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Morpho-physiological and Biochemical Fingerprints of the Soybean Agroforestry System in Different Crop Rotation Models

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ABSTRACT

Background: Soybean cultivars grown on various crop rotation models in the agroforestry system with *kayu putih* (*Melaleuca cajuputi*) have shown different yields per hectare. However, no information related to morpho-physiological and biochemical fingerprints affecting soybean yield has been found. Thus, this study aimed to determine the morpho-physiological and biochemical fingerprints and their effect on the soybean agroforestry system in different crop rotation models through multivariate analysis.

Methods: The study was conducted during the dry season (March-June 2021) and the wet season (November 2021-February 2022) in Menggoran Forest Resort, Playen Forest Section, Yogyakarta Forest Management District, Indonesia. The morpho-physiological and biochemical variables of 15 soybean cultivars were evaluated using four crop rotation models. Observations included 22 morphological, physiological and biochemical variables of soybean. Data were analyzed using ANOVA, PCA-biplot, heatmap cluster, factor analysis, SEM-PLS and standardized stepwise regression.

Result: Results showed four groups of soybean cultivars and three groups of crop rotation models based on morpho-physiological and biochemical fingerprints. Morpho-physiological and biochemical fingerprints of soybean can be differentiated based on root surface area, nitrogen content and superoxide dismutase.

Key words: Agroforestry, Crop rotation, Fingerprints, Multivariate analysis, Soybean.

INTRODUCTION

Soybean is an essential commodity in Indonesia (FAO, 2017). The Ministry of Agriculture (2022) reported that the soybean production in 2021 is only 200 thousand tons, while the demand for soybeans is around 1 million tons per year. One alternative solution is intensifying rainfed areas, particularly in an agroforestry system with *kayu putih* (*Melaleuca cajuputi*). Soybean cultivation in an agroforestry system with *kayu putih* has no competition for sunlight, nutrients and water (Alam *et al*., 2021; Faridah *et al*., 2021; Suryanto *et al*., 2022; Taryono *et al*., 2022).

The main problem of soybean cultivation among *kayu putih* stands is low productivity caused by soil moisture depending on rainfall and relatively low soil fertility (Alam *et al*., 2021; Nurmalasari *et al.,* 2020; Suryanto *et al*., 2022; Taryono *et al*., 2022). Soybean productivity in the agroforestry system with *kayu putih* can be increased by using superior soybean cultivars and crop rotation arrangements. The cultivar is a cheap and easy-to-apply technology by farmers to maximize productivity (Piepho *et al*., 2016).

Crop rotation can significantly increase crop yields by improving soil's physical, chemical and biological properties. Continuous legume cropping can reduce productivity and soil health (Ashworth *et al.,* 2017; Bowles *et al.,* 2020). Taryono *et al*. (2022) conducted a study of 15 soybean cultivars on various crop rotation models. The Dering I cultivar had the highest yield per hectare in crop rotation models of soybean planting after fallow (F-S) and rice (R-S), whereas the Grobogan soybean cultivar had the lowest yield after maize (M-S) and continuous soybean planting (S-S). However, no information regarding the morpho-physiological and biochemical variables that affect soybean yield has been found, including the fingerprints of each cultivar.

Fingerprint analysis was used to assess the morphological characteristics of novel rice lines to flooded stress (Aristya *et al*., 2021). This study aimed to determine the morpho-physiological and biochemical fingerprints and their effect on the soybean agroforestry system in different crop rotation models through multivariate analysis.

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MATERIALS AND METHODS

The study was conducted during the dry season (March-June 2021) and the wet season (November 2021-February 2022) in Menggoran Forest Resort, Playen Forest Section, Yogyakarta Forest Management district, Indonesia. The total rainfall, average air temperature, relative humidity, sun hours and sun radiation were 586 mm, 24.80°C, 85.00%, 7.78 h and 98.25 watt m^{-2}, respectively (Taryono *et al*., 2022). The geographical locations of the experimental study was 7°52′ 59.5992″S to 7°59′41.1288″S (latitude) and 110°26′ 21.462″E to 110°35′7.4868″E (longitude). The soil type was Lithic Haplusterts with a clay texture and slow drainage (Alam *et al*., 2019). The CEC, pH H_2O and SOC were categorized as very high, alkaline and low, respectively. Total N, P and K availability was classified as very low, medium and low, respectively (Taryono *et al*., 2022).

The trial was laid out in a randomized complete block factorial design with three blocks as replications. The first factor was crop rotation models consisting of soybean planting after fallow (F-S), after maize (M-S), after rice (R-S) and continuous soybean planting (S-S). The second factor was soybean cultivars, including Anjasmoro (G1), Argomulyo (G2), Baluran (G3), Biosoy I (G4), Burangrang (G5), Dega I (G6), Dena I (G7), Dena II (G8), Dering I (G9), Dering II (G10), Dering III (G11), Devon I (G12), Grobogan (G13), Mahameru (G14) and Tanggamus (G15).

Soil tillage was carried out with minimum tillage. Plant spacing was 40 cm × 20 cm with two seeds per planting hole. The fertilizer was composed of urea, SP-36 and KCl of 50, 100 and 150 kg ha^{-1}, respectively, which was applied once a week after planting (wap). No organic fertilizer application in this study. Irrigation was not carried out because it is situated in rainfed areas.

The morphological variables included root surface area (RSA), root length (RL), root dry weight (RDW), shoot dry weight, leaf dry weight (LDW), leaf area (LA), number of pods (NP) and weight of 1000 seeds (WS). The physiological variables included intercellular CO_2 content (CO_2), stomata conductivity (SC), leaf transpiration rate (LTR) and leaf photosynthetic rate (LPR). The biochemical variables included nitrogen content in the leaf tissue (NC), phosphorus content in the leaf tissue (PC), potassium content in the leaf tissue (KC), nitrate reductase activity (NRA), total chlorophyll (TC), superoxide dismutase (SOD), peroxide dismutase (POD), superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Beyer and Fridovich, 1987; Bouazizi *et al.,* 2007; Gross, 2012; Jones Jr, 2001; Krywult and Bielec, 2013; Li-Cor Bioscience Inc, 2014; Malecka *et al.,* 2014; Saravananet *et al*., 2004).

Morphological variables were observed at harvest time, whereas physiological and biochemical variables were examined during the maximum vegetative phase. The observations were carried out at the study site and the Laboratory of Crop Production Management, Faculty of Agriculture and Laboratory of Integrated Research and Testing, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Data were evaluated for normality and homogeneity using a Q-Q plot and residual vs. value graph (Welham *et al.,* 2015). Soybean cultivars and crop rotation models were classified on the basis of fingerprint identification using PCA-biplot and heatmap cluster (Widyawan *et al.,* 2020). Screening variables that affect soybean yield were analyzed using analysis of variance (ANOVA, $P<0.05$), factor analysis, structural equation modeling-partial least square (SEM-PLS) and standardized stepwise regression (Alam *et al.,* 2019; Andrews *et al.,* 2002; Suryanto *et al.,* 2020; Suryanto *et al.,* 2020). ANOVA, factor analysis and standardized stepwise regression were performed using SAS 9.4 with PROC GLM, PROC FACTOR and PROC REG (SAS Institute Inc., 2013). Bar plots with error bars, PCA-biplot and heatmap clusters were visualized using Rstudio with FactoMineR, ggplot2, GGally and pheatmap packages (Galili *et al.,* 2015; Maechler *et al.,* 2016; Le *et al.,* 2008; R Core Team, 2017; Wickham, 2009). SEM-PLS was analyzed with SmartPLS 3 (Smith *et al.,* 1993).

RESULTS AND DISCUSSION

Yields of soybean cultivars with different crop rotation models

The results of Taryono *et al*. (2022) showed an interaction between soybean cultivars and crop rotation models in the agroforestry system. The best linear unbiased prediction (EBLUP) revealed that the Dering I cultivar had the highest yields in F-S and R-S by 1.267 and 1.375 tons ha^{-1}, respectively, whereas the Grobogan cultivar in M-S and S-S obtained 1.200 and 1.349 tons ha^{-1}, respectively (Fig 1). This result is due to the interaction phenomenon between genotype and environment and each cultivar is adaptable and stable in different environments (Kasno and Trustinah, 2015).

Morpho-physiological and biochemical fingerprints of soybean cultivars and crop rotation models

Heatmap clusters are generally used to assess proximity and classify soybean cultivars based on morpho-physiological and biochemical variables. The heatmap cluster contains two main clusters based on the soybean cultivars. The first cluster consisted of G3, G15, G4, G14, G5 and G8, whereas the second cluster consisted of G9, G13, G10, G1, G2, G6, G11, G7 and G12 (Fig 2). Most of the genotypes belonging to the same cluster are also grouped in the cluster heatmap. Cantelli *et al*. (2016) reported similar results for soybean (*Glycine max*). Soybean cultivar grouping with each fingerprint identification can be continuously visualized using PCA-biplot.

The PCA-biplot showed morpho-physiological and biochemical differences that serve as fingerprints for each soybean cultivar and crop rotation model. The PCA-biplot classified soybean cultivars into four major groups based on morpho-physiological and biochemical fingerprints. The first group (quadrant I) was composed of G2, G4, G14 and G15, with H_2O_2 and O_2^- fingerprints. The second group (quadrant II) was composed of G6, G10, G11 and G12, with

LTR, SC, CO_2, TC, PC, KC and NP fingerprints. The third group (quadrant III) included G1, G5 and G8, with RL fingerprint, whereas the fourth group (quadrant IV) was G7, G9 and G13, with WS, RDW, STW, RSA, POD, SY, NC, SOD, LA and LDW fingerprints (Fig 3a).

Based on the PCA-biplot of crop rotation models, three groups were identified. The first group (quadrant I) included S-S, with O_2^- fingerprint. The second group (quadrant III) was composed of F-S and M-S, with RL fingerprint, whereas the third group (quadrant IV) comprised R-S, with NRA and TC fingerprints (Fig 3b).

PCA was used to simplify the relationship among the correlated variables. This analysis generates new variables, also known as principal components, which include important information from the original data set (Lever *et al.,* 2017). Each of the main components can be interpreted in more detail to identify the variables that have the most significant contribution to being fingerprints for each soybean cultivar and crop rotation model.

Screening of morpho-physiological and biochemical fingerprints that affect soybean yield

The ANOVA results showed that all morpho-physiological and biochemical variables showed significant differences (P<0.001), with a coefficient of variance of <40%. Therefore, all variables could be continuously analyzed using factor

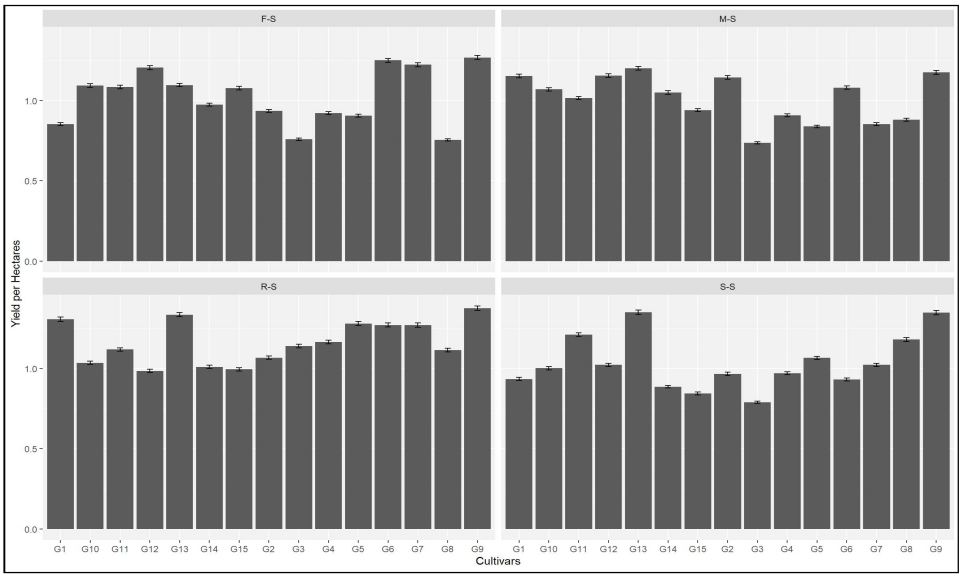

Fig 1: Visualization of EBLUP with a bar plot and error bars of soybean cultivars on various crop rotation models.

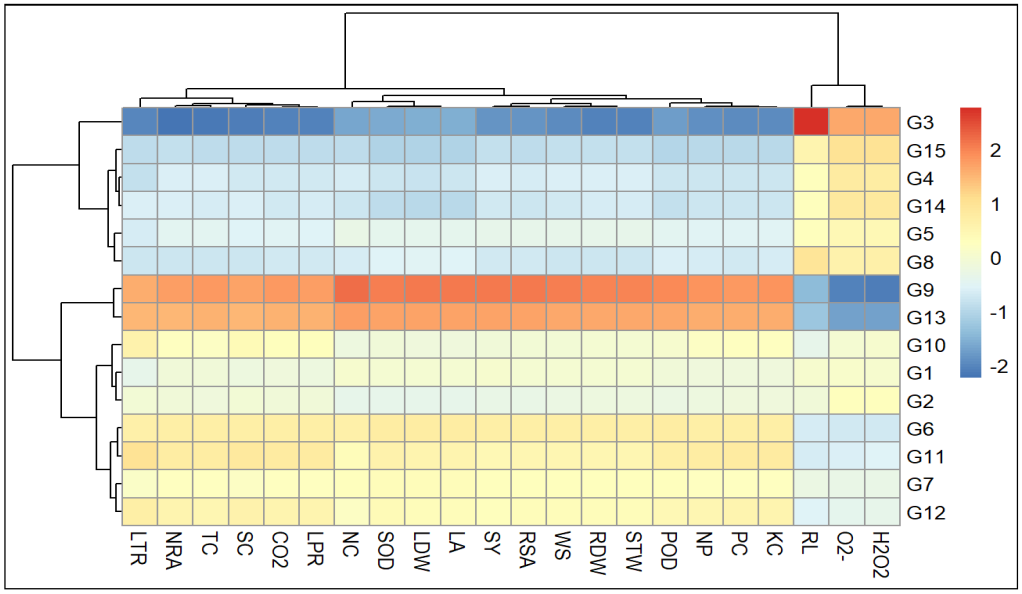

Fig 2: Heatmap cluster for classifying soybean cultivars.

analysis (Alam *et al*., 2019; Andrews *et al*., 2002; Suryanto *et al.,* 2020; Suryanto *et al.,* 2020). Factor analysis indicated two sets of factors (Table 1). Factor 1 consisted of RSA, RL, RDW, STW, LDW, LA, NP, CO_2, LPR, NC, PC, KC, NRA, SOD and H_2O_2. Factor 2 consisted of LA, WS, CO_2, SC, LTR, LPR, TC, POD, O_2^- and H_2O_2. Variables with a value and communality of >0.500 can be continued with SEM-PLS and standardized stepwise regression. Factor analysis is a multivariate technique used for high data. Varimax rotation improves the ability to interpret uncorrelated components (Govaerts *et al*., 2006; Yao *et al*., 2013).

In general, the results of SEM-PLS showed that the variables that directly affect soybean yield were physiological (-0.487**) and morphological (1.211**, Fig 4). Based on standardized stepwise regression, the variables that affected soybean yield included RSA, SOD and NC. The

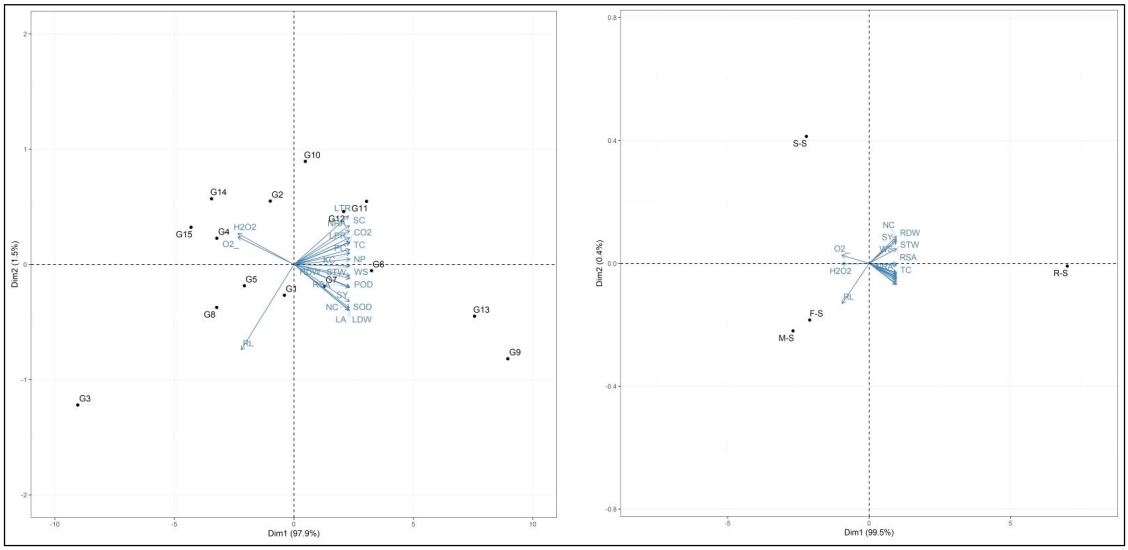

Fig 3: PCA-biplot to classify morpho-physiological and biochemical fingerprints based on soybean cultivar and crop rotation models. (a) Soybean cultivars and (b) Crop rotation models.

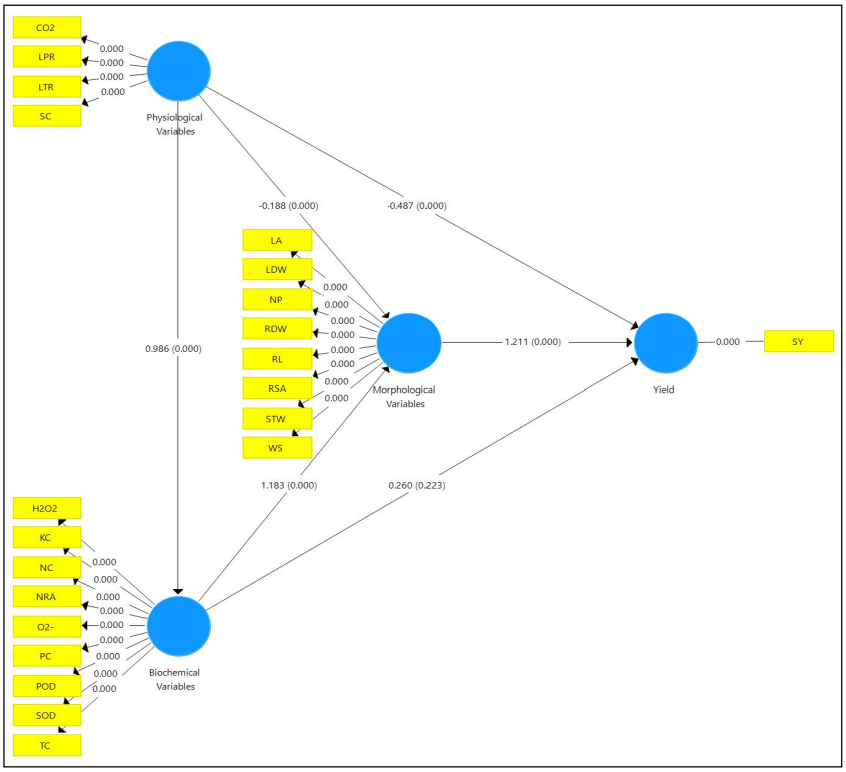

Fig 4: Relationship between morphological, physiological and biochemical variables with soybean yield.

Table 1: Factor analysis with varimax rotation of morphological, physiological and biochemical variables.

Variables	Factor 1	Factor 2	Communality
RSA	0.996*	0.031	0.990
RL	-0.940*	-0.027	0.999
RDW	0.995*	0.027	0.999
STW	0.993*	0.032	0.996
LDW	0.989*	0.029	0.999
LA	0.825*	-0.555*	0.999
NP	0.969*	0.220	0.999
WS	0.200	0.979*	0.999
CO_2	0.517*	-0.854*	0.999
SC	-0.092	0.996*	0.999
LTR	-0.105	0.994*	0.999
LPR	0.838*	-0.533*	0.999
NC	0.971*	-0.161	0.999
PC	0.969*	0.220	0.999
KC	0.976*	0.183	0.999
NRA	0.918*	-0.376	0.999
TC	0.377	0.924*	0.999
SOD	0.989*	-0.106	0.999
POD	0.387	0.918*	0.996
O_2^-	-0.189	0.982*	0.999
H_2O_2	-0.545*	-0.838*	0.999
Eigen value	12.797	7.891	

Notes- * Variables had a significant value in each group of morpho-physiological and biochemical factors.

standard stepwise regression equation was calculated as follows:

$$Y= 0.060^{**} + 0.005\ RSA^{**} + 0.051\ SOD^{**} + 0.050\ NC^{**}\ (R^2=0.999^{**})$$

Increasing the value of RSA, SOD and NC significantly increased soybean yield. Based on this, the morpho-physiological and biochemical fingerprints of soybean can be differentiated based on RSA, NC and SOD.

RSA plays a role in the absorption of nutrients in soil (Gregory, 2020). SOD is an enzymatic antioxidant enzyme that plays a role in plant defense against reactive oxygen species (Miller *et al*., 2010). Nitrogen is a nutrient needed in large quantities for photosynthesis, particularly in the formation of chlorophyll (Marschner, 2011).

Neupane *et al*. (2021) present that the soybean yield planted after maize is higher than continuous soybean. This is because planting after maize has higher soil microbial content than after rice or others. In addition, planting maize and leguminous crops in rotation can improve soil physical and chemical properties and microbial activity.

CONCLUSION

Soybean cultivars have four groups and crop rotation models have three groups based on morpho-physiological and biochemical fingerprints. Morpho-physiological and biochemical fingerprints of soybean can be differentiated based on root surface area, nitrogen content and superoxide dismutase.

Conflict of interest: None.

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