Mega-Environment Analysis and Identify Stable and High-Yielding of New Promising Black Soybean Lines in Indonesia

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Abstract

Stable and high-yielding are the major goals of black soybean breeding. Testing new lines in a mega-environment is one of the development processes in black soybean breeding. The aims of the research were (i) to identify the effects of genotype, environment, and GEIs on the grain yield of soybean lines in Java Island; (ii) to select stable and high yielding soybean lines; and (iii) to determine the discriminative environments, and (iv) to determine the concept of stability measurements on black soybean grain yields. Field trials of 10 new F8 promising lines and three check varieties were conducted under eight different environments during four years (2016–2019). The measurement results showed that the grain yield was influenced by genotype (8.35%), environment (59.49%), and GEIs (32.16%). Grain yield stability measurements showed that the four newly lines was identified had high yields and stable in eight environments, they were A-5A-PSJ (S2), DB-96-CTY (S5), UP 161 (S6), and UP 162 (S7). The Ngawi (2017) followed by Bogor (2019) and Banyuwangi (2016) has the strongest interactive capabilities, and was suitable for used as a trial environments. Grain yield (Y) was identified as having a positive and significant correlation ($p < 0.05$) with $S^{(3)}$, $S^{(6)}$, $NP^{(2)}$, $NP^{(3)}$, $NP^{(4)}$, KR, and YSI stability measurements, which indicated that they were included in the concept of dynamic stability measurement.

Introduction

Black Soybeans contain many nutrients that are beneficial for humans. The current research of black soybean leading to the results of secondary metabolites that are beneficial to health. There were many secondary metabolites of black soybeans that are beneficial to humans, one of which is isoflavones. Isoflavones are a group of flavonoid compounds that produce natural antioxidants¹. The benefits of isoflavones for humans include preventing cancer, heart attack, and climateric syndrome^{2,3}. Isoflavones in black soybeans were found in all plant organs, especially soybean seeds. Sumardi et al.⁴ reported that the largest types of isoflavones contained in black soybean seeds were daedzein and genistein. The important role of black soybeans for food and human health, makes this commodity increasingly needed by the community so that its demand was predicted to increase.

In Indonesia, black soybeans were still needed as raw materials for production in soy sauce industries. The advantage as a raw material for soy sauce was to improved the quality of the color to dark chocolate. Soy sauce made from black soybeans "Merapi" has a protein content of 3.20%, higher than the soy sauce from the yellow soybean "Argomulyo" (2.37%)⁵. The large role of black soybeans for functional foods, makes the demand for black soybeans increase. Triyanti⁶ reported that soybean production in 2020 had increased to 632,333 tons from 2019 which produced 424,190 tons. However, this amount was still not able to meet the higher demand for black soybeans, so it was predicted that the import value of s black oybeans to meet domestic needs was still high.

One of the efforts that can be done is by assembling new superior black soybean that have high grain yields. The stages that must be passed in the assembly of promising lines are testing in a variety of environments (multilocation trial). Multi-location trials was carried out to obtain information on stable and adaptive lines in all environments as well as specific adapted lines with high yields. The more environments used for testing (mega-environment), the higher the accuracy of stable lines⁷. According to Srivastava et al.⁸, mega-environment analysis using the GGE biplot provides information on suitable sites for extensive adaptation and suitable sites for specific adaptation testing. Silva et al.⁹ reported that mega-environment trials could lead to complex GEIs and obtained information about the relationship between trial environments. Information obtained from mega-environment trials was very helpful for plant breeders in manages of GEIs and extracting them into the same agro-climatic concept¹⁰. According to Zdiarski et al.¹¹, mega-environment trial provides information on specific adapted lines based on regional targets and regional climate. In addition, based on these environment groupings, lines that show high yield stability and high yielding in suitable locations for recommended lines. Based on this description, the objectives of this study were (i) to identify the effects of genotype, environment, and GEIs on the grain yield of soybean lines in Java Island; (ii) to select stable and high yielding soybean lines; and (iii) to determine the discriminative environments, and (iv) to determine the concept of stability measurements on black soybean grain yields. The results information was useful in the development of soybean lines in the future.

Result

Combined Analysis of Variance (ANOVA) to identify GEIs

AMMI ANOVA (Table 1) revealed that the effect of genotype, environment, and their interactions (GEIs) had a significant effect ($P < 0.05$) on soybean grain yield variations. The main effect of genotype accounted for 8.35% of the total variation, while environment and GEIs accounted for 59.49% and 32.16%, respectively. These results indicated that grain yields were significantly affected by environmental changes, followed by effects of GEIs and genotypes (Table 1). Highly significant environmental effects and high variance components can be attributed to large differences between test environments such as elevation, and differences in the amount and distribution of annual rainfall (Table 2). The GEIs were further divided into seven IPCAs, of which the first two IPCAs showed significant contributions. IPCA1 contributed 42.66% to the number of squared GEIs. The first two IPCAs accounted for 69.30% of the total variation in GEIs indicating the accuracy of the data. The magnitude of the GEIs effects was also be seen in the box plot (Figure 1) and the GEIs heat map (Figure 2). In Figure 1 the distribution of the average grain yield in each environment showed high variation. The GEIs heatmap (Figure 2) showed the relationship between each environment and the average yield. In Figure 2, the environment was divided into three main clusters, namely environmental groups that have high average grain yields (E4 and E5), low grain yield (E2, E6, and E7), and medium grain yield (E1, E3, and E8). This data showed that the influence of the environment was very strong on soybean grain yields.

Parametric and non-parametric stability measurements on soybean grain yields

The stability of soybean grain yields in multi-environmental trials based on parametric and non-parametric measurements was presented in Table 3. Linear regression measurements on environmental indexed showed that grain yields of the two stability parameters b_i and S^2_{di} were inconsistent in assessing the

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(1), on the contrary, some lines also showed a significant deviation from the regression value (S^2_{di}) greater than zero (Table 3). Based on the regression coefficients, all lines had an average response in all test environments. Thus, the lines UP161 (S6), UP 162 (S7), and UP 163 (S8) were declared the most stable according to this measurements. The other lines, Detam 1 (S11) and Detam 3 (S12), had a regression deviation (S^2_{di}) which was significantly greater than zero and a bi value that was not different from one, indicating that these two lines were better adapted to environments that produce high yields (favorable). The Wi^2 , σ^2 , dan θ stability measurements, showed the same results in determining the stability of soybean grain yields. Based on these three stability measurements, the A-5A-PSJ (S2) was declared the most stable line, followed by the UP 161(S6), DB-96-CTY (S5), and UP163 (S8) lines. CVI measurement indicated A-8A-PSJ (S4) as the most stable, followed by A-4A-PSJ (S1) and UP 162 (S7). θ stability measurement indicated the Detam 3 (S12) line as the most stable, followed by A-7A-PSJ (S3) and GH Detam 5 (S13). These three lines have a high average yield. ASV measurement revealed UP 161 (S6), UP 162 (S7), and A-5A-PSJ (S2) lines as the most stable. YSI measurement indicated UP 162 (S7) as the most stable line, followed by DB-96-CTY (S5), and A-5A-PSJ (S2). All three lines had high average grain yields across the eight test environments. In non-parametric stability measurements, DB-96-CTY (S5) was declared the most stable by $S^{(3)}$ and KR measurements. The UP 161 (S6) line was declared the most stable by NP(1) measurement. The UP 162 (S7) line was declared the most stable by $S^{(1)}$, $S^{(2)}$, NP⁽²⁾, NP⁽³⁾, and NP⁽⁴⁾ measurements. The GH Detam 5 (S13) line was declared the most stable by $S^{(1)}$ and $S^{(2)}$ measurements.

The grouping of soybean lines tested in this study was presented in Figure 3. The grouping was the output of Hierarchical Clustering Analysis (HCA) on the stability rank's of the tested lines based on parametric and non-parametric measurements. In the Figure 3, soybean lines were divided into three main groups, namely the unstable low yield cluster containing the A-4A-PSJ (S1), UP 165 (S10), A-8A-PSJ (S4), and GH Detam 5 (S13) lines; unstable high yield cluster containing Detam 1 (S11), Detam 3(S12), A-7A-PSJ(S3), and UP 164 (S9) lines; and a stable high yield cluster containing DB-96-CTY (S5), UP 162 (S7), UP 161 (S6), UP 163 (S8), and A-5A-PSJ (S2). The third group was preferred because it has stable and high grain yields than the overall average.

The relationship between parametric and non-parametric stability measurements with soybean line grain yields

The relationship between stability measurements was estimated using Spearmans correlation to the stability rank's of each measurement. The results of the correlation analysis was presented in Table 4. The grain yield (Y) was identified as having a positive and significant correlation ($p < 0.05$) with the $S^{(3)}$, $S^{(6)}$, NP⁽²⁾, NP⁽³⁾, NP⁽⁴⁾, KR, and YSI stability measurements. Another positive and significant correlation was shown by $S^{(1)}$ with $S^{(2)}$ ($p < 0.01$); NP⁽¹⁾ with bi ($p < 0.01$); KR with Wi^2 , σ^2 , S^2_{di} , θ , ASV, YSI ($p < 0.01$), and θ ($p < 0.05$); and CVI with ASV ($p < 0.05$). To classify the relationship between each measurement, Principal Component Analysis (PCA) was used on the grain yield stability rank's. The results of the PCA measurement showed that the first four PCs was identified produced an eigenvalue > 1 , with an effect of 93.14% of the total variation (Table 5). PCA biplots regarding the relationship between stability measurements and grain yield were drawn based on the PC1 and PC2 values (Figure 4). Based on Figure 4, the grain yield (Y) was in one group with NP⁽²⁾, NP⁽³⁾, NP⁽⁴⁾, $S^{(3)}$, $S^{(6)}$, YSI, and KR measurements. The $S^{(1)}$ grouped with $S^{(2)}$. NP⁽¹⁾ grouped with bi . ASV grouped with S^2_{di} , θ , σ^2 , and Wi^2 . The CVI measurement was weakly correlated with the last group, while the θ measurement was not correlated with all other measurements.

AMMI biplot analysis of soybean grain yield

The first two principal components (IPCA1 and IPCA2), accounted for 69.2% of the total variation in GEIs (Figure 5). The length of the environment vector from the center of the biplot was proportional to the number of GEIs. An environment with a longer vector has a strong interaction, while an environment with a shorter vector has a weak interaction. The Ngawi (2017) environment has the strongest interactive power followed by Bogor (2019) and Banyuwangi (2016). Malang (2018) environment has the weakest interactive. The lines that clustered close to the biplot axis had stable yields across the environment. Thus, the A-4A-PSJ (S1), A-5A-PSJ (S2), A-7A-PSJ (S3), DB-96-CTY (S5), and UP 162(S7) lines had almost the same grain yield appearance (stable). Lines and environments with the same arrow pointing from the center of the biplot had positive GEIs, and vice versa. Thus, the lines UP 163 (S8), UP 164 (S9), and UP 165 (S10) have a positive GEIs with Banyuwangi (2016), Ngawi (2018), and Bantul (2019) environments, respectively.

The first IPCA (IPCA1) was plotted against the line and environmental mean (Figure 6). The lines were distributed below and above the mean IPCA1 values of -40 to +60. The environments and lines on the left hand side of the vertical line have grain yields below the overall average, while those on the right side of the origin have grain yields above the overall average. The environments of Ngawi (2016), Ngawi (2017) and Probolinggo (2018) have large positive IPCA1 scores and have above-average grain yields. The Ngawi (2017) environment was classified as having the highest average yield compared to other environments, and the lines that had the same IPCA sign and close to Ngawi (2017) were S5 and S7. The Malang (2018) environment was identified as having the lowest average grain yield and a small positive IPCA value close to zero, while Banyuwangi (2017) had a small negative IPCA value close to zero with an average grain yield less than the overall average. Lines that are close to the horizontal line have stable grain yields. Overall, of the 13 lines tested, six lines (46%) produced grain yields above the overall average grain yield with three lines being close to the horizontal line, and seven lines (54%) produced grain yields below the overall average grain yields.

Mega-environments analysis of 13 soybean lines in eight environments

The genetic correlations between the eight environments was presented in Table 5 and reflected in the GGE biplot (Figure 7). In the GGE biplot analysis in 2016–2019, PC1 explained 46.28% of the total variation and PC2 explained 23.56% of the total variation, accounting for 67.84% of the total variation for grain yields (Figure 7). Biplots based on average data during four years showed that the eight test environments had five sectors with different winning lines (vertex). The vertex lines were A-7A-PSJ (S3), UP 164 (S9), GH Detam 5 (S13), A-8A-PSJ (S4), and Detam 3 (S12). Figure 7 represents the four mega-environments. The first mega-environment (ME-I) consisted of Ngawi (2016), Bogor (2019), Ngawi (2017), and Malang (2018) with the A-7A-PSJ (S3) line as the winner. The second mega-environment (ME-II) consisted of Banyuwangi (2016) and Probolinggo (2018) with the Detam 3 (S12) line as the winner. Bantul (2019) and Banyuwangi (2017) were the two individual mega-environments (ME-III and ME-IV) with A-8A-PSJ (S4) and UP 164 (S9) as vertex lines,

environment in its sector, so it's not a line that has a high average grain yield in all environment.

Discussion

The development of stable and high yielding black soybean lines is one of the goals of the current breeding program. Effects of GEIs are frequent for many quantitative traits including grain yield¹², reproductive fitness, harvest time, and biotic and abiotic resistance¹³. The emergence of GEIs effects in multi-environment experiments makes it difficult to select the superior line^{12,14}. In this study, the combined ANOVA showed that the effects of environment (E) and GEIs explained 59.49 and 32.16% of the total variability of the grain yield, respectively (Table 1). The box plot (Fig. 1) and heatmap GEIs (Fig. 2) also showed that the tested environments and lines had high grain yield variations. Since combined ANOVA cannot explain the effect of GEIs, other statistical models such as AMMI are more useful. Therefore, parametric and non-parametric measurements were used to estimate the adaptability of the lines to a wide range of environments. The magnitude of the environmental and GEIs effects showed that the test environment had a significant effect on grain yields. Differences in rainfall, humidity, and light in each environment during the experiment had a significant effect on soybean grain yields¹⁵. In this study, the Ngawi (2017) had the highest average grain yield (2.54 t/ha) compared to other environments. The average daily rainfall during the experiment in Ngawi (2017) was 15.31 mm (Table 2). According to Mandic et al.¹⁶, rainfall was related to soybean grain yields. In other studies, rainfall also affects in sweet potato yields¹⁷. In addition, the humidity during the experiment also showed suitable conditions for growth and development of soybean.

Stability and adaptability analyzes were used when the results of the combined ANOVA indicated the presence of GEIs. In this case, we used various stability measurements, namely parametric, non-parametric, AMMI, and GGE biplot. The use of various measurements aims to increase accuracy in selecting stable and high yielding lines tested^{12,18,19}. The parametric and non-parametric measurements presented in Table 4, showed that each measurement selects a different stable line. This is because each measurement has different assumptions in terms of calculating stability and adaptability. However, there were several measurements showing uniform output, namely Wi^2 , σ^2 , and θ . In other studies, it was also reported that there were parametric measurements that had similar results, including Vaezi et al.¹² on barley in Iran. According to Karuniawan et al.¹⁷, if there were stability measurements that have similar outputs, one of them can be chosen to estimate the stability of the yield. Ruswandi et al.²⁰ showed different things, all parametric and non-parametric measurements showed differences in selecting stable maize. This difference in output indicates that another approach was needed to classify the stable lines based on parametric and non-parametric measurements. The approach commonly used in cases like this was Hierarchical Cluster Analysis (HCA)^{12,17}. Based on the HCA results, the tested lines were divided into three main groups, namely unstable low yield, unstable high yield, and stable high yield groups (Fig. 3). Lines belonging to the unstable low yield group include A-4A-PSJ (S1), UP 165 (S10), A-8A-PSJ (S4), and GH Detam 5 (S13). This first group was less favored because of low grain yields, which will result in low economic income. The second group, namely unstable high yield, consists of the Detam 1 (S11), Detam 3 (S12), A-7A-PSJ (S3), and UP 164 (S9) lines. The second group can be used for specific adapt. Meanwhile, the third group, namely stable high yields, consists of DB-96-CTY (S5), UP 162 (S7), UP 161 (S6), UP 163 (S8), and A-5A-PSJ (S2). The third group was the expected ideal group. According to²¹, a stable and high yielding lines is one of the targets for soybean breeding. In addition, lines with high and stable yields are expected to increase farmers' income¹⁹. Thus, this group was strategically developed for a further sustainable soybean breeding program.

To classify parametric and non-parametric stability measurements, the principal component analysis (PCA) was used. The results of PCA (Fig. 4) and correlation analysis (Table 4) showed that the grain yield (Y) in the same group with $NP^{(2)}$, $NP^{(3)}$, $NP^{(4)}$, $S^{(3)}$, $S^{(6)}$, KR , and YSI . This group belongs to the concept of dynamic stability^{12,18}. Another group that correlates with each other were $S^{(1)}$ with $S^{(2)}$; bi with $NP^{(1)}$; S^2di with ASV , θ , σ^2 , and Wi^2 ; While CVi and θ were not grouped with other measurements. These groups were included in the concept of static stability¹². Thus, dynamic stability groups can be used to select lines in favorable environments¹⁸. While the static stability group can be used to select lines in a unfavorable environment.

AMMI biplots showed that the first two principal components (IPCA1 and IPCA2), explained 69.2% of the total variation of GEIs (Fig. 5). In Fig. 5, the lines A-4A-PSJ (S1), A-5A-PSJ (S2), A-7A-PSJ (S3), DB-96-CTY (S5), and UP 162 (S7) were closest to the biplot axis point. According to several researchers, the lines that were close to the biplot axis point were the most stable^{22,23}. The Ngawi (2017) environment has the longest vector length followed by Bogor (2019) and Banyuwangi (2016), while Malang (2018) has the shortest vector. According to²⁴, the environmental vector length from the center of the biplot was proportional to the number of GEIs. This shows that the Ngawi (2017), Bogor (2019), and Banyuwangi (2016) have a strong interaction, while the Malang (2018) environment has a weak interaction.

In the AMMI biplot PC1 vs Yield (Fig. 6), the first IPCA biplot (IPCA1) was plotted against the line and environmental mean. The environments and lines tested on the left side of the vertical line had grain yields below the overall average grain yield, while those on the right had above the overall average grain yield^{19,23}. Ngawi (2017) environment was classified as had the highest average yield compared to other environments, and the lines that close to this environment were DB-96-CTY (S5) and UP 162 (S7). The Malang (2018) environment was identified as had the lowest average yield and a small positive IPCA value close to zero while Banyuwangi (2017) has a small negative IPCA value that was close to zero with an average grain yield of less than the overall average grain yield. These two environments were not suitable for testing and development of soybeans. Lines that were close to the horizontal line have stable grain yields²⁴. Overall, of the 13 lines tested, six lines (46%) produced grain yields above the overall average grain yield, namely A-5A-PSJ (S2), A-7A-PSJ (S3), DB-96-CTY (S5), UP 162 (S7), UP 164 (S9), and Detam 3 (S12), with three lines that were close to the horizontal line (stable), namely A-5A-PSJ (S2), DB-96-CTY (S5), and UP 162 (S7). While the other seven lines (54%) had grain yields below the overall average grain yield. According to several researchers, the lines that were on the right side and close to the horizontal line were potential lines and can provide economic income^{19,23}.

The mega-environment trials uses the GGE biplot "which won where" (Fig. 7). The results of the GGE biplot analysis provided an opportunity to detect differences between environments with different characteristics related to the performance of the tested lines in these environments^{12,25}. The line at the top of the sector (vertex) has the highest yield for the environment in that sector. One of the important features of this biplot was the clustering of environments, which suggests the possibility of different mega-environments⁷. Our results showed that during 2016–2019, the environments fell into different clusters with varied environmental clustering patterns. The first two PCs accounted for 67.84% of the total variability attributable to the effects of genotype (G),

environment (E), and their interactions (GEIs) over 4 years (Fig. 7). Based on 4 years of average data, we found four mega-environments with different vertex lines. This suggests a specific adaptation of the line to the mega-environment and positive exploitation of GEIs^{12,25}. Our findings revealed that some of the new lines were better adapted to different environments on the Java island (stable) than other lines and check varieties. Based on our results, both numerical (parametric and non-parametric) and graphical (AMMI and GGE biplot) measurements resulted the same pattern for the identification of stable soybean lines. For example, parametric and non-parametric stability measurements identified the DB-96-CTY (S5), UP 162(S7), UP 161 (S6), UP 163 (S8), and A-5A-PSJ (S2) lines as the most stable and high yielding than others. The AMMI biplot identified A-5A-PSJ (S2), DB-96-CTY (S5), UP 161 (S6), and UP 162 (S7) lines, as the most stable and high yielding. The GGE biplot identified A-5A-PSJ (S2), DB-96-CTY (S5), UP 161 (S6), UP 162 (S7), and Detam 1(S11) as the most ideal lines. Similarly, Karuniawan et al.¹⁷ used a numerical measurements, AMMI, and GGE biplot to select stable sweet potato in West Java, Indonesia. Several other studies also reported the relative contribution of these various measurements to identify the ideal line, including Vaezi et al.²⁶ on barley, Oyekunle et al.²⁷ on maize, Jamshidmoghaddam & Pourdad²⁸ on safflower. From our test results, there were four new soybean lines that were identified as stable and high yielding by various stability measurements, namely A-5A-PSJ (S2), DB-96-CTY (S5), UP 161 (S6), and UP 162 (S7). They were can be further proposed as candidates for superior black soybean lines in Indonesia.

Conclusion

The effects of genotype (G), environment (E), and their interactions (GEIs) have an influence on black soybean grain yield variations with contributions of 8.35%, 59.49%, and 32.16% of the total variation, respectively. Four new black soybean lines were identified as having high and stable grain yields in diverse environments on the Java island, namely A-5A-PSJ (S2), DB-96-CTY (S5), UP 161 (S6), and UP 162 (S7). The Ngawi (2017) followed by Bogor (2019) and Banyuwangi (2016) environments has the strongest interactive. The stability measurements NP⁽²⁾, NP⁽³⁾, NP⁽⁴⁾, S⁽³⁾, S⁽⁶⁾, KR, and YSI were included in the concept of dynamic stability that can select the best lines in a favorable environment.

Materials And Methods

Plant Material and Field Experiments

Planting materials used include 10 new promising F8 black soybean lines, namely, A-4A-PSJ (S1), A-5A-PSJ (S2), A-7A-PSJ (S3), A-8A-PSJ (S4), DB- 96-CTY (S5), UP 161 (S6), UP 162 (S7), UP 163 (S8), UP 164 (S9), and UP 165 (S10), as well as three check varieties as control, namely Detam 1 (S11), Detam 3 (S12), and GH Detam 5 (S13). The experiment was carried out in eight environments in Java Island, Indonesia during four years (2016–2019) including Ngawi (2016), Banyuwangi (2016), Banyuwangi (2017), Ngawi (2017), Probolinggo (2018), Malang (2018), Bantul (2019), and Bogor (2019). The climate condition of eight environments was presented in Table 2. The field experiment used a randomized completed block design which was repeated four times. Each line was planted on a plot 3 x 5 m² with a spacing of 20 cm x 30 cm.

Grain yield measures

A total of 13 black soybean lines were observed for their grain yield following the standard Descriptor for Soyabean²⁹. Grain yield was harvested when the pods are brown. The grain yield data per plot was converted to ton/ ha.

Statistical Analysis

The combined ANOVA statistical model to estimate GEIs follows the equation

$$Y_{opqr} = \mu + G_o + E_p + GE_{op} + R_{q(p)} + B_{r(q)} + \varepsilon_{opqr}$$

where Y_{opqr} is the value of line o in plot r, and the value in location p of each replication q; μ is the grand mean; G_o is the effect of line o; E_p is the effect of the environment p; GE_{op} is the effect of genotype by environment interactions on line o and environment p; $R_{q(p)}$ is the effect of replicate q on location p; $B_{r(q)}$ is the effect of replication q on plot r; and ε_{opqr} is the error effects from line o in plot r and repeat q of location p, respectively. To calculate the combined ANOVA used Genstat 12th .

Grain yield stability was estimated using parametric and non-parametric measurements (Table 6). To calculate grain yield stability based on parametric and non-parametric measurements used STABILITYSOFT³⁰.

Identification of stable lines among the selected genotypes was conducted using AMMI following the study of³¹:

$$Y_{ijk} = \mu + G_i + E_j + \sum_{k=1}^m (\lambda_k \alpha_{ik} \gamma_{jk}) + \rho_{ijr}$$

where: Y_{ijk} is the yield in location j from line i of replication k, μ is the average of grand yield, G_i is the influence of line i, E_j is the influence of the location j, λ_k is the value of primer component k, α_{ik} and γ_{jk} were the vector score for the line i and location j to component k, ρ_{ijr} is a mistake from line i and location j

While ASV was estimated following the study of³²:

$$ASV = \sqrt{\frac{SS_{IPCA1}}{IPCA1} (IPCA1)^2 + (IPCA2)^2}$$

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ss IPCA1, ss IPCA2 were the sum of square in IPCA 1 and 2, which shows the score of the main component because of the high contribution in genotype by location interactions. IPCA1 and IPCA2 were the first and second from IPCA scores for each genotype from the AMMI analysis.

The model for GGE biplot following³³ with the formula:

$$\bar{Y}_{mn} - \mu_m = \beta_n + \sum_{k=1}^r \lambda_o \alpha_{mo} \gamma_{no} + \varepsilon_{mn}$$

where $\bar{Y}_{mn}$; μ_m ; β_n ; k ; λ_o ; α_{mo} and γ_{no} ; ε_{mn} are the performance in location 'n' from line 'm'; overall average grain yield; the influence of location 'n'; number of primer components; the singular value from primer component 'o'; value of line 'm' and location 'n' for primer component 'o'; and the error of the line 'm' in location 'n', respectively. Visualization of AMMI and GGE biplots using R program.

Declarations

Ethical Approval: All experiments on black soybean plants were carried out according to the guidelines of ILETRI – Indonesian Legumes and Tuber Crops Research Institute³⁴. The plant material used in the study is a cultivated plant, not-lethal collecting, and not threatened species. In addition, the material used was black soybeans resulted cross-bred from the plant breeding laboratory of Universitas Padjadjaran (UNPAD) and ILETRI as an effort to increase crop production to meet domestic needs. The use of this plant materials has also complied with the provisions of Law Number 11 of 2013 in Indonesia in accordance with the Ratification of the Nagoya Protocol on access to genetic resources, fair and balanced profit sharing arising from its use.

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Authors Contribution

G.W.A.S., P.H.P., A.K., conceived, planed, and designed the study; G.W.A.S., P.H.P., and A.A.W. conducted experiments and collected the data. H.M. and A.K., Analyzed the data and wrote the manuscript. H.M. prepared Figure 1-7 and Table 1-6. G.W.A.S., P.H.P., and A.A.W. critically edited the manuscript. All authors reviewed the manuscript.

Competing Interests Statement

The author(s) declare no competing interest.

Data Availability

The data used to support the findings of this study are included within the article.

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Tables

Table 1. Combined ANOVA and AMMI analysis of new promising soybean lines in eight environments

Source	df	SS	MS	F	% Treatment	% GEIs
Total	415	108.39	0.26	*	*	
Treatments	103	50.66	0.49	2.97	**	
Genotypes	12	4.23	0.35	2.13	*	8.35
Environments	7	30.13	4.31	10.21	**	59.49
Block	24	10.12	0.42	2.55	**	
Interactions	84	16.29	0.19	1.17	*	32.16
IPCA1	18	6.95	0.39	2.33	**	42.66
IPCA2	16	4.34	0.27	1.64	*	26.64
IPCA3	14	2.93	0.21	1.27		17.99
IPCA4	12	1.32	0.11	0.66		8.10
IPCA5	10	0.46	0.05	0.28		2.82
IPCA6	8	0.23	0.03	0.17		1.41
IPCA7	6	0.06	0.01	0.06		0.37
Error	288	47.62	0.17			
Minimum (ton/ha)			0.72			
Mean (ton/ha)			2.11			
Maximum (ton/ha)			3.68			
SD			0.51			
CV (%)			19.25			

df = degree freedom; SS = sum of square; MS = mean of square; GEIs = genotype by environment interactions; *p<0.05; **p<0.01; SD = Standard of deviation

Table 2. Climate conditions in the eight environments

Locations	Ngawi (2016)	Banyuwangi (2016)	Banyuwangi (2017)	Ngawi (2017)	Probolinggo (2018)	Malang (2018)	Bantul (2019)	Bogor (2019)
tavg (°C)	27.95	28.28	27.53	26.10	26.58	23.24	26.26	21.38
RH (%)	67.50	92.00	79.00	85.00	77.60	71.42	78.75	90.13
DR (mm)	18.18	31.96	33.04	15.31	17.50	4.65	2.44	14.67
ss (hour /day)	6.37	7.91	7.54	7.11	4.71	7.89	7.63	2.19
Altitude (masl)	55.00	168.00	168.00	55.00	85.00	335.00	45.00	120.00

tavg = temperature average (°C); RH = humidity (%); DR = daily rainfall (mm); ss = solar radiation (hour /day); masl = meters above sea level

Table 3. The result of stability analysis on soybean lines based on parametric and non-parametric measurements.

Genotypes	Y	s^2d	b	W^2	σ^2	CVi	θ_{000}	θ	ASV	YSI	S^{*1}	S^{*2}	S^{*3}	S^{***}	NP^{*1}	NP^{*2}	NP^{*3}
A-4A-PSJ	2.01	0.03	0.94	0.23	0.03	40.63	0.04	0.04	0.27	15.00	4.67	17.03	29.90	6.73	3.56	1.19	0.81
A-5A-PSJ	2.15	0.01	1.05	0.12	0.01	41.83	0.05	0.03	0.25	9.00	4.33	12.86	13.04	3.44	2.56	0.30	0.38
A-7A-PSJ	2.28	0.08	1.09	0.62	0.09	42.59	0.04	0.07	0.83	14.00	4.22	13.36	12.03	3.03	4.11	0.34	0.51
A-8A-PSJ	1.98	0.05	0.89	0.38	0.05	39.64	0.04	0.05	0.41	20.00	3.72	9.75	14.63	4.25	3.33	0.78	0.79
DB-96-CTY	2.20	0.03	1.07	0.20	0.03	41.85	0.05	0.04	0.30	8.00	3.56	9.00	8.00	2.22	3.11	0.31	0.42
UP 161	2.08	0.03	1.00	0.18	0.02	41.39	0.05	0.04	0.17	10.00	3.94	10.75	12.90	3.50	2.11	0.38	0.43
UP 162	2.19	0.03	1.03	0.22	0.03	40.73	0.04	0.04	0.22	6.00	3.39	10.50	8.69	2.07	2.56	0.27	0.32
UP 163	2.08	0.03	0.99	0.20	0.03	41.37	0.05	0.04	0.35	14.00	4.11	12.78	15.59	4.07	3.11	0.57	0.53
UP 164	2.22	0.04	1.06	0.33	0.04	41.71	0.04	0.05	0.59	11.00	4.39	13.75	12.22	3.11	3.33	0.36	0.43
UP 165	2.05	0.03	0.96	0.24	0.03	40.77	0.04	0.04	0.40	17.00	4.78	17.86	24.26	5.55	4.00	1.00	0.66
Detam 1	2.11	0.06	1.00	0.42	0.06	42.25	0.04	0.05	0.65	18.00	4.28	13.11	17.16	4.07	2.44	0.54	0.55
Detam 3	2.18	0.09	1.01	0.62	0.09	41.81	0.04	0.07	0.75	17.00	4.11	11.86	13.34	3.50	2.33	0.35	0.44
GH Detam 5	1.92	0.06	0.91	0.42	0.06	42.37	0.04	0.05	0.62	23.00	3.39	8.25	16.50	5.50	3.78	1.00	1.10
Rank	Y	s^2d	b	W^2	σ^2	CVi	θ_{000}	θ	ASV	YSI	S^{*1}	S^{*2}	S^{*3}	S^{***}	NP^{*1}	NP^{*2}	NP^{*3}
A-4A-PSJ	11	6	9	6	6	2	6	8	4	8	12	12	13	13	10	13	12
A-5A-PSJ	6	1	7	1	1	9	1	13	3	3	10	8	6	5	4	2	2
A-7A-PSJ	1	12	12	12	12	13	12	2	13	6	8	10	3	3	13	4	7
A-8A-PSJ	12	9	13	9	9	1	9	5	8	12	4	3	8	10	8	10	11
DB-96-CTY	3	2	10	3	3	10	3	11	5	2	3	2	1	2	6	3	3
UP 161	9	3	2	2	2	6	2	12	1	4	5	5	5	6	1	7	5
UP 162	4	5	5	5	5	3	5	9	2	1	1	4	2	1	4	1	1
UP 163	8	4	3	4	4	5	4	10	6	7	6	7	9	8	6	9	8
UP 164	2	8	8	8	8	7	8	6	9	5	11	11	4	4	8	6	4
UP 165	10	7	6	7	7	4	7	7	7	9	13	13	12	12	12	11	10
Detam 1	7	11	1	10	10	11	10	4	11	11	9	9	11	9	3	8	9
Detam 3	5	13	4	13	13	8	13	1	12	10	6	6	7	7	2	5	6
GH Detam 5	13	10	11	11	11	12	11	3	10	13	1	1	10	11	11	11	13

Y = yield; SR=Sum of rank; AR=Average of rank; SD = Standard deviation

Table 4. correlation of parametric and non-parametric stability measurements to soybean grain yield

	Y	S ¹	S ²	S ³	S ⁴	NP ¹	NP ²	NP ³	NP ⁴	K R	W ²	σ ²	s ² d	b	CVi	θ ¹	θ ²	A
Y	1.00																	
S ¹	-0.06	1.00																
S ²	-0.16	0.95	1.00															
S ³	0.76	0.45	0.38	1.00														
S ⁴	0.86	0.35	0.26	0.96	1.00													
NP ¹	0.15	0.27	0.32	0.26	0.33	1.00												
NP ²	0.81	0.29	0.24	0.86	0.94	0.44	1.00											
NP ³	0.76	0.15	0.12	0.82	0.90	0.55	0.92	1.00										
NP ⁴	0.87	0.31	0.22	0.96	0.98	0.29	0.94	0.90	1.00									
K R	0.63	-0.09	-0.10	0.62	0.72	0.31	0.67	0.83	0.69	1.00								
W ²	-0.01	-0.02	0.07	0.23	0.25	0.35	0.24	0.49	0.21	0.75	1.00							
σ ²	-0.01	-0.02	0.07	0.23	0.25	0.35	0.24	0.49	0.21	0.75	1.00	1.00						
s ² d	-0.01	0.04	0.13	0.25	0.26	0.28	0.25	0.48	0.23	0.74	0.99	0.99	1.00					
b	0.05	-0.13	-0.18	-0.15	0.02	0.71	0.10	0.27	-0.03	0.22	0.25	0.25	0.15	1.00				
CVi	-0.40	-0.11	-0.13	-0.25	-0.30	0.05	-0.33	-0.10	-0.21	-0.05	0.31	0.31	0.28	0.05	1.00			
θ ¹	-0.01	-0.02	0.07	0.23	0.25	0.35	0.24	0.49	0.21	0.75	1.00	1.00	0.99	0.25	0.31	1.00		
θ ²	0.01	0.02	-0.07	-0.23	-0.25	-0.35	-0.24	-0.49	-0.21	-0.75	-1.00	-1.00	-0.99	-0.25	-0.31	-1.00	1.00	
ASV	-0.17	0.11	0.14	0.16	0.15	0.37	0.15	0.40	0.13	0.59	0.91	0.91	0.90	0.24	0.52	0.91	-0.91	1
YSI	0.64	0.06	0.00	0.74	0.80	0.31	0.74	0.88	0.78	0.94	0.70	0.70	0.70	0.14	0.03	0.70	-0.70	0

Bold numbers correlate positively and significantly at the 5% level

Table 5. Eigenvalue, percent, and cumulative of stability measurements

PC	1	2	3	4
Eigenvalue	9.13	4.79	2.21	1.56
Percent (%)	48.07	25.23	11.62	8.22
Cumulative (%)	48.07	73.30	84.92	93.14

PC = Principal Component

Table 6. Parametric and non-parametric stability measurements

Parametric stability measurements		
Linear regression	$bi - 1 = \frac{\sum_i (x_{ij} - \bar{x}_i - \bar{x}_j + \bar{x}_{..})(\bar{x}_j - \bar{x}_{..})}{\sum_j (\bar{x}_j - \bar{x}_{..})^2}$	Eberhart and Russel ³⁵
	$s_{di}^2 = \frac{1}{N-2} \left[\sum_j (\bar{x}_{ij} - \bar{x}_i - \bar{x}_j + \bar{x}_{..}) - (bi - 2)^2 \sum_j (\bar{x}_j + \bar{x}_{..})^2 \right]$	
Mean variance component (θ_i)	$\theta_i = \frac{p}{2(p-1)(q-1)} \sum_{j=1}^q (x_{ij} - \bar{x}_i + \bar{x}_j)^2 + \frac{SSGE}{2(p-2)(q-1)}$	Plaisted and Peterson ³⁶
GE variance component ($\theta_{(i)}$)	$\theta_{(i)} = \frac{-p}{(p-1)(p-2)(q-1)} \sum_{j=1}^q (x_{ij} - \bar{x}_i - \bar{x}_j + \bar{x}_{..})^2 + \frac{SSGE}{(p-2)(q-1)}$	Plaisted ³⁷
Ecovariance value (W_i^2)	$W_i^2 = \sum_j (x_{ij} - \bar{x}_i - \bar{x}_j + \bar{x}_{..})^2$	Wricke and Weber ³⁸
Shukla's stability variance ($\sigma^2 i$)	$\sigma_i^2 = \left \frac{p}{(p-2)(q-1)} \right W_i^2 - \frac{\sum W_i^2}{(p-1)(p-2)(q-1)}$	Shukla ³⁹
Coefficient of variance (CV _i)	$CV_i = \frac{SD_i}{\bar{x}} \times 100$	Francis and Kannenberg ⁴⁰
Yield Stability Indexed (YSI)	YSI = RASV + RGY	Kang ⁴¹
Non-parametric stability measurements		
Nassar and Huehn	$S_i^{(1)} = 2 \sum_j^{n-1} \frac{\sum_{j'=j+1}^n r_{ij} - r_{ij'} }{[N(n-1)]}$	Huehn; Nassar and Huehn ^{42,43}
	$S_i^{(2)} = \frac{\sum_{j=1}^n (r_{ij} - \bar{r}_i)^2}{(N-1)}$	
	$S_i^{(3)} = \frac{\sum_{j=1}^n (r_{ij} - \bar{r}_i)^2}{\bar{r}_i}$	
	$S_i^{(6)} = \frac{\sum_{j=1}^n r_{ij} - \bar{r}_i }{\bar{r}_i}$	
Thenarasu	$NP^{(1)} = \frac{\sum_{j=1}^n r_{ij}^* - M_{di}^* }{N}$	Thenarasu ⁴⁴
	$NP^{(2)} = \frac{[\sum_{j=1}^n r_{ij}^* - M_{di}^* / M_{di}]}{N}$	
	$NP^{(3)} = \sqrt{\frac{\sum (r_{ij}^* - r_i^*)^2}{N \bar{r}_i}}$	
	$NP^{(6)} = \frac{2x [\sum_{j=1}^{n-1} \sum_{j'=j+1}^n r_{ij}^* - r_i^* / \bar{r}_i]}{N(N-1)}$	
Kang Rank	KR = RGY + R $\sigma^2 i$	Kang ⁴⁵

X_{ij} : grain yield total in of the i^{th} line in j^{th} environment, $\bar{x}_i$: average of the grain yield total from i^{th} line at all (three) environments, $\bar{x}_j$: mean of the grain yield in the j^{th} environment, $\bar{x}_{..}$: average of the grain yield total, p and q : numbers of environments and lines; SD_i : standard deviation of GEIs. r_{ij} : stability rank of the i^{th} line in the j^{th} environment; $\bar{r}_i$: average rank if i^{th} line in all environments; and N : number of environment. r_{ij}^* : stability rank of the i^{th} line in the j^{th} environment (adjusted data); M_{di}^* : adjusted data (median rank); M_{di} : unadjusted data (median rank's of the same parameters). RGY: rank of grain yield; $R\sigma^2 i$ = Rank of Shukla stability measurement.

Figures

Figure 1

GEIs Box plot from eight environments on 13 soybean lines in Indonesia. E1 = Ngawi (2016), E2 = Banyuwangi(2016), E3 = Banyuwangi (2017), E4 = Ngawi (2017), E5 = Probolinggo (2018), E6 = Malang (2018), E7 = Bantul (2019), E8 = Bogor (2019).

Figure 2
 GEIs heatmap from eight environments on 13 soybean lines in Indonesia. E1 = Ngawi (2016), E2 = Banyuwangi(2016), E3 = Banyuwangi (2017), E4 = Ngawi (2017), E5 = Probolinggo (2018), E6 = Malang (2018), E7 = Bantul (2019), E8 = Bogor (2019)

Clustering of soybean lines based on parametric and non-parametric measurements in Indonesia

Figure 4

Principal Component Analysis (PCA) of parametric and non-parametric stability measurements on soybean lines in Indonesia

Figure 5

AMMI biplot PC1 vs PC2 on 13 soybean lines in Indonesia during 2016-2019

Figure 6

AMMI biplot PC1 vs Yield on 13 soybean lines in Indonesia during 2016-2019

Figure 7

Mega-environments on 13 soybean lines in Indonesia during 2016-2019