RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
51 (4) 405-418, 2019

STABILITY ANALYSIS FOR SOYBEAN IN AGROFORESTRY SYSTEM WITH *KAYU PUTIH*

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SUMMARY

Yield stability testing is an important component of the breeding before a cultivar is recommended and released for farmers. The objective of this study was to provide the best estimates of genotype mean yields in every soil types for soybean cultivars recommendations in an agroforestry system with *kayu putih* across three locations, which have three different soil types, i.e., Lithic Haplusterts, Ustic Epiaquerts, and Vertic Haplustalfs. The experiment was conducted in agroforestry system with *kayu putih* at Menggoran Forest Resort, Gunungkidul Regency, Special Province of Yogyakarta, Indonesia. The stability analysis was conducted using Shukla model and the effects of soil types on soybean cultivars were predicted using empirical best linear unbiased prediction (EBLUP). Thus, cultivars were treated as random effects to select and obtain the EBLUPs of the best cultivars in each soil types. Three soybean cultivars, i.e., Anjasmoro, Argomulyo, and Burangrang were categorized as fairly stable cultivars, while two cultivars, viz., Dering-I and Gema were categorized as relatively unstable based on the stability analysis. The EBLUPs revealed that cultivar Dering-I showed the highest yield at two soil types, i.e., Lithic Haplusterts and Ustic Epiaquerts of 1.33 and 1.25 tons.ha^{-1}, respectively. However, soybean cultivar Burangrang in the Vertic Haplustalfs yielded 1.06 tons.ha^{-1}. Therefore, this study can be used to provide recommendations of soybean cultivars, which had high stability and best performance for specific soil type.

Key words: Soybean, yield, stability analysis, cultivars, soil types

Key findings: Stability analysis revealed that soybean cultivars revealed significant differences about stability, i.e., fairly stable and relatively unstable. Furthermore,

all the soybean cultivars performed differently in three different soil types based on the EBLUPs.

Manuscript received: May 13, 2019; Decision on manuscript: July 20, 2019; Accepted: August 28, 2019.
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Communicating Editor: Dr. Sanun Jogloy

INTRODUCTION

Soybean is one of the main commodities in Indonesia after rice and maize (Ministry of Agriculture, 2015). During 2015-2018, the average domestic soybean production was 836.04 thousand tons while average import of soybean was 2.48 million tons (Ministry of Trade, 2018). Mulyani *et al*. (2017) stated that the rate of national conversion of fields was 96,512 ha.year^{-1}, and so the existing rice fields covering an area of 8.10 million hectare will be decreased to only around 5.10 million hectares in 2045. The decrease in land for rice production had a direct effect for soybean production because farmers have the habit of planting soybeans after rice planting (Mejaya *et al*., 2015). One alternative to improve soybean production is by utilizing space between *kayu putih* stands. This method is possible since *kayu putih* trees are pruned routinely to harvest the leaves. Hence, the shade factor does not affect the annual crops. Agroforestry with *kayu putih* can be carried out continuously for 30 years (Suwignyo *et al*., 2015).

Utilization of new cultivars is one of the leading technologies in agriculture that can increase of crop productivity and farmer income. These new cultivars are also the most accessible technology adopted by farmers because it is affordable and applicable (Indonesian Agency for Agricultural Research and Development, 2007). Makarim and Las (2005) stated that in order to achieve maximum yield from new cultivars, an appropriate growing environment is needed, and so the yield and superiority can be obtained. Cultivars selection for yield stability across diverse environmental conditions is a crucial part of a breeding programme to recommend the best genotype across environments (Gauch, 2006; Piepho *et al*., 2016). It is very important to do before giving recommendation of these cultivars to farmers (Piepho *et al*., 2016).

The genotype-by-environment interaction (GEI) is a phenomenon that cultivars perform differently across diverse environment. Thus, GEI will make the selection ineffective and cause difficulties in the selection of ideal and stable genotypes for all environments (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Yan *et al*., 2007). To assess GEI and to select stable genotypes, multi-environmental trials (MET) are conducted. A genotype is considered stable if it has the least interaction with the environments (Gauch, 2008; Piepho *et al*., 2016). Stability analysis aims to characterize the performance of genotypes in various environments. Cultivar stability is not only important for breeders but also important for farmers since a cultivar should be able to adapt different growing conditions in order to reduce the risk of fluctuation of yield due to unpredictable environmental changes (Baihaki and Wicaksana, 2005).

Several methods have been applied for stability analysis in soybean, i.e., Eberhart-Russel (Hossain *et al.,* 2003), Finlay-Wilkinson (Primomo *et al.,* 2002), site regression genotype plus genotype-by-environment interaction (GGE) biplot analysis (Asfaw *et al.* 2009), Kang yield-stability statistic (Pazdernik *et al.,* 2013), Shukla model (Ghiday 2016), and additive main effects and multiplicative interaction (AMMI) analysis (Yan *et al.,* 2007). However, stability analysis of soybean cultivars under agroforestry system has never been conducted. Krisnawati and Adie (2018) reported that G2 and G6 genotypes were considered as high yielding and stable promising lines of soybean across environments in which their soil types, seasonal rainfall, and altitude are different. Jandoung *et al.,* (2011) reported that soybean cultivars 'Kyado' and 'Sebore' have a good performance in soil with pH ranged from 5.5 to 6.5 and have a relative tolerance to moderate acidic soil.

The objective of this study was to perform a stability analysis and provide best predictions of yield for genotypes in each soil type for soybean recommendation in agroforestry system with *kayu putih* by using empirical best linear unbiased prediction (EBLUP). To best of our knowledge, this is the first study that report stability analysis of soybean cultivars under agroforestry system. This study provided information and options for farmers, scientist, and policy makers regarding soybean cultivars that are stable and have high yield in agroforestry system with *kayu putih*.

MATERIALS AND METHODS

Characteristics of location

This experiment was conducted during May to August, 2018 at Menggoran Forest Resort, Gunungkidul Regency, Special Province of Yogyakarta, Indonesia. This area is located ±43 km to the South-East of Yogyakarta City (Figure 1). The location had an ustic moisture regime. It was a soil regime containing limited moisture but is suitable for growing plants when the environmental conditions favor (Boettinger *et al*., 2015). The altitude of the study was ±100 meters above sea level. The average air temperature was 25.6 °C and relative humidity was 84.2 %. The total rainfall in the study location was 2,005 mm.year^{-1} (Alam and Kurniasih, 2018). The macro and micro climates in the study site were highly suitable for soybean cultivation (Djaenudin *et al*., 2011).

Soil type Lithic Haplusterts was included into the Vertisols soil type that has a shallow solum and rock contact of 50 cm from the surface. Second soil type Vertic Haplustalfs was Alfisols soil type with vertic characteristic. The third soil type, i.e., Ustic Epiaquerts was a Vertisols soil type that has fracture of >5 mm and thickness of >25 cm for 90 days each year in a reasonable condition when it is not irrigated (Soil Survey Staff, 2014). General soybean was suitable to be planted in Lithic Haplusterts and Vertic Haplustalfs. However, it was marginally suitable to be planted in Ustic Epiaquerts because the land is flooded during the wet season (Djaenudin *et al*., 2011).

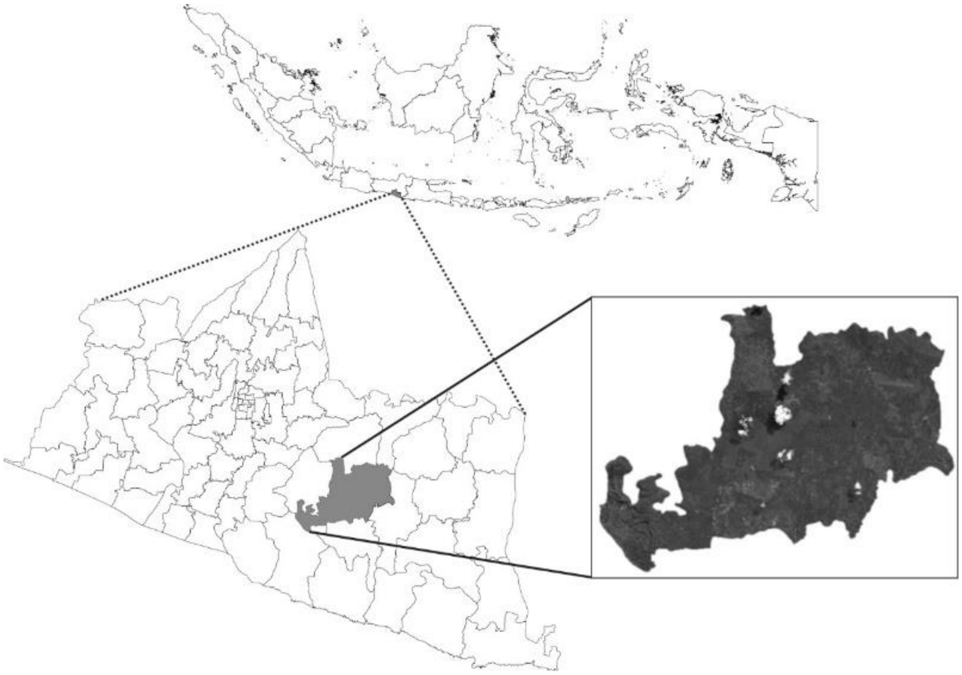

Figure 1. Geographical locations of the study area (latitude 7º 52` 59.5992`` S to 7º 59` 41.1288`` S and longitude 110º 26` 21.462``E to 110º 35` 7.4868`` E).

Previous findings revealed general information that there was no difference in the physical properties of soil (Alam and Kurniasih, 2018). The three types of soil were included in the category of clay texture with very slow permeability. Thus, they have differences in their chemical properties. The three types of soil that were significantly different from the CEC, EC, NH_4^+, P, Mn, and Zn. Similarity in terms of pH value of H_2O, soil organic matter, NO_3^-, K, Ca, Mg, Na, Cu, and Fe.

Multi-environmental trials (MET) setup

All the trials were laid out in a randomized complete block design (RCBD) with five replications. The present study was conducted to evaluate eight soybean cultivars across three soil types. Genetic materials used in this study were eight major soybean cultivars that mostly used by farmers in Indonesia. Seeds were obtained from Indonesian Legumes and Tuber Crops Research Institute in Malang Regency, Province of East Java, Indonesia. Eight soybean cultivars comprising Anjasmoro, Argomulyo, Burangrang, Demas-I, Dering-I, Devon-I, Gema, and Grobogan were used in the study. The more details regarding yield characteristics, resistant level to pest and disease, and their pedigree are presented in Table 1. The three soil types consisted of Lithic Haplusterts, Ustic Epiaquerts, and Vertic Haplustalfs. The detailed information about the properties of each soil types were presented in the Table 2.

The experimental plots cover an area of 24 m^2 (6 X 4 m) in the area between *kayu putih* stands and the harvest area of 20 m^2, excluding the

Table 1. Eight soybean cultivars (with some features) used in this study.

No.	Cultivars	Pedigree	Yield potential (tons.ha^{-1})	Pest/Disease Resistance	Specific Features
1	Anjasmoro	Mass selection for 'Mansuria' pureline	2.03-2.25	Moderate resistance to leaf rust	Resistance to pod shattering
2	Argomulyo	Introduction from Thailand	1.5-2.0	Tolerant to leaf rust	Suitable for soy milk ingredient
3	Burangrang	Pureline selection from Jember landrace	1.6-2.5	Tolerant to leaf rust	Suitable for soy milk, Tempeh, and Tofu
4	Demas-I	Derrived from 'Mansuria' × 'SJ'	2.5	Resistant to leaf rust and pod borer	Adaptive to drought and acidic soil
5	Dering-I	Single cross of 'Davros' × MLG 2984	2.8	Resistant to pod borer Resistant to leaf rust	Resistant to drought on reproductive phase
6	Devon-I	Derrived from 'Kawi' × IAC100	2.75	Resistant to leaf rust Moderate resistance to pod sucker	High isoflavon content (2,219.8 μg.g^{-1})
7	Gema	Derrived from 'Shirome' × 'Wilis'	3.06	Moderate resistance to leaf rust Moderate resistance to *S. litura*	-
8	Grobogan	Pureline selection from 'Malabar' in Grobogan	2.77	-	Less pod shattering

Source: Mejaya *et al*. (2015)

Table 2. Soil types (with properties) used in this study.

No.	Environment Parameters	Unit	Soil Types		
			Lithic Haplusterts	Ustic Epiaquerts	Vertic Haplustalfs
	Soil Physic Characters				
1	Soil Texture	-	Clay	Clay	Clay
2	Bulk Density	$g.cm^{-3}$	1.15	1.08	1.14
3	Available Soil Moisture	$mm.cm^{-1}$	3.77	3.73	2.28
4	Permeability	$cm.h^{-1}$	0.01	0.01	0.01
	Soil Chemistry Characters				
1	Cation Exchange Capacity (CEC)	$cmol^{(+)}.kg^{-1}$	58.83	65.30	32.65
2	Soil Organic Matters (SOM)	%	2.6	2.8	2.7
3	pH H_2O	-	8.2	7.8	7.7
4	Soil Nutrient Available				
	– Ammonium (NH_4^+)	ppm	39.4	56.8	51.0
	– Nitrate (NO_3^-)	ppm	86.2	143.2	82.7
	– Phosporus (P)	ppm	6.9	18.8	2.5
	– Potassium (K)	$cmol^{(+)}.kg^{-1}$	0.8	0.9	0.9
	– Sodium (Na)	$cmol^{(+)}.kg^{-1}$	0.6	0.8	0.7
	– Calcium (Ca)	$cmol^{(+)}.kg^{-1}$	5.9	5.8	5.7
	– Magnesium (Mg)	$cmol^{(+)}.kg^{-1}$	0.3	0.3	0.3
	– Iron (Fe)	ppm	12.2	12.6	9.2
	– Manganese (Mn)	ppm	32.5	32.9	35.2
	– Copper (Cu)	ppm	3.2	3.4	1.7
	– Zinc (Zn)	ppm	1.4	1.5	4.2
	Climate Characters				
1	Total Rainfall	$mm.year^{-1}$	2,005	2,005	2,005
2	Air Temperature	ºC	26.40	24.80	25.20
3	Relative Humidity	%	81.90	86.50	83.40

Source: Alam and Kurniasih (2018)

Table 3. Factors for analysis of soybean under agroforestry system with *kayu putih* by linear mixed model.

Factor	Number of Levels	Symbol
Soil type	3	S
Cultivar	8	C
Replicate	5	R

border rows. The plant spacing was 40 X 20 cm. No fertilization and pesticide were applied in this study. Irrigation was not performed due to the field used in this study was rainfed area.

Soybean variables

The observation of soybean yield was done on the seed dry weight. Soybean seeds were dried under the sunlight to the 11% of moisture level (Suryanto *et al*., 2017b).

Statistical analysis

The factors of analysis of this study were tabulated in Table 3. The baseline of the fitted model is as follows:

Cultivar × (Soil/Rep) = Soil: Rep + Cultivar + Cultivar • Soil

The fixed effect was specified before the colon and the random effects were specified after the colon. The nesting operator (/) specifies that replicate (R) was nested within factor soil types (S), the crossing operator (×) defines a full factorial model, the dot (•) between two factors indicates a crossed effect. For example, Cultivar • Soil (C•S) represents the cultivar-by-soil type interaction. The replicate was nested in the soil type. The response variable (i.e., yield), grand mean and the residual error term were implicit. The covariance structures for each factor in random effects are as follows:

i. The covariance structure for R (replicate) is $\mathbf{G}_R = \bigoplus_{j=1}^{J} \mathbf{G}_{R(j)}$,

where $\mathbf{G}_{R(j)}$ was a diagonal matrix with diagonal elements $\sigma^2_{R(j)}$, where j was the level of soil type, *j* = 1, 2, …, *J*. In other words, soil type-specific variances were assumed.

ii. The covariance structure for cultivar effects was the identity structure, i.e., $\mathbf{G}_C = \sigma^2 \mathbf{I}$.

iii. The residual covariance structure was heterogeneous with soil type-specific,

$$\mathbf{R} = \bigoplus_{j=1}^{J} \mathbf{R}_j,$$

where $\mathbf{R}_j$ was diagonal matrix with diagonal elements $\sigma^2_{\varepsilon(j)}$.

We made the model based on a generalization of the stability of variance model proposed originally by Shukla (1972) to assess the stability of cultivars. To obtain the estimates of cultivars effects per soil type with borrowing information across soil type, we fitted the effect of cultivar and cultivar • soil as random effects. Since the cultivar was assigned as random effects, the estimation was called empirical best linear unbiased prediction (EBLUP) (Littell *et al.*, 2006). All analyses were performed using PROC MIXED in SAS 9.4 (SAS Institute, 2013). The EBLUPs graphic was made in RStudio (RStudio Team, 2015) using ggplot2 package (Wickham, 2009).

RESULTS AND DISCUSSION

Stability variance estimates

The variance parameter estimates for stability with the Shukla model are presented in Table 4. The Shukla's stability variance for the effect C•S varies considerably among cultivars, which indicates stability differences. Anjasmoro, Argomulyo, and Burangrang were considered as fairly stable cultivars, while Dering-I and Gema I tended to be relatively unstable. Variance-covariance (VCOV) model of C•S term were imposed using re-parameterization of the baseline model by excluding the main

Table 4. Stability variance estimates ($10^{-3}.kg^{2}.ha^{-2}$) for the Shukla model (reporting cultivar-specific stability variances for C•S), taking C and C•S as random effects.

Cultivars	Stability Variance Estimate for C•S
Anjasmoro	0.00
Argomulyo	0.00
Burangrang	3.65
Demas-I	7.43
Dering-I	54.68
Devon-I	31.00
Gema	78.45
Grobogan	0.21

Table 5. Akaike Information Criterion (AIC) for variance–covariance structures fitted to cultivar-by-soil.

Model	Akaike Information Criterion
Identity	1549.5
Compound symmetry	1548.1
Compound symmetry heteroscedastic	1549.1
Unstructured	1552.1

effect of cultivar and imposing different VCOV models for the effect C•S using C as the subject effect. Therefore, the soil-type specific genetic effects C•S for the same cultivar were correlated between soil types, and so it allows BLUPs for a specific environment to borrow strength/information from the other environments, in this case, soil-types (Buntaran *et al.*, 2019; Piepho and Möhring, 2005; Przystalski *et al.*, 2008; Kleinknecht *et al.*, 2013; Piepho *et al.*, 2016). Piepho *et al.* (2016) noted that any lack of genetic correlation between environments corresponds to genotype-by-environment interaction. Several VCOV models for C•S were fitted: independent (ID), compound symmetry (CS), heterogeneous compound symmetry (CSH) and unstructured (UN).

The values of the Akaike Information Criterion (AIC) for different variance structures fitted to the C•S are shown in Table 5. The CS model fitted the best since its AIC was the smallest among other VCOV structures. The unstructured model, which allows having different variance and covariance, had larger AIC, means that this model was not parsimonious enough and can be considered over-fitted. Moreover, the low number of cultivars does not support more complex heteroscedastic model like the unstructured (UN) model. In the interest of searching a parsimonious model, the simpler model was generally considered preferable (Littell *et al.*, 2006).

Since the smallest AIC was the compound symmetry (CS) model and the genetic variance was estimated based on only eight cultivars, we chose to present BLUPs for the CS model (Table 6). The CS model assumes homogeneity of genetic variance between the zones and,

Table 6. Variance estimates (10^{-3}.kg^{2}.ha^{-2}) for the CS model.

Source of variation†	Group	Variance Estimate
R	Lithic Haplusterts	10.17
	Ustic Epiaquerts	3.48
	Vertic Haplustalfs	17.28
C‡		10.28
C•S‡		9.74
E	Lithic Haplusterts	16.25
	Ustic Epiaquerts	23.10
	Vertic Haplustalfs	9.91

†R, replicate; C, cultivar; C•S, cultivar-by-soil interaction; E, residual error term

‡ Obtained by fitting CS model

Table 7. Estimates of cultivar variance (on the diagonal), correlation (above the diagonal), and covariance (below the diagonal) with CS structure

Soil	1	2	3
1	20.02	0.49	0.49
2	10.28	20.02	0.49
3	10.28	10.28	20.02

therefore, the yield stability estimates the genetic variances and covariances (Table 7). Kleinknecht *et al*. (2013) also used the CS model and to analyse a zoned dataset for maize in India. As shown in Table 6, genetic variances under the CS model are relatively large compared to the other terms (excluding error term), means that the cultivars performed differently. The genetic correlation between these three soil-types is 0.49, which was relatively small (Table 7). This small genetic correlation explains that the performance of cultivars (seed dry weight) was largely different across the three soil types. Therefore, the cultivars ranking can be considered very distinct among three soil types. The replicates variance within Vertic Haplustalfs was considerably larger than the other two soil types. However, the residual of Vertic Haplustalfs was much smaller than the other soil types.

Ranking and EBLUP of eight cultivars in each soil type

The soil-pairwise scatter plot of each cultivar prediction (EBLUP) of C•S effect is presented in Figure 2. Figure 2A shows the pairwise cultivar predictions/estimates of C•S effect of Lithic Haplusterts and Ustic Epiaquerts. Figure 2B presents the pairwise cultivar predictions/estimates C•S effect of Lithic Haplusterts and Vertic Haplustalfs and Figure 2C shows the pairwise cultivar estimates C•S effect of Ustic Epiaquerts and Vertic Haplustalfs. In general, all three figures present a wide-spread of soil-pairwise cultivar predictions means that the ranking between two environments (soils) are not similar. Only in Figure 2C the scatter plot is a bit narrow than the other two. Furthermore, in Figure 2A (Lithic Haplusterts and Ustic Epiaquerts) shows lack of cultivar peformance

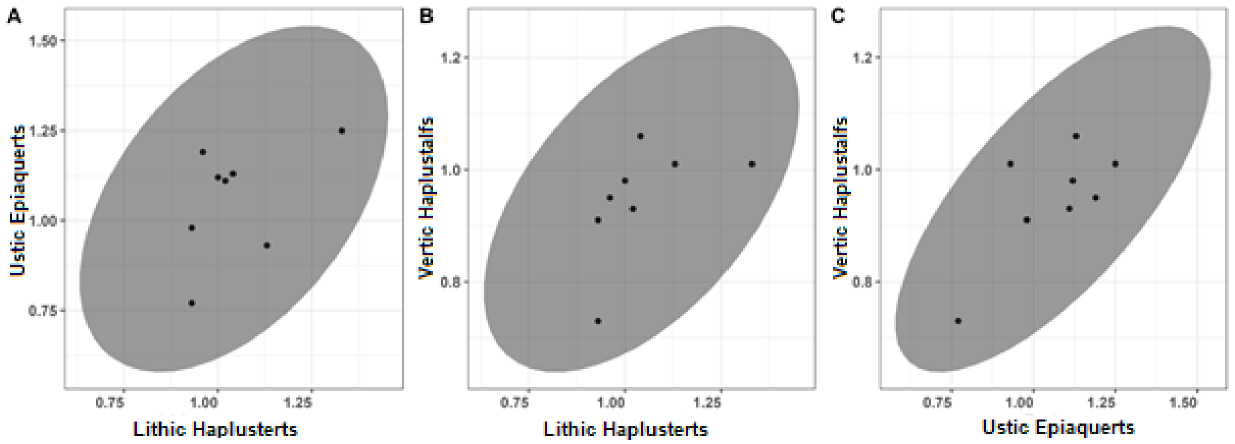

Figure 2. Soil-pairwise scatter plot of cultivar estimates of cultivar-by-soil interaction effects. (A) Estimates cultivar-by-soil between Lithic Haplusterts and Ustic Epiaquerts. (B) Estimates cultivar-by-zone between Lithic Haplusterts and Vertic Haplustalfs. (C) Estimates cultivar-by-zone between Ustic Epiaquerts and Vertic Haplustalfs.

similarity because the dots are spread widely. Thus, it can be seen that in each soil-type, each cultivar performed differently so the C•S effect was not negligible.

The EBLUPs and ranking of cultivars was showed in Table 8. The cultivars rankings were different among the three soil types, which explain the low genetic correlation. Since the C and C•S terms are random, it is possible to borrow the strength across different environments, in this case, soil types. The CS model reduced the degree of shrinkage compared to other models. The ID model will have more shrinkage than the CS model. The degree of shrinkage was reduced in the CS model since it allows borrowing strength across soil types, whereas the ID model only uses the information of the targeted soil types. We preferred to assign cultivar as random effect since the objective of the analysis was to select and predict the best cultivar in each soil type. As stated by Smith *et al*. (2005), when the objective was to select the best cultivar, the rankings of the estimated cultivar effects need to be as accurate as the true cultivar effects, which require the best prediction for the true effects.

Cultivar Dering-I showed the highest yield of soybean per hectare when getting planted in Lithic Haplusterts (1.33 tons.ha^{-1}) and Ustic Epiaquerts (1.25 tons.ha^{-1}). Burangrang showed the highest yield of soybean when getting planted in Vertic Haplustalfs (1.06 tons.ha^{-1}). The interaction between environmental factors and genotypes had the highest influence on crop yields (Jeromela *et al*., 2011). Crop yields are generally inconsistent in various locations and seasons. This is due to existence genotype × season × location interaction (Kasno and Trustinah, 2015).

Soybean cultivars showed the differences in yield per hectare when grown in Lithic Haplusterts, Ustic Epiaquerts and Vertic Haplustalfs. The yield depends on the genetics of

Table 8. The ranking and EBLUPs (10^3 kg.ha^{-1}) of eight cultivars in each soil types

Ranking	Lithic Haplusterts		Ustic Epiaquerts		Vertic Haplustalfs	
	Cultivars	EBLUP	Cultivars	EBLUP	Cultivars	EBLUP
1	Dering-I	1.33	Dering-I	1.25	Burangrang	1.06
2	Devon-I	1.13	Grobogan	1.19	Dering-I	1.01
3	Burangrang	1.04	Burangrang	1.13	Devon-I	1.01
4	Anjasmoro	1.02	Argomulyo	1.12	Argomulyo	0.98
5	Argomulyo	1.00	Anjasmoro	1.11	Grobogan	0.95
6	Grobogan	0.96	Demas-I	0.98	Anjasmoro	0.93
7	Demas-I	0.93	Devon-I	0.93	Demas-I	0.91
8	Gema	0.93	Gema	0.77	Gema	0.73

each plants (Klee and Tieman, 2013). Giller *et al*. (2011) suggested that each plant has a different response in absorbing nutrients, fertilizers, and lime applications in a site. This shows that the soil has a high heterogeneity that affects plant growth.

Suryanto *et al*. (2017a) reported that the *kayu putih* forest in Menggoran Forest Resort has two soil orders, namely Vertisols and Alfisols. Both types of soil have different soil quality which causes differences in rice yields. Alam and Kurniasih (2019) informed that Dering-I was sensitive to Manganese (Mn). The increase in Mn significantly reduced the yield of soybean. The Mn content of Lithic Haplusterts and Ustic Epiaquerts was 32.5 and 32.9 ppm, respectively, lower than that of Vertic Haplustalfs (35.2 ppm). This caused the yield of Dering-I to be higher when getting planted in Lithic Haplusterts and Ustic Epiaquerts compared to Vertic Haplustalfs.

Silva *et al*. (2017) reported that Mn poisoning in corn may reduce chlorophyll content, plant biomass and plant antioxidants. The translocation of Mn from the root to leaf triggering a decrease in chlorophyll content. High Mn concentrations cause an increase in reactive oxygen species (ROS) accompanied by higher levels of antioxidant enzyme activity and lipid peroxidation.

Burangrang is very responsive to the content of ammonium nitrogen (NH_4^+) in the soil. The increase in soil NH_4^+ would significantly increased the yield of Burangrang. The content of NH_4^+ in Vertic Haplustalfs (51.0 ppm) was higher than in soil types (Alam and Kurniasih, 2018). N is a macronutrient needed for plant growth although N compounds (i.e., NH_4^+, NO_2^-, and NO_3^-) contribute < 5% of total N in the soil (Brady and Weil, 2008). Nitrogen can be a limiting factor for plant growth after fixed carbon (Marschner, 2012). In physiology process, urea is an essential internal and external source of N which converted to ammonia for N assimilation (Wang *et al*., 2008).

CONCLUSION

Three soybean cultivars, i.e., Anjasmoro, Argomulyo, and Burangrang were found fairly stable, while two cultivars Dering-I and Gema I tended to be relatively unstable. Cultivar Dering-I with soil types Lithic Haplusterts and Ustic Epiaquerts was recommended for agroforestry with *kayu putih* at Menggoran Forest Resort, Gunungkidul Regency, Special

Province of Yogyakarta, Indonesia. However, cultivar Burangrang was recommended for soil type Vertic Haplustalfs in Indonesia.

ACKNOWLEDGEMENT

Authors like to thank the Directorate of Research of University of Gadjah Mada, Indonesia for funding this research through the University grant-in-aid scheme (No: 3129/UN1/DITLIT/DIT-LIT/LT/2018). Our special thanks goes to Mr. Harimurti Buntaran (Doctoral Student at the Swedish University of Agricultural Sciences, Uppsala, Sweden, and univeristät Hohenheim, Stuttgart, Germany) for his assistance in data analysis and his constructive comments on the manuscript.

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