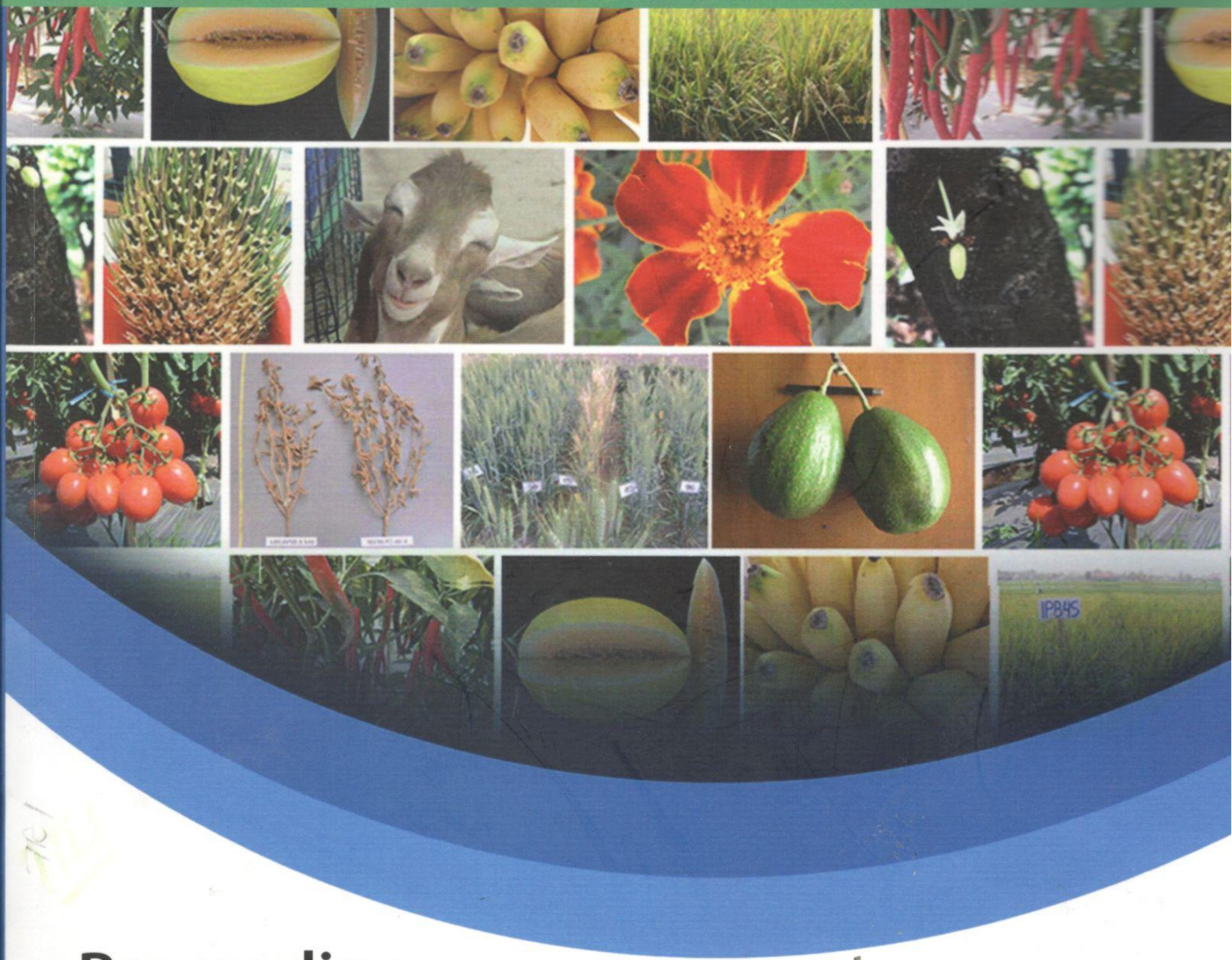

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Genetic Improvement for Productivity and Quality

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STABILITY ANALYSIS OF PUTATIVE MUTANT SOYBEAN LINES (*Glycine max* (L.) Merr) in Four Environments

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SUMMARY

Breeding program for high yielding varieties of soybean through mutation breeding conducted by BAU has produced M9 generation of putative mutant lines. The aim of this research was to evaluate the stability and adaptability of putative mutant lines in some environments. Evaluation was done on 10 putative mutant lines and two national varieties as control, i.e. Argomulyo and Tanggamus. The research was conducted in Bogor and Purwakarta, West Java in Randomized Complete Block Design (RCBD) with three replications nested in each environment. The results showed that M200-13-47-7 and M200-93-49-6 were stable lines with general adaptability. M100-46-44-6, M100-96-53-6, and M150-7B-41-10 had specific adaptation on marginal environment, meanwhile M200-39-64-4 had specific adaptation on optimum environment. M150-29-44-10 and M200-93-49-13 had the specific adaptations in the dry season in Bogor and Purwakarta, respectively.

Keywords: AMMI, Eberhart-Russell, Finlay-Wilkinson, putative mutant, stability

INTRODUCTION

Soybean is one of the major commodity with a high demand in Indonesia because it is an important source of vegetable protein. Efforts to obtain high yielding soybean varieties and stable or environmental specific is done by evaluating the multi environmental conditions (Mattjik, 2005; Mattjik and Sumertajaya, 2013). Testing in various environments need to be done because the growing environment in Indonesia is very diverse in types of land use, way of cultivation, cropping and the planting season (Sulaeman, 2012). The performance of the plant is influenced by genetic and environmental factors and their interaction. Differences in the performance of genotypes in different environments causes changes in the ranking and known as the interaction of genotype and environment (Roy 2002). Interaction of genotype and environment becomes very important if a genotype should be grown in different environments (Falconer and Mackay, 1996).

The influence of the interaction of genotype and environment on the results suggest two approaches, i.e. (1) environmental stratification into several more homogeneous environment by developing varieties that are specifically adapted to the environment; and (2) the development of a stable varieties (Trustinah and Iswanto, 2013). Stability analysis is a further analysis of genotype and environmental analysis in a combined analysis of variance. The expected results of analysis of stability is getting high yield and stable genotype in the range of diverse environments and or stable production over time, and makes it easier to predict the level of production of a certain genotypes or varieties for some time to come (Jusuf *et al.,* 2008; Wardiana and Pranowo, 2010; Purbokurniawan, 2013). The interaction of genotype and environment on soybean plants continued with the analysis of stability and adaptability has been observed by Arsyad and Nur (2006), Sudaric *et al.* (2006), Aremu *et al.* (2007), Rasyad and Idwar (2010), Susanto and Adie (2008), and Suhartina *et al.* (2012). Interaction of genotype and environment influence the grain yield per plot, harvesting age, number of pods pithy (Rasyad and Idwar, 2010), where soybean yield is closely related to soybean yield components which include the number of pithy pod, weight of 100 seeds, and pod length (Arshad *et al.,* 2006).

Breeding programs to improve soybean yield and adaptation has been done through mutation breeding by using mutagen gamma ray irradiation in Bogor Agricultural University. The research has produced some putative mutant lines that need to evaluate for yield and stability analysis. Stability analysis is an important step after selection to determine the promising lines to be released as high-yielding varieties. Genotype stability is the genotype's ability to have a similar phenotypic performance in a variety of diverse environment. A stable genotype is a genotype that has a consistent performance in different environment, while unstable genotype only perform well in a specific location. Approach to stability analysis is used to determine performance varieties in various environments and to assist plant breeders in selecting varieties. Stability analysis used in this the research is the analysis of Finlay-Wilkinson, Eberhart-Russell, and AMMI methods.

Estimation of Finlay-Wilkinson and Eberhart-Russsell yield stability based on coefficients and regression deviation. The approach of Finlay-Wilkinson and Eberhart-Russell stability analysis based on linear components of the effects of the interaction, so that if the pattern of interaction of genotype and environment are not linear it will leave considerable influence (Sumertajaya, 2005). AMMI method evolved to answers the weaknesses of the previous stability method which is more effective to explain the interaction of genotype and environment (Mattjik and Sumertajaya, 2013).

This study aimed to study the performance and stability and adaptability of putative mutant soybean lines in some environments.

MATERIALS AND METHODS

The research had been done from Oktober 2012 until June 2013 at four environments. The four environments are Bogor with altitude of 250 m above sea level and Purwakarta with altitude of 258 m above sea level in rainy and dry season. The genetic materials used for this experiments were 10 putative mutant lines M9 and M10 generations (M100-29A-42-14, M100-33-6-11, M100-46-44-6, M100-96-53-6, M150-29-44-10, M150-7B-41-10, M200-13-47-7, M200-39-64-4, M200-93-49-6 dan M200-93-49-13) and 2 national varieties (Argomulyo and Tanggamus).

The experiment was conducted in a randomized complete block design with three replications in each environments. The putative mutant soybean lines were planted in 3 m x 3 m plot with 30 cm x 20 cm. Maintenance and control of pest / weed done when pest/ weed begins to interfere with the growth of soybean plants. Plants are harvested when more than 95% brown pods, leaves turn yellow and fall, and the trunk has dried. Observation of the dry seed weight character carried out on tile (2.4 x 1.0 m) which is then converted into hectares.

The data obtained was subjected to analysis of variance and t-Dunnett. Data analysis to testing of yield stability was using the stability method according Finlay-Wilkinson and AMMI.

RESULTS AND DISCUSSION

Interaction of Genotype and Environment

Analysis of the combined variance of the four environmental tested showed that the environment and the genotype and environment interactions significantly affect the dry seed weight putative mutant lines of soybeans. However, the genotype did not significantly affect to the dry seed weight putative mutant lines of soybeans (Table 1). Based on the Table 1 sum of squares shows that the environment contributes the largest influence to the results diversity. Environmental factors as well as the interaction of genotype and environment contribute to the diversity of results for 75.1 and 8.3%, respectively.

Table 1. Analysis of the combined variance for yield parameters at different lines and environments

Source of variation	db	SS	MS	F-value	Contribute to the diversity (%)
Environment (E)	3	65.7	21.9	177.3^{**}	75.1
Rep/E	8	2.0	0.3	2.1	2.3
Genotype (G)	11	1.6	0.1	1.2^{ns}	1.8
G x E	33	7.3	0.2	1.8^{*}	8.3
Eror	88	10.9	0.1		12.4
Total	143	87.4			

*, ** Significant at p<0.05 and 0.01, respectively; ns = not significantly

Interaction of genotype and environment showed qualitative interaction and become a major factor in supporting the recommendation and genotype selection (Purbokurniawan, 2013; Stagnari *et al.,* 2013). Qualitative interaction is indicated by a change in the ranking of a genotype at each environment test (Chahal and Gosal, 2002). The results showed that the rank of putative mutant lines changed in each environment (Table 2). Several putative mutant lines of soybean grow well in certain locations, such as Bogor in dry season but not so well when planted in other locations, such as Purwakarta in rainy season.

Tanggamus varieties have the highest of dry seed weight per hectare in Bogor in rainy season (2.11 ton ha^{-1}), but the lowest in Purwakarta in rainy season (0.49 ton ha^{-1}). Weight of dry seed per hectare of entire environment ranged from 0.49 – 3.11 tons ha^{-1} (Tabel 2). Dry seed weight is highest M200-39-64-4 line at Bogor in dry season, while the lowest in Tanggamus varieties at Purwakarta in rainy season. Purwakarta in rainy season is an environment with the lowest average weight of dry seeds (0.81 ton ha^{-1}), while the Bogor in dry season is an environment with the highest average weight of dry seed (2.66 ton ha^{-1}). The low weight of dry seeds in Purwakarta in rainy season due to pod sucking pests (*Nezara viridula* and *Riptortus linearis*), pod borer (*Etiella* spp.) and the withches broom disease (phytoplasmas).

Table 2. Average and ranks putative mutant lines yield potential of soybeans and varieties on four test environment

No	Lines	B1	rank	P1	rank	B2	rank	P2	rank	Average
		ton ha^{-1}								
1	M100-29A-42-14	1.19	10	0.92	4	2.59	8	1.13	10	1.46
2	M100-33-6-11	1.46	5	0.62	10	2.53	9	1.32	7	1.48
3	M100-46-44-6	1.18	11	1.17	1	2.60	7	1.89	1	1.71
4	M100-96-53-6	1.51	4	0.88	5	2.40	11	1.74	2	1.63
5	M150-29-44-10	1.40	7	0.87	6	2.84	4	0.86	12	1.49
6	M150-7B-41-10	1.19	9	1.06	2	2.30	12	1.18	9	1.43
7	M200-13-47-7	1.64	2	1.04	3	2.94	2	1.38	5	1.75
8	M200-39-64-4	1.58	3	0.64	9	3.11	1	1.23	8	1.64
9	M200-93-49-6	1.45	6	0.61	11	2.84	3	1.38	6	1.57
10	M200-93-49-13	1.18	12	0.66	8	2.40	10	1.72	3	1.49
11	Argomulyo	1.28	8	0.81	7	2.77	5	1.11	11	1.49
12	Tanggamus	2.11	1	0.49	12	2.61	6	1.48	4	1.68
	Average	1.43		0.81		2.66		1.37		1.57

rank = the order of the potential yield of the putative mutant lines of soybeans from highest to lowest, B1 = Bogor in rainy season, P1 = Purwakarta in rainy season, B2 = Bogor in dry season, P2 = Purwakarta in dry season

Interaction of genotype and environment is a common phenomenon in plant breeding activities, as reported by Aremu *et al.* (2007), Sudaric *et al.* (2006), and Rasyad and Idwar (2010). Aremu et al. (2007) stated that the genotype instability is caused by the planting site which has very different environmental characteristics while Sudaric et al. (2006) divides the soybean genotypes planted at several locations in Croatia into three types of adaptation that is widely adapted genotypes, genotypes adapted to an optimal environment, and genotype are well adapted to the environment. It is similar to Rasyad and Idwar (2010) which divides the soybean genotypes in widely adapted genotypes and specific locations based on soybean yield components per plot.

Sucking pest and pod borer attacks cause a decrease in the quality and quantity of yield (Susanto and Adie, 2008). Damage done by sucking pest and pod borer cause low soybean seed yield per plot in North Bengkulu (Suryati *et al.,* 2010), while the attack on Gajah varieties of groundnut damage to 26.64% of pods (Apriyanto *et al.,* 2009) and generally increases based on age of the plant (Apriyanto *et al.,* 2010). Symptoms of attack of the pod borer drill a hole in the skin round pods (Marwoto and Hardaningsih, 2007; Srinivasan, 2014). Witches broom disease mainly occurs in M200-39-64-4, M200-93-49-6, M200-93-49-13 lines, Argomulyo, and Tanggamus varieties. Witches broom symptoms at the affected plants form new shoots growing from the armpit leaves in large quantities and small sizes, grow upright like a broom, the formed pods are very small. Symptoms of soybean broom are similar to the symptoms of broom *Arachis pintoi* caused by phytoplasmas in Cikabayan reported by Budiyarto and Mutaqin (2012).

Analysis Stability of Soybean

The interaction of genotype and environment that are qualitative cause high levels of uncertainty in identifying lines which have broad adaptability and difficult selecting stable genotypes (Sulaeman, 2012; Purbokurniawan, 2013). The interaction of genotype and environment requires breeders to evaluate genotypes in various environments in order to determine the exact genotype genetic potential (Chahal dan Gosal, 2002). Stability is the key word for plant breeders to analyze the interaction of genotype and environment (Mattjik, 2005). Stability below or above the average basically shows the pattern of adaptability of lines tested (Sulaeman, 2012).

The stability method gives information wheter a genotype is categorized as relatively stable or requesting specific environment. Finlay & Wilkinson's (1963) method used regression coefficient (b_i) for stability parameter. An increase to the regression coefficient (b_i >1.0) means that the genotype adaptability decreases to the changing environment, while a decrease (b_i < 1.0) means that the genotype adaptability increases. Stability analysis putative mutant soybean lines according to Finlay-Wilkinson method had a regression coefficient ranges between 0.72–1.35 (Table 1).

Calculation showed there are some lines that are stable according to Finlay-Wilkinson method. Stability analysis Finlay-Wilkinson method of producing six putative mutant soybean lines and two varieties that have a value b_i not significantly different from 1.0, and that are categorized into lines and varieties stable. M100-29A-42-14, M100-33-6-11, M150-29-44-10, M200-93-49-6 lines and Argomulyo varieties have an average weight of dry beans per hectare is lower than the average total, thus grouped into stable lines and varieties with low adaptability. M200-13-47-7, M200-93-49-6 lines and Tanggamus varieties have an average weight of dry beans per hectare is higher than the average total, thus grouped in a stable lines and varieties with high adaptability. M200-39-64-4 line stability is below the average value of bi 1.35, while M100-46-44-6, M100-96-53-6, and M150-7B-41-10 lines have stability above average -rata with b_i value respectively 0.78, 0.77, and 0.72.

Putative mutant lines were stable, the estimated range of potential yields are almost always follow the index environment and is able to sustain production growth is proportional to changes in the environment and does not give much fluctuation in the performance although grown in different environments. The higher of environmental index, the lower of expected weight of dry seed per hectare putative mutant lines. When the environmental index 1 ton ha^{-1} lines that have predictive value per hectare of dry seed weight index above environmental index, but the environmental index of 2-5 tonnes ha^{-1} had a predictive value of dry seed weight per hectare is lower than the environment index (Table 3). This shows that the M100-46-44-6, M100-96-53-6, and M150-7B-41-10 lines has the ability of adaptability on marginal soils. Environmental index 2 ton ha^{-1} in this study can be used as a standard for determining the critical point whether or not a marginal environment.

Table 3. Stability of yield 10 putative mutant soybean from the four environment based methods Finlay-Wilkinson and Eberhart Russell

No.	Lines	b_i		Mean yield	$\hat{Y}_i$ in environment					Stability criteria	
					1	2	3	4	5	Eberhart-Russell	Finlay-Wilkinson
				-------------- ton ha^{-1} --------------							
1	M100-29A-42-14	0.96^{ns}	0.00^{ns}	1.46	0.91	1.87	2.83	3.79	4.75	stable	stable/PA
2	M100-33-6-11	1.01^{ns}	-0.03^{ns}	1.48	0.91	1.92	2.93	3.94	4.95	stable	stable/PA
3	M100-46-44-6	0.78^{**}	0.10^{*}	1.71	1.27	2.05	2.82	3.60	4.38	unstable	RME
4	M100-96-53-6	0.77^{**}	0.01^{ns}	1.63	1.20	1.96	2.73	3.50	4.26	unstable	RME
5	M150-29-44-10	1.14^{ns}	0.08^{*}	1.49	0.84	1.99	3.13	4.27	5.41	unstable	stable/PA
6	M150-7B-41-10	0.72^{**}	-0.01^{ns}	1.43	1.02	1.74	2.46	3.18	3.90	unstable	RME
7	M200-13-47-7	1.05^{ns}	-0.02^{ns}	1.75	1.15	2.20	3.26	4.31	5.37	stable	stable/GA
8	M200-39-64-4	1.35^{**}	-0.02^{ns}	1.64	0.88	2.22	3.57	4.91	6.26	unstable	ROE
9	M200-93-49-6	1.19^{ns}	-0.04^{ns}	1.57	0.89	2.08	3.28	4.47	5.66	stable	stable/GA
10	M200-93-49-13	0.89^{ns}	0.08^{*}	1.49	0.98	1.87	2.76	3.65	4.54	unstable	stable/PA
11	Argomulyo	1.11^{ns}	-0.01^{ns}	1.49	0.86	1.97	3.08	4.19	5.30	stable	stable/PA
12	Tanggamus	1.04^{ns}	0.23^{**}	1.68	1.08	2.12	3.16	4.20	5.24	unstable	stable/GA
	Total mean	1.00	0.03	1.57	1.00	2.00	3.00	4.00	5.00		

b_i = regression coefficient, S^2_{di} = deviation from regression, ** = significant at b_i = 1.0; * = significant at S^2_{di} = 0.0; ns = not significant at b_i = 1.0 and S^2_{di} = 0.0, $\hat{Y}_i$ = perkiraan hasil, PA = poorly adaptability, GA = general adaptability, RME = recommended for marginal environment, ROE = recommended for optimum environment

Improved predictive value of yield M200-39-64-4 line comparable with improved environmental index. On the environmental index 1 ton ha^{-1}, M200-39-64-4 line have predictive value of dry seed weight is lower than the environmental index. Increased yield began to occur in the environmental index 2 ton ha^{-1} with the predictive value weight of dry seeds per hectare always exceed the value environmental index. It shows the line sensitivity to environmental changes. In the marginal environment M200-39-64-4 line have results below average while the optimum environment will be able to have results above average. Thus, M200-93-49-6 line including line specifically adapted to the optimal environment. Thus lines are sensitive to environmental change and provide good results in an environment that is conducive (Trustinah and Iswanto, 2013).

Eberhart and Russell (1966) extending regression approach and suggested that stability should be used to describe the unexpected deviations as a result of the interaction of genotype and environment were measured by deviation of regression. The level of adaptability genotypes measured by three parameters, namely the average yield, genotype responsive to the environment (regression coefficient), and measuring the stability (deviation regression) (Chahal and Gosal, 2002). Yield stability analysis of variance combined with Eberhart-Russell method showed that most of the putative mutant lines have stability results were not significantly different, except M100-46-44-6, M150-29-44-10, and M200-93- 49-13 and Tanggamus varieties (Table 3).

Putative mutant lines and Argomulyo varieties were not significantly different mean value deviation (deviation) regression mean squares (S^2_{di}) is relatively close to zero. Regression deviation value putative mutant lines and varieties such Argomulyo 0.00, -0.03, 0.01, -0.01, -0.02, -0.02, -0.04, and -0.01, respectively. Stable lines according to the method of stability Eberhart-Russell is a lines with a value of deviation regression (S^2_{di}) is close to zero and the regression coefficients close to one. Lines M100-29A-42-14, M100-33-6-11, M200-13-47-7, M200-93-49-6, and Argomulyo varieties as stable based on the method of stability Eberhart-Russell (Table 3).

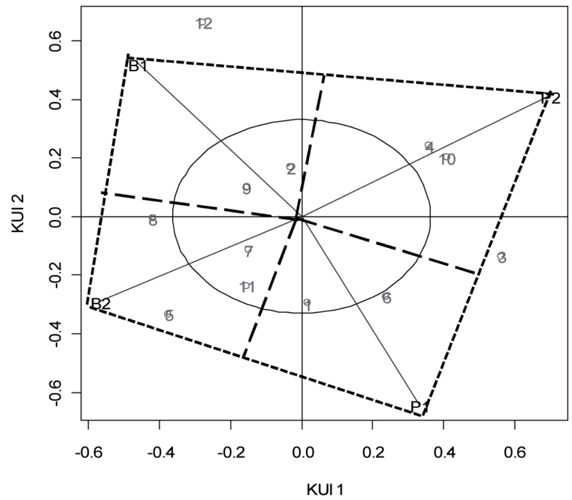
Eberhart-Russell and Finlay-Wilkinson method quite effective in selecting stable genotypes and specific. However, these approaches still leave a sizable diversity interaction because that approach only describes linear components and the interaction effect so that if the pattern of interaction of the environment is not linear genotype will leave considerable diversity. AMMI method very effectively describes the interaction of genotype by environment interaction effect decomposition performed by bilinear models, so that the suitability of a place to grow for genotype can be mapped clearly (Mattjik, 2005).

Table 4. Analysis of variance AMMI method 10 putative mutant soybean lines yield potential parameters on four environments

Source of variation	db	SS	MS	F-value	Contribute to the diversity (%)
Environment (E)	3	65.66	21.89	86.47**	
Rep/E	8	2.03	0.25	2.05	
Genotype (G)	11	1.56	0.14	1.15tn	
G x E	33	7.29	0.22	1.79*	
PCI1	13	3.82	0.29	2.38**	52.33
PCI2	11	2.63	0.24	1.94*	36.07
PCI3	9	0.85	0.09	0.76	11.60
Eror	88	10.86	0.12		
Total	143	87.41			

*, ** Significant at p<0.05 and 0.01, respectively; ns = not significantly

Results of analysis of variance AMMI showed that the number of Principal Component Interactions (PCI) that can be considered in building the model AMMI is a three component i.e PCI1-PCI3, with only two PCI significantly (Table 4), in order to obtain that the model AMMI2 as the best model to explain the stability the results of putative mutant lines of soybeans. Effect of interaction with the use of models AMMI2 reduced to two components, ie components that explain the diversity of interactions of 88.40% and components that can not be explained by a model of 11.60%. Biplot that can be made is biplot AMMI1 and AMMI2.



1: M100-29A-42-14, 2: M100-33-6-11, 3: M100-46-44-6, 4: M100-96-53-6, 5: M150-29-44-10, 6: M150-7B-41-10, 7: M200-13-47-7, 8: M200-39-64-4, 9: M200-93-49-6, 10: M200-93-49-13, 11: Argomulyo, 12: Tanggamus, B1: rainy season Bogor, B2: dry season Bogor, P1: rainy season Purwakarta, P2: dry season Purwakarta DS

Figure 1. AMMI2 biplot for dry seed weight parameter of putative mutant soybean lines in four environment

Biplot AMMI2 describe the influence the interaction between genotype and environment (Hadi and Sa'diyah, 2004). Biplot AMMI2 can be used to determine the description of genotype adaptability (Jaya and Hadi, 2008). Based on AMMI2 biplot, M100-29A-42-14, M100-33-6-11, M200-13-47-7, M200-93-49-6, and Argomulyo are widely adapted genotypes in four locations (Fig 1). Otherwise, other lines adapted to specific environments. Tanggamus variety was specific for rainy season Bogor; M150-29-44-10 was specific for dry season Bogor, and M200-93-49-13 was spesific for dry season Purwakarta.

CONCLUSION

Finlay-Wilkinson, Eberhart-Russell, and AMMI analysis revealed that M200-13-47-7 and M200-93-49-6 were stable with high adaptability, meanwhile M100-29A-42-14, M100-33-6-11, and Argomulyo were stable lines with low adaptability in all environment. M100-46-44-6, M100-96-53-6, and M150-7B-10 had specific adaptation on marginal environment and M200-39-64-4 had specific adaptation on optimum environment. Based on AMMI analysis, Tanggamus showed specific adaptation in rainy season Bogor; M150-29-44-10 and M200-39-64-4 had specific adaptation in dry season Bogor; M150-7B-41-10 had specific adaptation in rainy season Purwakarta; M100-46-44-6, M100-96-53-6, and M200-93-49-13 had specific adaptation in dry season Purwakarta. Putative mutant soybean lines M200-13-47-7 and M200-93-49-6 with high potential and stable could be proposed to be released as candidate for a new superior variety.

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