij}{}^2 - \frac{1}{p+2}\sum(x_{i.}+x_{jj})^2 + \frac{1}{(p+1)(p+2)}X\ldots^2$

m = df of error from statistical analysis.

p = Total number of parent line used in cross.

$x_{i.}$ = Sum of the mean of all hybrid's pairs obtained by interbreeding of lines i and the rest of the species = $x_{i1} + x_{i2} + x_{i3} +\ldots\ldots+ x_{in}$

$x_{j.}$ = Sum of the mean of all hybrid's pairs obtained from crossing between line j and the rest of the line = $x_{j1} + x_{j2} + x_{j3} +\ldots\ldots+ x_{jn}$

x_{ij} = Mean of hybrids resulting from self-pollinated of line i

x_{jj} = Mean of hybrids resulting from self-pollinated of line j

$x\ldots$ = The sum of the mean of all hybrids pairs obtained by interbreeding i or j and the rest of the lines plus the mean of hybrids resulting from i or j self-pollination.

For testing the differences due to combined ability, do the following:

General combining ability test (GCA) $F_{[(p-1),\,m]} = MS_g/MS_{e'}$

Specific combining ability test (SCA) $F_{[p(p-1)/2,\,m]} = MS_s/MS_{e'}$

For calculating the effect of GCA in each parent or the effect of SCA in each pair, it can be done as follows:

$g_i = \frac{1}{p+2}[x_{i.} + x_{ii} - \frac{2}{p}x\ldots]$

$s_{ij} = x_{ij} - \frac{1}{p+2}[x_i + x_{ij} + x_j + x_{jj}] + \frac{2}{(p+1)(p+2)}x\ldots$

3.6.3 The study heterosis of F_1 hybrid

Measured by comparison with the mean of the parent lines.

$$\text{Heterosis (\%)} = \frac{\overline{F1}-\overline{MP}}{\overline{MP}} \text{ x } 100$$

where, F_1 = mean of the hybrids.

MP = mean of the parent lines.

Testing for significance by comparing the values of t-statistics as follow:

$$t_{(MP)} = \frac{\overline{F1}-\overline{MP}}{\overline{S1}}$$

$$S1 = \sqrt{\frac{(n_{P1}-1)MS_{P1}+(n_{P2}-1)MS_{P2}}{(n_{P1}+n_{P2})[(n_{P1}-1)+(n_{P2}-1)}} + \sqrt{\frac{MS_{F1}}{n_{F1}}}$$

Where:

MS_{P1} = the mean square of the parent.

MS_{P2} = the mean square of the parent.

MS_{F1} = the mean square of the F_1 hybrids.

n = the number of trees in that generation.

Measured by comparison with the mean of the better parent lines.

$$\text{Heterobeltiosis (\%)} = \frac{\overline{F1}-\overline{HP}}{\overline{HP}} \text{ x } 100$$

where, F_1 = mean of the hybrids.

HP = mean of the better parent lines.

Testing for significance by comparing the values of t-statistics as follows:

$$t_{(HP)} = \frac{\overline{F1}-\overline{HP}}{\overline{S2}}$$

$$S2 = \sqrt{\frac{MS_{F1}}{n_{F1}}} + \sqrt{\frac{MS_{HP}}{n_{HP}}}$$

Where:

MS_{F1} = the mean square of the F_1 hybrids.

MS_{HP} = the mean square of the better parent

n = the number of plants in that generation.

3.6.4 Correlation Coefficient Analysis

Use data from the F_5 hybrids to analyze phenotypic correlation, to study phenotypic correlation according to the method provided by Briggs and Knowles (1967) as follow:

$$r = \sqrt{\frac{\sum XiYi - \frac{(\sum Xi)(\sum Yi)}{n}}{\left[\sum X_i^2 - \frac{(\sum X_i^2)}{n}\right]\left[\left[\sum Y_i^2 - \frac{(\sum Y_i^2)}{n}\right]\right.}}$$

Where: X_i = the observed value X at i

Y_i = the observed value Y at i

when i = 1, 2, 3,.. n (n = Amount of the observed value)