

CHAPTER 3

MATERIALS AND METHODS

Half-diallel mating design was manipulated in four azuki bean varieties, Kamuidainagon (K), Hondawase (H), Akatsukidainagon (A) and Erimo (E) to develop all possible six F1 hybrid crosses. These six F1 crosses are Kamuidainagon x Hondawase (K x H), Kamuidainagon x Akatsukidainagon (K x A), Kamuidainagon x Erimo (K x E), Hondawase x Akatsukidainagon (H x A), Hondawase x Erimo (H x E) and Akatsukidainagon x Erimo (A x E). These parental lines of crosses were selected primarily on the basis of their diversities for seed yield and yield component properties. Basic six populations; P1, P2, F1, F2, BC1 and BC2 were developed for studying. These six populations of each cross were evaluated in a randomized complete block design with four replicates at three parallel locations which are different in altitudes, namely, Inthanon Royal Project Research Station (1,300 m above sea level, ASL), Khunpae Royal Project Development Center (1,200 m ASL) and Pangda Royal Agricultural Station (700 m ASL). The crosses, each consisting of six populations, were randomly assigned to the blocks. All six populations were then randomly assigned to six plots within a block. The plots of various populations had different number of rows, i.e., each F1 and backcross generation consisted of one row, while each parent had two rows and F2 had three rows. Each row was 2.0 m with a row-to-row distance of 50 cm. One seed was planted in hill with space 20 cm apart. The data were recorded on the seed yield and yield components of each individual plant in all six populations.

The experiment was conducted for two consecutive growing seasons in 2005-2006 on the highland areas in Chiang Mai province, Thailand. The growing period was in late rainy season, during August to November.

The data obtained were analyzed in the following topics:-

1) Number of gene control. The number of gene control was calculated by Jacobs and Broers (1989) formula.

$$K = (1/2 d^2 + 1/2 h^2) / V_{F_2}$$

Where, K is the number of independently-segregating effective factors (genes)

$$d = 1/2\bar{P}_1 + 1/2\bar{P}_2$$

$$h = 6\bar{BC}_1 + 6\bar{BC}_2 - 8\bar{F}_2 - \bar{F}_1 - 1.5\bar{P}_1 - 1.5\bar{P}_2$$

\bar{P}_1 is the mean observation of the female parent

\bar{P}_2 is the mean observation of the male parent

\bar{F}_1 is the mean observation of the F1 hybrid

\bar{F}_2 is the mean observation of the F2 generation

\bar{BC}_1 is the mean observation of the backcross to female parent

\bar{BC}_2 is the mean observation of the backcross to male parent

V_{F_2} is the variance of F2 generation

By using this equation, the underlying assumptions of the equation which were given by Wright (1968) are (1) no systematic relation between mean and variance, (2) no linkage of genes, (3) no epistasis, (4) the relevant genes are of equal effect, (5) one parent supplies only plus alleles of those genes in which the two parents differ, whereas the other parent supplies only minus alleles, and (6) an equal degree of dominance for all plus alleles.

2) Combining ability analysis. The data of parents and their six F1 hybrid crosses of each trait were subjected to the Model I Method 2 proposed by Griffing (1956). DIALLEL-SAS program developed by Zhang and Kang (2003) was used for analyzing diallel-cross data to obtain both general and specific combining ability.

3) Generation mean analysis. The data of six basic generations; P1, P2, F1, F2, BC1 and BC2 of each cross were used to calculate means and variances for each generation and environment by using the standard statistical method. These variances were used to compute the standard error for each generation mean and environment. The joint-scaling test and additive-dominance model were used to estimate the generation means as

proposed by Mather and Jinks (1982). Six genetic parameters (m, d, h, i, j and l) were estimated by non-allelic interaction model, according to Hayman (1958). By using this model, assuming the inheritance of the trait under study must present non-allelic interaction of genes. Matrix algebra was used for calculating each model.

The joint-scaling test identifies the type of gene action (additive vs. non-additive gene action). The joint-scaling test is classified as follows.

$$\begin{aligned} A &= 2\overline{BC}_1 - \overline{P}_1 - \overline{F}_1 & V_A &= 4V_{\overline{BC}_1} + V_{\overline{P}_1} + V_{\overline{F}_1} \\ B &= 2\overline{BC}_2 - \overline{P}_2 - \overline{F}_1 & \text{and } V_B &= 4V_{\overline{BC}_2} + V_{\overline{P}_2} + V_{\overline{F}_1} \\ C &= 4\overline{F}_2 - 2\overline{F}_1 - \overline{P}_1 - \overline{P}_2 & V_C &= 16V_{\overline{F}_2} + 4V_{\overline{F}_1} + V_{\overline{P}_1} + V_{\overline{P}_2} \end{aligned}$$

Where,

\overline{P}_1 is the mean observation of the female parent

\overline{P}_2 is the mean observation of the male parent

\overline{F}_1 is the mean observation of the F₁ hybrid

\overline{F}_2 is the mean observation of the F₂ generation

\overline{BC}_1 is the mean observation of the backcross to female parent

\overline{BC}_2 is the mean observation of the backcross to male parent

$V_{\overline{P}_1}$ is the variance of female parent

$V_{\overline{P}_2}$ is the variance of male parent

$V_{\overline{F}_1}$ is the variance of F₁ hybrid

$V_{\overline{F}_2}$ is the variance of F₂

$V_{\overline{BC}_1}$ is the variance of BC₁

$V_{\overline{BC}_2}$ is the variance of BC₂

For testing of significance; if all values of A, B and C are not significantly different from zero by *t*-test, it means that genetic effect is either additive or dominance gene action. In this case, analysis of genetic effect can be analyzed by using additive and dominance model. If any value of A, B and C is significantly different from zero, non-additive dominance model will be used.

(A) Additive-dominance model, joint-scaling test suggested that A, B and C were not significantly different from zero, calculated by weight least square method (Mather and Jinks, 1982).

$$M = J^{-1} \times S$$

$$J = B_1' \times B$$

$$S = B_1' \times C$$

$$V = D \times V_1$$

$$SE = \sqrt{V}$$

Where, M is the matrix of the parameters

J is the information matrix

B is the matrix of parameter coefficients

B_1 is the matrix of weight

S is the matrix of score

C is the matrix of six generation means

V is the matrix of parameter variances

V_1 is the matrix of six generation variances

D is the matrix of squared elements of the matrix [$J^{-1} \times B_1'$]

SE is the standard error of parameters

(B) Non-allelic interaction model, six parameters, approach by Hayman (1958).

$$M = B^{-1} \times C$$

$$V = E \times V_1$$

$$SE = \sqrt{V}$$

Where, M is the matrix of the parameters

B is the matrix of parameter coefficients

C is the matrix of six generation means

V is the matrix of parameter variances

V_1 is the matrix of six generation variances

E is the matrix of squared elements of the matrix [B^{-1}]

SE is the standard error of parameters

(C) **Non-allelic interaction model**, omitting parameter(s) were not significantly different from zero, calculated by least square method (Hayman, 1958).

$$M = (B' \times B)^{-1} \times (B' \times C)$$

$$V = F \times V_1$$

$$SE = \sqrt{V}$$

Where, M is the matrix of the parameters

B is the matrix of parameter coefficients

C is the matrix of six generation means

V is the matrix of parameter variances

V₁ is the matrix of six generation variances

F is the matrix of squared elements of the matrix $[(B' \times B)^{-1} \times (B')]$

SE is the standard error of parameters

For all model, testing for significance of each parameters from zero by *t*-test.

4) Heterosis (H) and heterobeltiosis (Hb). Both heterosis (H) and heterobeltiosis (Hb) were calculated as percent increase or decrease over mid- and better-parent values, respectively. Significant level of H and Hb were determined by *t*-test as described by Xin *et al.* (2003).

$$\% \text{ Heterosis (H)} = (\bar{F}_1 - \bar{MP}) \times 100 / \bar{MP}$$

$$\% \text{ Heterobeltiosis (Hb)} = (\bar{F}_1 - \bar{P}_i) \times 100 / \bar{P}_i \quad (i = 1 \text{ or } 2)$$

where, F₁ = mean observation of the F₁ hybrid taken from n₃ plants

\bar{MP} = mean observation of both parents taken from n₁ + n₂ plants

\bar{P}_i = mean observation of one parent to the cross taken from the total of n₁

plants for P₁, or n₂ plants for P₂

Null hypothesis; Ho : H = Hb = 0. Significances of H and Hb were determined by *t*-test as follow:

$$H = \frac{\bar{F1} - \bar{MP}}{SH} \quad ; \quad SH = \sqrt{\text{variance } H}$$

$$df = (n_1 - 1) + (n_2 - 1) + (n_3 - 1)$$

$$\text{variance } H = \frac{SSF1}{n_3(n_3-1)} + \frac{SSP1}{4n_1(n_1-1)} + \frac{SSP2}{4n_2(n_2-1)}$$

$$Hb = \frac{\bar{F1} - \bar{P}_i}{SHb} \quad ; \quad SHb = \sqrt{\text{variance } Hb}$$

$$df = (n_3 - 1) + (n_i - 1)$$

$$\text{variance } Hb = \frac{SSF1}{n_3(n_3-1)} + \frac{SSP_i}{n_i(n_i-1)}$$

Where, $i = 1$ or 2 (compared with female or male parent, respectively)

SH = standard error of the estimate of H

SHbi = standard error of the estimate of Hb

SSF1 = sum of square of F1 hybrid

SSP1 = sum of square of female parent

SSP2 = sum of square of male parent

n_1 = number of individual plant of female parent

n_2 = number of individual plant of male parent

n_3 = number of individual plant of F1 hybrid

5) Generation variance analysis. Generation variance analysis is evaluated by using Kearsey and Pooni (1996) method which was adapted from the matrix algebra. Calculation is indicated as follows;

Kearsey and Pooni (1996) formula, $V_A^* = 2V_{F2} - V_{BC1} - V_{BC2}$

$$V_D^* = V_{BC1} + V_{BC2} - V_{F2} - V_E$$

$$V_{AD} = 1/2 (V_{BC2} - V_{BC1})$$

$$V_E = 1/3 (V_{P1} + V_{P2} + V_{F1})$$

Where, V_A^* is the additive variance
 V_D^* is the dominance variance
 V_{AD} is the covariance
 V_E is the environmental variance
 V_{P1} is the variance of female parent
 V_{P2} is the variance of male parent
 V_{F1} is the variance of F1 hybrid
 V_{F2} is the variance of F2
 V_{BC1} is the variance of BC1
 V_{BC2} is the variance of BC2

Matrix algebra method,

$$C = (A' \times A)^{-1} \times (A' \times B)$$

Where,

A is the matrix of parameter coefficients

B is the matrix of six generation variances

C is the matrix of the parameter of variances

6) Heritability estimation. Two types of heritability were estimated; broad-sense heritability, h^2_b and narrow-sense heritability, h^2_n . Each type was calculated by generation variance analysis of Kearsy and Pooni (1996) method.

$$\text{Broad-sense heritability } (h^2_b) = (V_A^* + V_D^*) / (V_A^* + V_D^* + V_E)$$

$$\text{Narrow-sense heritability } (h^2_n) = (V_A^*) / (V_A^* + V_D^* + V_E)$$

Where, V_A^* is the additive variance
 V_D^* is the dominance variance
 V_E is the environmental variance

7) Genetic advance from selection (genetic gain). This genetic gain was calculated by Falconer (1989) formula:

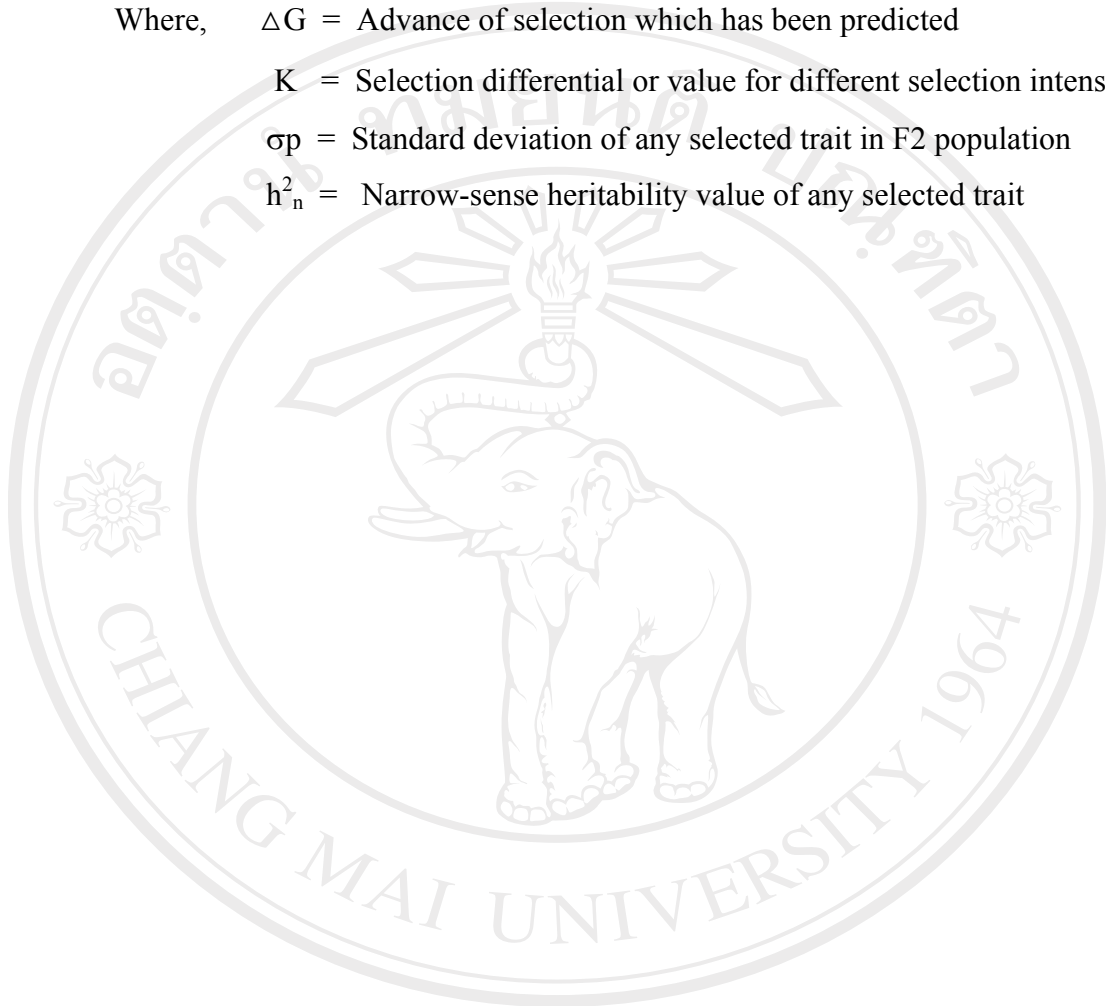
$$\Delta G = K\sigma_p h_n^2$$

Where, ΔG = Advance of selection which has been predicted

K = Selection differential or value for different selection intensity

σ_p = Standard deviation of any selected trait in F2 population

h_n^2 = Narrow-sense heritability value of any selected trait



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