Vegetable Soybean Line Development for Black Seed Coat Color Trait in F_2, BC_1F_2 and BC_2F_1 Generations

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ABSTRACT

This study was aimed to develop vegetable soybean lines in vegetable soybean breeding program for black seed coat color by crossing among a 'Black Seed' variety (black seed coat color) with two aromatic vegetable soybeans, 'Kaori' and 'Chiang Mai 84-2', in dry season 2013 at Chiang Mai Field Crops Research Center, Chiang Mai Province, Thailand. The F_2, BC_1F_2 and BC_2F_1 populations were grown together with their parents. Some agronomic and yield component traits related to black seed coat color were recorded in parents F_2, BC_1F_2 and BC_2F_1 of the two crosses. The results of generation mean analysis (GMA) in F_2, BC_1F_2 and BC_2F_1 showed multiple gene controlling, with additive and non-additive effects, in seed coat color, number of pod bearing branches, number of 2-seed pods, number of pods per plant, 100-dry seed weight and seed weight per plant. The two parents, 'Chiang Mai 84-2' and 'Kaori' had different genetic control in these traits but both could be used in breeding program between black seed coat and aromatic varieties.

Key words: black seed coat, backcross, vegetable soybean, generation mean analysis, segregating population, polygene, gene effect

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INTRODUCTION

Vegetable soybean was grown differently from dry seed type soybean and harvested at R_6 stage , in which the pod containing a green seed that filled the pod capacity at one of the four uppermost nodes on the main stem (Fehr *et al.,* 1971). The standard pod of vegetable soybean for export market should be dark green, is at full seed pod stage (R_6), with gray or light brown pubescence on the pod, no bruises, no marks of pest infestation in pod with 2-3 seeds per pod, pod length not less than 4.5 cm., not less than 1.5 cm wide. and not less than 0.8 cm thick. (Department of Agriculture, 2007). Vegetable soybean seeds are considered as food crops with high nutritional value (Masuda,1991). Anthocyanin is one group of phenolic compounds as flavonoid pigments which are found in all parts of plants including flowers and fruits (Matin, 2017). It could reduce cancer, help heal wounds, strengthen immunity and lower the risk of cardiovascular disease (Wallace, 2011). Anthocyanin colors found in plants were purple to black, such as those found in blueberries, black berry, cherry, purple grape, purple corn and also in seed coat of black soybean (Choung *et al*., 2001; Park *et al*., 2011). The phenolic compounds were also reported as antioxidant (Zhang *et al.,* 2011; Zou and Chang, 2011). Traditionally, black soybean seed was used in Chinese medicine for both treatment of some ailments and health maintenance (Huang *et al*., 2011). Todd and Vodkin (1993) found that anthocyanins accumulated in epidermis palisade layer of seed. Different anthocyanins contents in the seed coat of black soybean variety were reported to be regulated by three genetic loci; i, R and T (Lee *et. al*., 2009). A study by Kim *et al.* (2006) found phenolic compounds and compounds of anthocyanins in the black seed coat of soybean rather than brown, yellow and green. Two major key factors for the successful breeding program were the suitable and effective germplasm selection and selection methods. Plant breeder needed to study for basic genetic and gene expression that influenced different characteristics. Generation mean analysis was a useful technique for plant breeder to estimate gene effect responsible for inheritance of quantitative traits. Srinives and Sutakom (1986) used generation mean analysis in yield per plant and yield components of 23 soybean crosses found that seed yield per plant and yield component were controlled by quantitatively inherited genes and most of heredity was responsible by additive gene effect. The objectives of this study were: 1) to determine the gene effects on black seed coat color and some agronomic traits related to vegetable soybean standard and 2) to develop vegetable soybean lines for black seed coat color trait.

MATERIALS AND METHODS

Plant materials

Three varieties of aromatic and black vegetable soybeans were used for crossing of the 2 crosses. Two varieties of aromatic vegetable soybean, 'Kaori' and 'Chiang Mai 84-2' were used as donor parents. And, a 'Black Seed' variety was used as recurrent parent. The procedure was asfollowed:

Season 1 June 2012 – September 2012: Hybridization of two crosses of cross between 'Chiang Mai 84-2' x 'Black Seed' and 'Kaori' x 'Black Seed' for F_1-seed

Season 2 November 2012 – February 2013: Hybridization of two crosses for F_1-seed F_1- plants x 'Black Seed' to BC_1F_1-seed and F_1 plants were self-pollination to F2-seed

Season 3 May 2013 – August 2013: Hybridization of two crosses for F_1-seedF_1-plants were self-pollination to F_2-seed, BC_1F_1-plants were self-pollination to BC_1F_2-seed and BC_1F_1-plants x 'Black Seed' to BC_2F_1-seed

Season 4 October 2013– February 2014: Parents,F_1-plants ,F_2-plants ,BC_1F_2-plants, BC_2F_1-plants

All progenies and theirs parents were grown together in the field experiment at Chiang Mai Field Crops Research Center, Chiang Mai,Thailand. The F_2, F_3, BC_1F_3 and BC_2F_2 seeds were harvested from F_1, F_2 and BC_1F_2 and BC_2F_1 plants at 95% of pod maturity stage (R_8) in January–February 2014 and were selected for black seed coat colors and some agronomic traits evaluation. The plots size was 1 x 5 m, the spacing was 50 x 20 cm., 1 plants/hill. The management in the field experiment followed the steps of Good Agriculture Practice of Vegetable soybean recommended by Department of Agriculture (DOA), Ministry of Agriculture and Cooperative, Thailand.

Data collections

Seed coat color was recorded at harvesting period. Number of pod bearing branches per plant, number of 2-seed pods, number of pods per plant and dry seed weight per plant (g) were determined from individual plants of progenies. The 100-dry seed weight was collected from randomly selected 100 seeds of each plant.

Experimental design and data analysis

Analysis of variance and mean comparison of characters were done using R-program software. Genetic analysis by generation mean analysis (GMA) from six populations of P_1, P_2, F_1, F_2, BC_1F_2 and BC_2F_1 from the Randomized complete block design (RCBD) with three replications was employed. The gene action was evaluated following the method of Mather and Jinks (1982) that could be determined for the allelic and non-allelic gene action. In this method the mean of each character was indicated as follows:

$$Y = m+\alpha[d]+\beta[h]+\alpha^2[i]+2\alpha\beta[j] +\beta^2[l]$$

Where: Y= the mean of one generation,
m = the mean of all generation,
d = the sum of additive effects,
h = the sum of dominance effects,
i = the sum of additive x additive interaction (complementary),
l = the sum of dominance x dominance interaction (duplicate).

and j = sum of additive x dominance. And $+\alpha$, 2α, β, β^2 were the coefficients of genetic parameters.

Results and Discussion

1. Morphological trait: Seed coat color

1.1 F_3-seeds derived from the F_2 plants of the 2 crosses

F_3 seeds of the cross 'Kaori' x 'Black Seed' showed variation of seed coat segregation (Figure 1). Similarly, F_3 seeds of the cross 'Chiang Mai 84-2' x 'Black Seed' also showed several of seed coat segregation (Figure 2).

Figure 1 Seed coat color of F_3-seeds of the cross 'Kaori' x 'Black Seed'

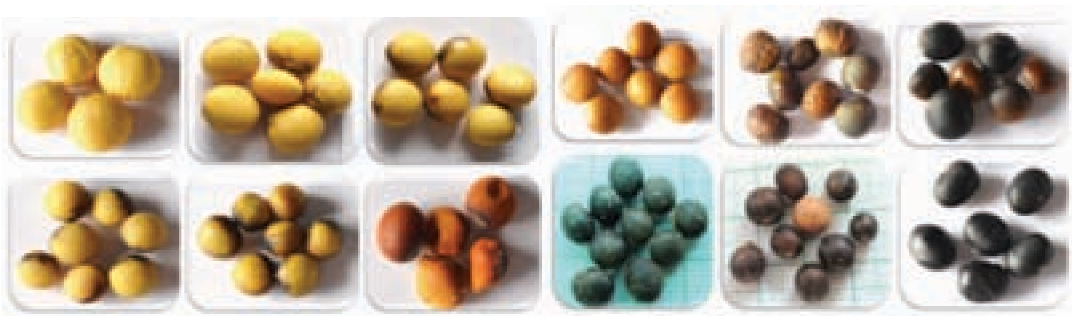

Figure 2 Seed coat color of F_3-seeds of the cross 'Chiang Mai 84-2' x 'Black Seed'

1.2 BC_1F_3-seeds derived from BC_1F_2

Seed coat color of BC_1F_3 ('Kaori' x 'Black Seed') was classified into 11 phenotypic groups: black, black with brown spot, blackish green, blackish yellow, yellowish green, yellow, buff, brownish black, green and brown (Figure 3). In contrast with seed coat color segregation of BC_1F_2 of the cross between 'Chiang Mai 84-2' x 'Black Seed', Seven phenotypic groups of black, black with brown spot, blackish yellow, yellow, buff, brownish black and brownish black with brown spot were observed (Figure 4).

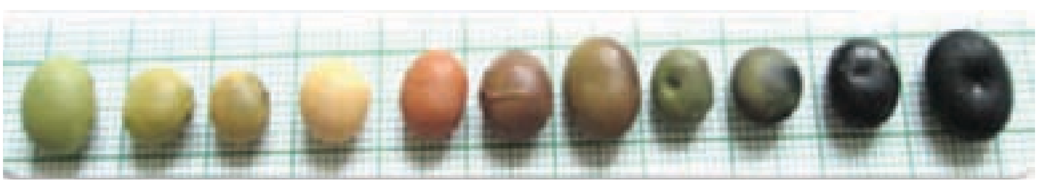

Figure 3 BC_1F_3-seeds of the cross 'Kaori' x 'Black Seed'

Figure 4 BC_1F_3-seeds of the cross 'Chiang Mai 84-2' x 'Black Seed'

2. Yield and yield components:

The average means of yield of the cross 'Kaori' x 'Black seed' in BC_2F_1 generation were no statistically significant different from Parents, F_2 and BC_1F_2 generations (Table 1). All of the traits in BC_2F_1 generation were lower than in F_1 generation. The average mean of BC_2F_1 generation of the cross between 'Chiang Mai 84-2' x 'Black Seed' was greater than both parents, F_2 and BC_1F_2 of number of 2-seed pod per plant (N2SPP). But all of the traits except the average mean of number of 2-seed pod per plant (N2SPP) of BC_2F_1 generation were lower than the average means of F_1 generation. (Table 2)

Table 1 Generation means and standard errors of number of pod bearing branches per plant (NPBBP), number of 2-seed pods per plant (N2SPP), number of pods per plant (NPP), 100-dry seed weight (100DSW) and dry seed weight per plant (DSWPP) of the cross 'Kaori' x 'Black Seed'

Generations	Traits (Mean ± SD.)				
	NPBBP	N2SPP	NPP	100DSW (g)	DSWPP (g)
P_1	1.29±0.27 b	11.69±0.64 b	15.68±0.53 b	29.26±0.43 b	8.92±0.28 b
P_2	1.34±0.20 b	8.55±0.37 b	13.78±0.37 b	29.84±0.40 b	7.10±0.18 b
F_1	4.50±0.14 a	17.76±1.01 a	29.44±1.15 a	35.76±0.93 a	20.15±0.88 a
F_2	1.33±0.33 b	10.33±0.58 b	15.13±2.16 b	26.88±1.02 b	7.77±1.40 b
BC_1F_2	2.10±0.14 b	10.82±0.53 b	16.51±0.70 b	28.27±0.45 b	8.82±0.43 b
BC_2F_1	2.56±0.53 b	9.56±1.17 b	15.56±1.82 b	27.97±1.21 b	8.18±1.20 b
Mean	2.1	11.45	17.68	29.66	16.37
C.V. (%)	26.67	14.30	14.19	6.23	33.24

Mean in the same column followed by a common letter are not significantly different at 5% by DMRT

Investigation of gene action from generation mean analysis of the cross between 'Kaori' x 'Black Seed' revealed that number of pod bearing branches per plant, number of pod per plant and dry seed weight per plant were controlled by additive, additive x dominance and dominance x dominance genes. The gene action of number of 2-seed pods per plant was controlled by additive x dominance and dominance x dominance genes. The trait of 100-seed dry weight per plant was only controlled by dominance x dominance genes. (Table 3). On the contrary of the cross of 'Chiang Mai 84-2' x 'Black Seed', the significant effect of additive, dominance, additive x dominance and dominance x dominance of number of pod bearing branches, number of pod per plant and dry seed weight per plant were identified. The 2-seed pod per plant trait was controlled by additive; additive x dominance and dominance x dominance genes. While, the gene effect on 100-dry seed weight of the cross Chiang Mai 84-2 x Black Seed had only dominance gene action (Table 4)

Table 2 Generation means and standard errors of number of pod bearing branches per plant (NPBBP), number of 2-seed pods per plant (N2SPP), number of pods per plant (NPP), 100-dry seed weight (100DSW) and dry seed weight per plant (DSWPP) of the cross 'Chiang Mai 84-2' x 'Black Seed'

Generations	Traits (Mean ± SE)				
	NPBBP	N2SPP	NPP	100DSW (g)	DSWPP (g)
P_1	1.46±0.23 b	8.35±0.58 b	13.05±0.56 b	29.22±0.44 abc	7.03±0.29 b
P_2	1.34±0.20 b	8.55±0.37 b	13.78±0.37 b	29.84±0.40 ab	7.10±0.18 b
F_1	4.84±0.23 a	19.47±0.98 a	30.50±1.34 a	32.30±0.45 a	19.74±0.90 a
F_2	1.34±0.09 b	7.98±0.29 b	12.14±0.39 b	25.46±0.26 c	6.08±0.23 b
BC_1F_2	2.04±0.12 b	9.82±0.38 b	15.44±0.50 b	26.82±0.32 bc	7.87±0.30 b
BC_2F_1	2.82±0.46 b	11.16±1.78 a	18.60±2.02 b	27.90±0.99 bc	9.37±0.97 b
Mean	2.31	10.89	17.25	28.59	9.52
C.V. (%)	33.30	21.05	18.17	5.72	15.23

Mean in the same column followed by a common letter are not significantly different at 5% by DMRT

Table 3 Generation mean analysis (GMA) of number of pod bearing branches per plant (NPBBP), number of 2-seed pods per plant (N2SPP), number of pods per plant (NPP), 100-dry seed weight (100DSW) and dry seed weight per plant (DSWPP) of the cross Kaori x Black Seed

Genetic effect[1/]	NPBBP	N2SPP	NPP	100DSW	DSWPP
m	1.33±0.19**	10.33±0.75**	15.13±1.22**	26.88±0.57**	7.77±0.79**
d	-4.48±0.91**	-4.90±2.70	-10.77±4.18**	-4.19±2.38	-8.40±2.72**
h	2.83±1.54	2.33±3.94	7.72±5.82	3.39±3.80	6.98±3.77
i	-0.35±1.53	-5.37±3.84	-7.03±5.74	-2.67±3.73	-5.12±3.73
j	-4.45±0.93**	-6.51±2.73*	-11.73±4.19**	3.91±2.41	-9.31±2.72**
l	7.00±3.26*	25.44±9.08**	42.02±3.65**	28.11±8.41**	35.38±8.93**

*, ** = significant at the 0.05 and 0.01 probability levels, respectively

[1/] Genetic effect: m = mean, d = additive gene action, h = dominance gene action, i = additive x additive, j = additive x dominance, l = dominance x dominance

Table 4 Generation mean analysis (GMA) of number of pod bearing branches per plant (NPBBP), number of 2-seed pods per plant (N2SPP), number of pods per plant (NPP), 100-dry seed weight (100DSW) and dry seed weight per plant (DSWPP) of the cross 'Chiang Mai 84-2' x 'Black Seed'

Genetic effect[1/]	NPBBP	N2SPP	NPP	100DSW	DSWPP
m	1.33±0.09**	7.88±0.29**	12.22±0.39**	25.91±0.26**	6.17±0.23**
d	-4.24±0.73**	-10.97±2.64**	-19.12±3.10**	-2.46±1.63	-11.47±1.60**
h	3.73±1.35**	8.78±5.06	16.39±5.86**	6.54±2.96**	11.79±2.96**
i	0.29±1.33	-2.45±4.99	-1.01±5.76	3.85±2.93	-1.01±2.87
j	-4.33±0.75**	-10.90±2.67**	-18.81±3.12**	-2.11±1.67	11.46±1.60**
l	6.73±2.77*	29.77±10.37**	41.85±2.13**	11.96±6.13	31.54±6.25**

*, ** = significant at the 0.05 and 0.01 probability levels, respectively

[1/]Genetic effect: m = mean, d = additive gene action, h = dominance gene action, i = additive x additive, j = additive x dominance, l = dominance x dominance

Seed coat color trait was controlled by polygenes of at least 5 loci (I, T W1, R and O) (Palmer *et al*., 2004). Such inheritance of trait corresponded to the finding of this study and explained why in F_3-seed and BC_1F_3-seed, various segregation of seed coat color were observed. This study aimed to select for black seed coat color with fragrance soybean lines. According to the breeding program, we aimed to select for black seed coat first, and followed by the selection of fragrance in the next generations because the fragrance trait was controlled by recessive gene (Arikit *et al*., 2011).

The result of agronomic and yield component traits were in accordance with reports published by Tawar *et al.,* (1989) who found that branches per plant and 100-seed weight in F_2 were controlled by additive gene effect. Similar to Ojo and Dashiell (2002) who reported that 100-seed weight was controlled by additive gene and additive effect was in general and larger than dominance gene effect. On the contrary with Maloo and Nair (2005) reported dominance effect rather than additive effect for number of pods per plant, seeds per pod, 100-seed weight and seed yield per plant in all crosses. The results of this study suggested that the breeding strategy for selection of black vegetable soybean and agronomic traits, related to vegetable soybean standard and development of vegetable soybean lines for black seed coat color trait with fragrance in the future could be done.

Conclusions

The results presented could be concluded as follows:

1. Seed coat color was controlled by polygenes and different segregation was depending on parents.

2. F_2, BC_1F_2 and BC_2F_1 showed the polygenes controlling with additive and

non-additive gene effect for seed coat color, number of pod bearing branches, number of 2-seed pods, number of pods per plants, 100-dry seed weight and seed weight per plant.

3. The donor parents, ‘Chiang Mai 84-2’ and ‘Kaori’ had different genetic control in seed coat color and some agronomic traits.

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