Evaluation of yield stability of black soybean mutant lines in ten environments

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Evaluation of yield stability of black soybean mutant lines in ten environments

W Puspitasari, Tarmizi and Arwin
Centre for Isotope and Radiation Application, National Nuclear Energy Agency (BATAN), Jakarta, Indonesia

Email: winda_p@batan.go.id

Abstract. To obtain national high yielding varieties, it is necessary to evaluate black soybean yield stability in various environments. This study aims to evaluate yield stability and to study its genotype and environment interaction. Genetic material used in this study was five mutant lines of black soybean derived from the irradiation of the Cikuray variety with a dose of 200 Gy. Evaluation of yield stability was carried out jointly with 10 black soybean breeding lines from Balitkabi and Padjadjaran University. Two control varieties of Cikuray and Detam 1 were included in the evaluation. The test was carried out in ten locations across Java Island. Estimation of stability was conducted by the method of Finlay-Wilkinson and Eberhart-Russell. The results showed that genotype and environment significantly contributed to yield variation respectively. Genotype by environment interactions were existed which could affect genotype performance in the testing environment. Four out of five mutant lines showed significant higher yield than other genotypes and control varieties, which ranged from 2.34-2.42 ton/ha. Stability analysis indicated genotypes of G8, G14 and control variety of Detam 1 were stable genotypes and high yield above the general average with average yields of 2.36, 2.09 and 2.21 tons / ha, respectively.

1. Introduction
Soybean is one of important food crops in Indonesia after rice and maize. Soybean based food has become one of tradition due to its rich protein content, not only for direct consumption but also for processed foods, such as tempeh, tofu and soy sauce. Therefore, soybean availability is a national issue to support food security in Indonesia.

Soybean with black seed coat, named black soybean, mainly used as raw materials for soy sauce industry, not only to increase the color quality, but also to raise the nutrition, especially its protein content [1]. In some Asian countries, black soybean has been utilized as functional food due to its nutrition and antioxidant content so that useful as healthy food and suitable to overcome several degenerative diseases [2, 3].

Breeding program on black soybean is relatively left behind compared to soybean. Merely around 13% of the total soybean varieties in Indonesia are black soybean. In fact, the history of soybean breeding in Indonesia has noted the released of first and second soybean varieties in 1918 and 1919 were Otau and No. 27, which are black soybean [4]. In addition, the farmers prefer to grow soybean than black soybean due to the availability of high yielding varieties of soybean. Therefore, black soybean research to increase productivity, shall be developed continuously.
The application of induced mutation, either using physical or chemical mutagens, has afforded genetic variability and has given significant role in plant breeding, with official release of more than 2,700 plant mutant varieties throughout the world [5]. Induced mutation has been used in crops improvements, such as wheat, rice, barley, cottons, peanuts, beans, which are seed propagated plants. In vegetative propagated plants, mutants of ornamentals, such as chrysanthemum, carnation, roses, and fruit plants, such as banana, apple, pineapple, were obtained by irradiation techniques [6].

In seed propagated plants, selection of recessive mutants are usually conducted in M2 generation after mutagenesis treatment. Several generations are required to homogenize selected plants of interest. The next important step is evaluation of selected plants in various locations to estimate genetic by environment interaction (GEI) which usually known as multi-environment trial. High GEI effect will reduce genetic contribution on plant performance. In addition, the multi-location testing is required because Indonesia consists of various soil types, cultivation methods and growing seasons, so evaluation of both high yielding and stable genotypes in diverse environments is necessary to be conducted. This study aimed to evaluate black soybean genotypes in ten locations and to study the genetic and environment interactions.

2. Experimental methods
A number of one thousand seeds of black soybean Cikuray variety were irradiated using gamma ray of Co-60 of 200 Gy dose in 2008. M1 plants were harvested individually and later were sown in M2 generation using pedigree system. Positive selection was conducted on M2, M3 and M4 generations to choose early mature and vigour traits. The selected mutant lines then were homogenized until M5 which show homogeneous phenotype. 28 mutant lines were selected to have better yield than the parents, resistant to pest and disease attack. After several preliminary tests in different location, then five mutant lines were selected to have better performance and adaptation. These five mutant lines were evaluated jointly with other ten lines from Balitkabi and University of Padjadjaran. The evaluation included two control varieties, Cikuray and Detam-1 as well (Table 1). The adaptation test of these 17 genotypes was conducted in 10 locations in dry and rainy season in 2012 (Table 2). Trials were carried out using randomized complete block design with four replicates, plot size was 3 x 4 m and plants distance was 40 x 15 cm.

| No. | Genotype | Origin     | No. | Genotype | Origin     |
|-----|----------|------------|-----|----------|------------|
| 1   | G1       | BALITKABI  | 10  | G10      | BATAN      |
| 2   | G2       | BALITKABI  | 11  | G11      | UNPAD      |
| 3   | G3       | BALITKABI  | 12  | G12      | UNPAD      |
| 4   | G4       | BALITKABI  | 13  | G13      | UNPAD      |
| 5   | G5       | BALITKABI  | 14  | G14      | UNPAD      |
| 6   | G6       | BATAN      | 15  | G15      | UNPAD      |
| 7   | G7       | BATAN      | 16  | Cikuray  | BALITKABI  |
| 8   | G8       | BATAN      | 17  | Detam-1  | BALITKABI  |
| 9   | G9       | BATAN      |      |          |            |

Table 1. The 17 tested genotypes and its origin

Data analysis was carried out including analysis of variance of yield performance across ten locations and replicates. Significant difference of yield performance was determined by using least significant difference. Estimation of stability was conducted by using the method of Finlay-Wilkinson and Eberhart-Russel [7, 8].
Table 2. Ten Environments of seed yield evaluation in 2012

| Environment         | Planting Season | Soils type | Elevation (m) |
|---------------------|-----------------|------------|---------------|
| Cianjur, West Java  | Rainy season    | Regosol    | 700           |
| Bogor, West Java    | Rainy season    | Latosol    | 50            |
| Majalengka, West Java| Rainy season   | Regosol    | 50            |
| Gunung Kidul, Yogyakarta | Rainy season | Latosol    | 200           |
| Banyuwangi, East Java| Rainy season   | Latosol/Entisol | 168        |
| Madura, East Java   | Dry season      | Vertisol   | 62            |
| Ngawi, East Java    | Dry season      | Grayregosol | 86           |
| Cirebon, West Java  | Dry season      | Regosol    | 50            |
| Indramayu, West Java| Dry season      | Regosol    | 20            |
| Karawang, West Java | Dry season      | Latosol    | 10            |

3. Results and discussion
The analysis of variance indicates that the sources of variation (genotype, environment and GEI) of seed yield test were significant (Table 3). This allows us to conclude that the genotypes varied and the environments were different. Presence of GEI indicated genotypes gave different response in the testing environment. These responses were indicated by seed yield fluctuation of each genotype in different environment (Fig. 1). The GEI might be a problem to identify superior genotypes because it obscures genetic contribution on phenotypic performance [9]. Breeders will have difficulty to determine the best genotype because performance rank changes across environments. The presence of GEI in soybean and black soybean was reported as well in Xavier et al., Freiria et al. and Adie et al. [10, 11, 12].

Table 3. Analysis of variance of seed yield of 17 genotypes of black soybeans in ten environments

| Source               | Df   | Sum squares | Mean squares | F-count |
|----------------------|------|-------------|--------------|---------|
| Environment (E)      | 9    | 126.4941    | 14.0549      | 119.26**|
| Rep:E                | 30   | 3.5354      | 0.1178       | 2.55**  |
| Genotype (G)         | 16   | 33.3547     | 2.0847       | 8.18**  |
| G x E                | 144  | 36.7159     | 0.2550       | 5.51**  |
| cv = 10.66%          |      |             |              |         |

The average seed yield of tested genotypes ranged from 0.97 tons/ha to 2.55 tons/ha in all testing environments. Control variety of Cikuray had an average seed yield of 1.89 tons /ha, while Detam 1 had 2.21 tons/ha. The highest seed yield average was mutant line G6 and the lowest was G5 which was 2.42 tons/ha and 1.70 tons/ha respectively. Four mutant lines (G6, G8, G9 and G10) had significant higher seed yield than other genotypes which ranged 2.34 -2.42 ton/ha. The tested genotypes showed higher yield in environment 4 (Jogjakarta) and 8 (Cirebon), whereas in environment 9 (Indramayu) had the lowest seed yield average. The variation of seed yield of each genotype was less than 20% showing experiment condition was favourable.
Figure 1. The phenotype of seed yield of 17 genotypes across ten environments

A genotype might perform different seed yield in different environments because the variability of macro-geophysical environments will provide large growing environmental diversity. Deviations of seed yield fluctuation in each of these environments were then analyzed to obtain the regression of the average genotype. Finlay and Wilkinson (1963) suggested genotypes that have regression slope ($b_i$) less than 1, equal with 1 and more than 1, respectively has stability below average, on average and above average. In accordance to Finlay and Wilkinson, Eberhart and Russel has determined regression coefficient parameters ($b_i$) and regression deviation ($s_{i2}$) to consider genotype stability in tested environments. Both methods are based on linear assumptions, resulting in relatively similar genotype stability in the experiment.

Table 4. Seed yield, coefficient of variation, regression slope ($b_i$) and regression deviation ($s_{i2}$) of tested genotypes

| Genotype | Seed yield (ton/ha) | CV (%) | $b_i$ | $s_{i2}$ |
|----------|---------------------|--------|-------|----------|
| G1       | 1.92 cde            | 6.42   | 0.95ns| 0.004    |
| G2       | 1.82 de             | 9.64   | 0.95ns| 0.019    |
| G3       | 1.96 cd             | 11.44  | 1.19* | 0.039    |
| G4       | 1.90 cde            | 13.74  | 1.01ns| 0.056    |
| G5       | 1.70 c              | 19.26  | 0.89ns| 0.096    |
| G6       | 2.42 a              | 13.63  | 1.17* | 0.097    |
| G7       | 1.91 cde            | 15.62  | 0.88* | 0.077    |
| G8       | 2.36 a              | 15.40  | 1.08ns| 0.121    |
| G9       | 2.35 a              | 10.15  | 1.20* | 0.045    |
| G10      | 2.34 a              | 11.60  | 1.24* | 0.062    |
| G11      | 1.86 cde            | 14.03  | 0.85* | 0.057    |
| G12      | 1.95 cd             | 8.18   | 0.93ns| 0.014    |
| G13      | 1.79 de             | 11.39  | 0.90ns| 0.030    |
| G14      | 2.09 bc             | 9.99   | 1.08ns| 0.032    |
| G15      | 1.91 cde            | 10.55  | 0.83* | 0.029    |
| Cikuray  | 1.84 de             | 11.87  | 0.75* | 0.036    |
| Detam 1  | 2.21 ab             | 11.84  | 1.08ns| 0.057    |

*ns= not significant; * = significantly different in 95% of confidence interval
The genotypes had bi values about 0.75 - 1.19 and regression deviation of 0.004 – 0.121 (Table 4). Based on Finlay and Wilkinson criteria, the genotypes of G1, G2, G4, G5, G8, G12, G13, G14 and Detam 1 had bi values which were not different from 1, classified as a stable genotype. Genotypes of G8, G14 and Detam 1 were stable genotypes with high yield above the general mean, while genotypes G1, G2, G4, G5, G12 and G13 were classified as low stable genotypes. Genotypes of G3, G6, G9 and G10 had bi values of more than 1, which were 1.19, 1.17, 1.20 and 1.24 respectively. These genotypes were classified as below-average stability, which were able to adapt to a favourable environment. High input cultivation is highly required for these genotypes to have maximum performance. Genotypes of G7 and G11 had bi, less than 1, which were 0.88 and 0.85, respectively, which categorized to have above average stability. Thus, the two genotypes are expected to have better adaptation to larger range environment, which will give relatively similar response to input.

4. Conclusion
Genotype by environment interaction was existed which contributed to genotypes performance. Four mutant lines showed significant higher yield than other genotypes and control varieties, which ranged from 2.34-2.42 ton/ha. The stability analysis indicates nine genotypes were stable and genotypes of G8, G14 and Detam 1 had higher seed yield above the general average with average yields of 2.36, 2.09 and 2.21 tons / ha, respectively.

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