Segregation ratio and heritability of agronomic character in yardlong bean F2 population

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Abstract. Yardlongbean is very popular vegetable in Indonesia. Genetic variability can be induced by crossing two parental. The segregation ratio in Fn generations specify whether the progeny have attained homozygosity or still in process of segregation. Heritability is the genetic variability degree which may be inherited to the progeny. The aim of the research was to determine segregation ratio and heritability of agronomic character from crossing of red immature pod and whitish green immature pod of yardlong bean. F2 population and their parental were planted at Agrotechnopark, located in Tembalang – Semarang, Central Java, Indonesia. Data were analyzed using Chi square test for suitability normal distribution. Broad sense heritability was also calculated. The results showed that frequency of character plant height, number of leaf, pod length and sweet taste follow a normal distribution, while the frequency distribution of pod weight did not follow normal distribution. Segregation ratio of pod weight was 12 : 3 : 1. Number of leaf and pod length had low broad sense heritability.

1. Introduction

One of the most popular legume crop in term of planting area is cowpea [1]. There are two major group of cowpea according to growing area, different uses and evolution history [2]. They are yardlong bean [Vigna unguiculata (L.) Walp. ssp. unguiculata cv.-gr. sesquipedalis and cowpea [Vigna unguiculata (L.) Walp. ssp. unguiculata cv.-gr. unguiculata]. The characteristic of yardlong bean is long (30 – 90 cm) and succulent pods, whereas cowpea has shorter pods (20 – 30 cm in length). Yardlong bean are consumed as vegetable in both cooked forms and fresh vegetable for its young, crisp, tender and fresh pods. Yardlong bean provides vitamin, proteins, minerals and fibers [1]. It can be cultivated yearround and the tender pods can be harvested two to four times. The harvest area of yardlong bean in Indonesia at 2015 was 63.172 ha with pod yield was 6.93 ton/ha [3]. In Thailand, marketable pods of seven yardlong bean varietas varies from 11 – 15 ton/ha [4].

In plant breeding program, scientists should study segregation ratio, gene action, heritability and genetic variability of the crop to arrange effisien selection strategies. The segregation ratio in Fn generations specify whether the progeny have attained homozygosity or still in process of segregation. Agronomic characters are classified into qualitative character and quantitative character. One or two
genes controlled qualitative characters, whereas many genes controlled quantitative characters. Quantitative character do not follow the segregating pattern or Mendelian ratio modification [5]. The segregation ratio of pod dehiscence was 9 : 7. This is indicating that pod dehiscence character in yardlong bean was governed by duplicated recessive genes. Seed coat color segregated fitted to 3 : 13 and also 1 : 3. Black seed coat colour is dominant over brown. These indicated that seed coat color may be controlled by dominant and recessive epistasis or by a single gene. The gene mapping supported that seed coat colour was governed by single gene [6]. Seed coat and flower colour are interesting traits in yardlong bean. F1 and F7:8 generation analysis of recombinant inbred lines (RILs) population indicated a monogenic inheritance of seed coat and flower colour. Brown seed coat is dominant over cream seedcoat, where as purple flower is dominant over white colour [7]. Seed coat and flower pigmentation is governed by the same locus. Colouring mechanism of both traits is dependent on the parental lines used [8].

The quantitative characters and genetic inheritance of yardlong bean have been extensively investigated included pod yield. Heritability is the genetic variability degree which may be inherited to the progeny. Broad sense and narrow sense heritability of pod weight were 84 % and 75 %, respectively [9]. Immature pod yield per plant has low heritability (4.03 – 25.30 %) [10]. Pod length, pod number per plant and pod yield per plant had rather high narrow sense heritability [11]. Narrow sense heritability was low for pod yield, pod weight, number of pod per plant and number of inflorescence per plant. Whereas, pod diameter and pod length had moderate narrow sense heritability [12]. High estimates of broad sense heritability were observed for pod length, pod girth, plant height, pod yields per plant and seed number per pod [13].

Immature pod colour of yardlong bean varies such green, whitish green red and purple. Agronomic character of those varietis was expectedly also differ. The aim of the research was to determine segregation ratio and heritability of agronomic character from crossing of red immature pod and whitish green immature pod of yardlong bean.

2. Materials and Methods
Research was conducted at Agrotechnopark, Tembalang, Semarang – Indonesia that located at 7° 2’ 42” - 7° 3’ 27” south latitude and 110° 25’ 55” - 110° 26’ 55” east longitude, 125 m above sea level and latosol soil type. Plant material was F2 population that selected from F1 crossing between red and whitish green immature pod. There were 12 plots for F2 progenies and 2 plots for each parent (red immature pod and whitish green immature pod). Each plot consist of 9 plants. Parameter measured were plant height, number of leaf, pod length, pod weight and sweet taste. Parameter were measured from all plant.

The data were analyzed using normal distribution suitability and chi square test to check the suitability between observed and expected value. The calculation stages was :
1. Observational data was stated in the frequency tabel
2. From the frequency table; average ( \( \bar{X} \) ), varians (\( s^2 \)) and standar deviation (\( s \)) were calculated using the following formula
\[
\bar{X} = \frac{\sum (f) (x_i)}{\sum f} \\
\sigma^2 = \frac{1}{\sum (f) (x_i)^2 - \left( \frac{\sum (f) (x_i)}{\sum f} \right)^2} \\
s = \sqrt{s^2}
\]
3. In each class, the standard Z value was calculated, one for the lowest limit and the other for the highest limit.

\[ Z_i = \frac{L_i - \overline{x}}{s} \]

\[ Z_h = \frac{L_h - \overline{x}}{s} \]

4. The probability for each class were determined based on the probability distribution hypothesis as follows: \[ P = P(Z_i < X < Z_h) \]

5. The expected frequency for i class was calculated using this formula

\[ F_i = (n) \left( P_i \right) \]

6. \( X^2 \) was calculated [14].

\[ X^2 = \sum_{i=1}^{k} \frac{(f_i - F_i)^2}{F_i} \]

Null hypothesis assumed that the suitability test for the normal distribution of agronomic characters from crossing between red and whitish green immature pod is normally distributed. Null hypothesis is accepted when \( X^2 \) calculated < \( X^2 \) table.

The frequency distribution for characters with a normal distribution is evaluated by curtosis test to determine whether the curve is symmetrical or not. The curve of distribution of characters is said to be normal if the curtosis deviation (SK) is between -3 and 3.

\[ SK = \frac{3(\overline{x} - \overline{X})}{s} \]

To estimate a simple control gene. The F2 population will be matched against several ratios depending on the shape of the graph obtained [15]. If the F2 population distribution graph show:

a. Two peaks, the ratio possibility is 3 : 1 or 9 : 7 or 13 : 3 or 15 : 1
b. Three peaks, the ratio possibility is 1 : 2 : 1 or 9 : 3 : 4 or 9 : 6 : 1 or 12 : 3 : 1
c. More than three peaks, the ratio possibility is 9 : 3 : 3 : 1 or 6 : 3 : 3 : 4
d. One peak and normal distribution, the character controlled by many genes.

Then, the ratio possibility was analyzed using the chi square test. Null hypothesis accepted when \( X^2 \) calculated < \( X^2 \) table.

\[ X^2 = \sum (\text{observed} - \text{expected})^2 / \text{expected} \]

Heritability was calculated based on following the formula [16]:

\[ H^2_{(bs)} = \frac{\sigma^2_{F2}}{\sigma^2_{P2}} \sqrt{\frac{(\sigma^2_{P1})(\sigma^2_{P2})}{\sigma^2_{P1} + \sigma^2_{P2}}} \times 100\% \]

where \( H^2_{(bs)} \) = broad sense heritability, \( \sigma^2_{F2} \) = variance of F2, \( \sigma^2_{P1} \) = variance of female parental, \( \sigma^2_{P2} \) = variance of male parental

3. Results and Discussion

3.1. Segregation ratio

The results of normal distribution analysis of F2 population from red and whitish green immature pod hybridization showed that plant height, number of leaf, pod length and taste of yardlong bean had normally distribution. Character did not follow the normal distribution was pod weight (Table 1).
Table 1. Chi square test for agronomic character distribution of F2 population from red and whitish green immature pod hybridization

| No. | Agronomic character | $X^2_{\text{calculated}}$ | $X^2_{\text{table}}$ | Distribution |
|-----|---------------------|-----------------|-----------------|--------------|
| 1   | Plant height        | $5.447^{\text{ns}}$ | 9.21            | Normal       |
| 2   | Number of leaf      | $4.450^{\text{ns}}$ |                 | Normal       |
| 3   | Pod length          | $2.327^{\text{ns}}$ |                 | Normal       |
| 4   | Pod weight          | $14.536^{*}$     |                 | Non-normal   |
| 5   | Sweetness           | $4.239^{\text{ns}}$ |                 | Normal       |

Note: ns : not significant at $p = 0.01$ ; * : significant at $p = 0.01$

Character that normally distributed at F2 population indicated that those character was controlled by many genes and as a quantitative character [17]. Plant height, number of leaf, pod length and sweetness of hybridization red and whitish green immature pod in yardlong bean were classified as quantitative traits that were controlled by many genes (Figure 1). Each gene in a quantitative character has a small contribution to the inheritance of a character so that the individual effects can not be detected by Mendelian genetics[18]. The expression of this quantitative character is significantly influenced by the environment. The character segregation pattern does not follow Mendelian ratio or its modification [5]. Quantitative character of pod length in this research in accordance to other research. Pod length of cowpea (Vigna unguiculata (L.) Walp) was classified as quantitative traits [19].

![Figure 1. Frequency distribution in F2 population of agronomic character](image-url)
Tabel 2. A Segregation pattern of pod weight in F2 population based on Chi Square Test

| Character ratio | Observed (O) | Expected (E) | X²calculated | X²table |
|-----------------|--------------|--------------|--------------|---------|
| Two classes     |              |              |              |         |
| 3:1             | 56:7         | 47.3:15.8    | 6.9*         |         |
| 9:7             | 56:7         | 35.4:27.6    | 27.5*        | 6.63    |
| 13:3            | 56:7         | 51.2:11.8    | 2.8ns        |         |
| 15:1            | 56:7         | 59.1:3.9     | 1.9ns        |         |
| Three classes   |              |              |              |         |
| 1:2:1           | 49:12:2      | 15.8:31.5:15.8 | 94,270*   |         |
| 9:3:4           | 49:12:2      | 35.4:11.8:15.8 | 17,195*   | 9.21    |
| 9:6:1           | 49:12:2      | 35.4:23.6:3.9 | 11,867*   |         |
| 12:3:1          | 49:12:2      | 47.3:11.8:3.9 | 1,023ns    |         |
| Four classes    |              |              |              |         |
| 9:3:3:1         | 42:14:5:2    | 35.4:11.8:11.8:3.9 | 6,502ns  |         |
| 6:3:3:4         | 42:14:5:2    | 23.6:11.8:11.8:15.8 | 30,618*  | 11.35   |

Note: ns : not significant at p = 0.01 ;  * : significant at p = 0.01

Selection based on plant height, number of leaf, pod length and sweetness at F2 population can not be done at earlier generation. Selection based on those character should be delayed to later generation. This is because those agronomic characters were controlled by many genes that contribute additively. The role of each gene was small on the phenotype, whereas the environment had great influence on the phenotype of those agronomic characters. Selection based on pod weight also can be done at earlier generation because of epistasis gene action. Pod weight character was not inherited directly from parents to offspring.

3.2. Heritability
Heritability is the genetic variability degree which may be inherited to the progeny. Estimates of broad sense heritability obtained in F2 population of two yardlong bean crosses were shown in Table 2. Heritability estimates of three agronomic character ranged from 0.2 % to 62.2 %. Sweetness had the highest heritability estimate of 62.2 followed by pod length of 18.3 %. This indicates that inheritance of sweetness immature pod in the next generation (F3) are high, and selection to obtain yardlong bean genotypes with sweetness immature pod in F3 is relatively easy.

Table 3. Estimates of broad sense heritability of agronomic character in F2 population

| Agronomic character | Heritability (%) | Criteria |
|---------------------|-----------------|----------|
| Number of leaf      | 0.2             | Low      |
| Pod length          | 18.3            | Low      |
| Sweetness           | 62.2            | High     |

Broad sense heritability of pod length in this research was low. Other research reported heritability of pod length was high (91.76 %) [22]. Heritability of pod length in green yardlongbean was 98.21. Green yardlong bean were from sixty local genotypes and commercial varieties in Kerala and Tamil Nadu [23]. The difference estimate of broad sense heritability because of the difference of yardlong bean varieties and the population.
4. Conclusion
Frequency of character plant height, number of leaf, pod length and sweet taste follow a normal distribution, while the frequency distribution of pod weight did not follow normal distribution. Segregation ratio of pod weight was 12 : 3 : 1. Number of leaf and pod length had low broad sense heritability.

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