Kadapi M · Salim PO · Sumadi

Evaluation of physiological quality of seed on yield of yellow and black soybean treated with biological seed coating

Abstract. The demand for soybeans as one of cereal commodities increases every year, however, to meet the production and demand level is still challenging in Indonesia. One of the causes of low soybean production is stress during cultivation, lack of nutrients, or poor quality of soybean seeds. One solution is to increase seed protection by coating the seeds with an environmentally friendly biological seed coating. This study aims to obtain the best biological seed coating for increasing soybean seed viability, vigor, and yield. The research was conducted at the Faculty of Agriculture Experimental Station of Universitas Padjadjaran, Jatinangor from August to November 2021. Two sets of experimental design were employed in this study, a Completely Randomized Design for the viability and vigor parameters in laboratory, and a Randomized Block Design with four replications for evaluation of the yield components in the field. The experiment was carried out with a combination of soybean cultivar and biological seed coating. The cultivars used in this study were Anjasmoro cv. (yellow) and Detam-4 cv. (black) and the biological seed coatings were *B. subtilis*, *Trichoderma* sp., and *Rhizobium* sp. Post-hoc test after F-test used Least Significance Different (LSD) with a significant level of 5%. The results showed that the application of the three biological seed coatings was not significantly affect seed physiological traits. However, the seed coating treatments increased the number of seeds per plant and seed weight per plant in yellow soybeans. Meanwhile, the application of *B. subtilis* and *Rhizobium* sp. in black soybeans increased the number of seeds per plant and the weight of seeds per plant. The best biological seed coating in this experiment was *B. subtilis*.

Keywords: *B. subtilis* • Biological Seed Coating • *Rhizobium* sp • Soybean • *Trichoderma* sp.

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Kadapi M[1]*, Salim PO[2], Sumadi[1]
[1]Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran. Jalan Raya Bandung Sumedang Km 21 Jatinangor, Sumedang 45363, Indonesia.
[2]Undergraduate Program of Agrotechnology, Faculty of Agriculture, Universitas Padjadjaran. Jalan Raya Bandung Sumedang Km 21 Jatinangor, Sumedang 45363, Indonesia.
*Correspondence: kadapi@unpad.ac.id

Introduction

In Indonesia, soybean is one of important food crops which is indicated by the high demand year by year. There are two types of soybean according to the color of the seed coat, namely yellow and black. Each type of soybean has different industrial purpose, in which the yellow soybean is needed as raw materials for milk, "tempe" and tofu "tahu", while the black soybean is the main ingredient for soy sauce (STATISTICS INDONESIA, 2018). Indonesia still needs to import about 2.5 million tons per year to meet its soybean needs from several countries such as the United States, Canada, China, France, and Malaysia (STATISTICS INDONESIA, 2018), while soybean production reaches 0.9 million tons per year.

Several factors contributed to low soybean production in Indonesia such as limited arable land and less precise cultivation practices leading to low productivity. Soybean productivity is only around 1.56 tons which potential yield of soybean is up to 3 tons per hectare (STATISTICS INDONESIA, 2021; Balitkabi, 2015; Susanto & Nugrahaeni, 2017). As mentioned above, the lack of productivity may be caused by two factors, environmental and cultivation practices factors. The environmental factors may be modified in the field by cultivation practices to improve or adjust biological, chemical, and physical quality, while the others need more effort to modify such as temperature, relative humidity, rain, and light intensity. One of the efforts that can be applied to improve soybean productivity to avoid environmental stress is seed coating. Using seed coatings can increase seed vigor and viability, protect from environmental stress, maintain moisture content, and extend seed storability (Marwan & Handayani, 2019). Even, seed coating may improve seed performance by using substances such as pesticides to avoid biotic stress, nutrition, and microbes to boost growth in the early stage (Copeland & McDonald, 2004).

Seed coating with biological seed coatings or commonly known as PGPM (Plant Growth Promoting Microorganism) has several advantages such as environmentally friendly, protection from stress, increasing nutrient absorption, and stimulating plant growth (Ma, 2019). Some of microorganism that can use as seed coating substances are Rhizobium sp, Trichoderma sp. and *Bacillus subtilis*. As reported by Sutariati et al. (2006) that *B. subtilis* is an antagonistic bacterium that can induce plant growth and resistance. Tavanti et al. (2020) suggested that the application of *B. subtilis* can increase vigor, viability, and seed yield up to 15%. Also, Rhizobium sp. can be symbiotic with legume plants and form root nodules that help nitrogen fixation (Sari & Prayudyaningsih, 2015).

Inoculation of Rhizobium sp. as a biological seed coating can increase seed yield (Purwaningsih et al., 2012). Sumadi (2015) proposed that Trichoderma sp. is a seed coating substance due to its role as a decomposing organism, biological agent, and plant growth stimulator. In addition, the application of microorganism as a seed coating can increase the number of seeds per plant, seed weight per plant, and 100 seed weight (Winara et al., 2018). Based on these reports, the evaluation of some of microorganism species on physiological quality of seeds and yield of soybean is required to obtain which microorganisms can be used as biological seed coatings that are effective in increasing viability, vigor, and yield in soybean.

Materials and Methods

The experiment was conducted at the Experimental Farm of the Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Sumedang Regency, West Java with an altitude of ±750 meters above sea level in August-November 2021. The materials used in this experiment were yellow soybean cv. Anjasmoro and black soybean cv. Detam 4 obtained from the Research Center for Miscellaneous Bean and Tuber Crops (BALITKABI) Malang, East Java. Biological seed coatings, Trichoderma sp. (Tricho-G), Rhizobium sp. (Rhizobium), *Bacillus subtilis* was isolated by Microbiology Laboratory of the Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran. Polybags 30x30 cm was filled with a well-mixed of Inceptisols soil and manure (volume ratio of 3:1) as planting media. The chemical fertilizer used in present experiment were urea (0.4 g/plant) as a nitrogen source, KCl (0.8 g/plant) as potassium source, and TSP (0.6 g/plant) as phosphor source. Crop maintenance consisted of watering, weeding, and mechanical pest control based on need.

Two sets of experiment was employed in this study, a randomized group design was used as the experimental design in the field with 8 treatments including control and three replications. The treatments were a combination of two soybean cultivars coated by *B. subtilis*, Rhizobium sp., and Trichoderma sp. Each treatment consisted of 2 plants per polybag. The dose for biological coating was prepared as follows: *B. subtilis* 3 ml/100 seeds (Tavanti et al., 2020), Rhizobium sp. (Rhizobium) 5g/100 seeds (Sari et al., 2015), Trichoderma sp. (Tricho-G) 3 g/100 seeds (Sumadi et al., 2015). In the laboratory, a completely randomized design was used to analyze physiological seed traits. Analysis of variance (ANOVA) by the F (Fisher) test at a real level of 5% was used for both experimental designs. In which, the Least Significance Difference (LSD) as post-hoc test at 5% significance level was applied for comparing each treatment with its respective control (Gomez & Gomez, 1976). Moreover, normality, transformation the data and the data analysis were calculated using SPSS ver. 21.

Plant growth indicated by plant height was measured at the end of vegetative stage from the soil surface to the tip of crop. Yield components of soybean such as the number of pods per plant, the number of filled pods per plant, the number of seeds per plant, 100-grain weight, and seed weight per plant measured after harvesting and drying the seed for 3 days.

Physiological quality evaluation was conducted by observing the seed viability (germination and vigor traits) using the rolled paper with plastic test method after being coated with biological seed coatings. The paper used in this study was "merang paper" as same as towel paper in retaining moisture. The normal seed germination was observed at 5 days for FDC (First Day Count) and at 8 days for LDC (Last Day Count) in germinator. To determine the normal dry weight of seedlings, the seedlings were dried in an oven for 3 days at 80^{o}C before weighing.

Results and Discussion

Climate, Plant Height, and Physiological of Seed Quality. Watering during cultivation is required due to the low rainfall intensity during the cultivation period, i.e., 0.3 mm in August, 0.8 mm in September, 2.9 mm in October, and 20.2 mm in November. The rainfall required by soybean plants during cultivation ranges from 120-135 mm/month (Sumarno & Manshuri, 2013). Soybean require enough water in the vegetative to early generative stage, particularly at pod filling. However, in the maturation stage, the soybean requires lower than the vegetative stage. Temperature data during the study from August to November were 23^{0}C, 23.6^{0}C, 23.6^{0}C, and 23.5^{0}C respectively. The ideal temperature for soybean growth and development is 22-27^{0}C (Sumarno & Manshuri, 2013). The temperature during the implementation of the study was in the rage optimum for the growth and development of soybean. Also, the temperature was optimum for the growth of *B. subtilis* and Rhizobium sp. The average humidity during the study ranged from 84 to 93% and indicated that the relative humidity was increased in November due to rainy season. In the growth and development phase, soybean requires optimal relative humidity ranging from 75-90%, while in the pre-harvest phase soybean require lower humidity of 60- 75% (Sumarno & Manshuri, 2013).

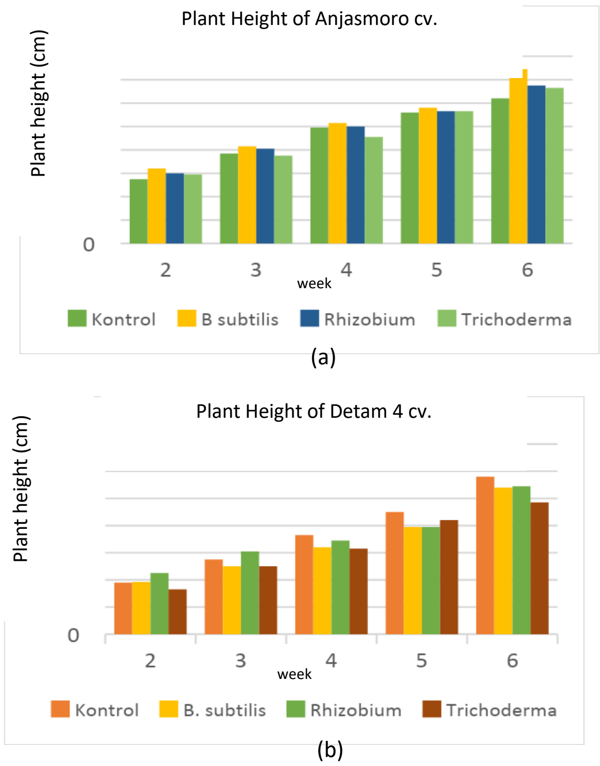

Figure 1. Plant height of yellow (a) and black (b) soybean at 2-6 weeks after planting.

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Plant height was measured at 2-6 weeks after planting in yellow soybean. Even though, these data were not calculated by statistical analysis, according to the descriptive presented in Figure 1, the application of microorganisms as bio-seed coating substances can enhance the plant height of Anjasmoro cultivars and *B. subtilis* treatment shows the highest plant height. This phenomenon is in accordance with previous research as reported by Bavaresco et al. (2020). *B. subtilis* produces phytohormones that increase plant growth. While, in Detam cultivar, the application of biological seed coatings has no direct effect on the increase in plant height.

Table 1. Effect of biological seed coatings on yellow and black soybean on seed viability and vigor.

Treatment	GP	VI	DWS (g)
Anjasmoro			
Control	90	8.55	0.14
B. subtilis	90.5	9.13	0.14
Rhizobium sp.	90.5	8.74	0.12
Trichoderma sp.	92	9.2	0.14
Detam 4			
Control	90	8.55	0.09
B. subtilis	83.5	8.26	0.10
Rhizobium sp.	84.5	8.08	0.09
Trichoderma sp.	92	8.95	0.09

note: GP = Germination Percentage, VI = Vigor Index, DWS = Dry Weight of Seedling (g)

Seed viability and vigor. The results of statistical analysis show that the physiological seed quality of two cultivars are in good condition, hence the effect of seed coating was not significantly noticed in these variables (Table 1).

Number of pods. The results of statistical analysis showed that the biological seed coating treatment did not affect the number of pods and filled pods per plant. However, there was an increase in the number of pods and the number of filled pods in yellow and black soybean due to the application of biological seed coatings. Li et al. (2021) reported that the application of *B. subtilis* can increase the availability of phosphate elements that are useful for pod formation and seed filling in soybean (Table 3).

The application of Rhizobium sp. can increase fixed nitrogen and indirectly can increase the formation of chlorophyll so that photosynthesis increases (Purwaningsih et al., 2012). Increased photosynthesis along with the production of photosynthates used for the formation and filling of pods. Harman (2006) informed that Trichoderma sp. fungus increases photosynthesis and solubility of phosphorus elements that are useful for the formation and filling of seeds.

Table 2. Effect of biological seed coatings on yellow and black soybean on number of seeds per plant and 100-grain weight.

Treatment	Number of seed	Weight of 100 seed (g)
Anjasmoro		
Control	66.25	15.65
B. subtilis	97.75*	15.33
Rhizobium sp.	81.5*	15.41
Trichoderma sp.	86.75*	15.60
Detam 4		
Control	114.75	10.38
B. subtilis	133.25**	10.41
Rhizobium sp.	127.75**	10.52
Trichoderma sp.	112	10.17

Note: The sign (*) on yellow soybean and (**) on black soybean shows that the treatment results are significantly different according to the 5% LSD test when compared to the control

Table 3. Effect of biological seed coatings on yellow and black soybean on number of pods and number of filled pods.

Treatment	Number of pod	Number of filled pod	%Filled pod
Anjasmoro			
Control	61	47.75	78.28
B. subtilis	82	60.5	73.78
Rhizobium sp.	69.25	57.5	75.81
Trichoderma sp.	69	58	84.06
Detam 4			
Control	75.5	62.5	82.78
B. subtilis	92	69.5	75.54
Rhizobium sp.	96.75	73	75.45
Trichoderma sp.	70.5	59.25	84.04

Number of Seed. The results of statistical analysis showed that the application of biological seed coatings influenced the number of seeds per plant (Table 2). This is indicated by significant differences in the application of the three seed coatings in yellow soybean, while Trichoderma sp. biological coating treatment indicated no effect in number of seed of black soybean. This is in line with several previous studies conducted by Tavanti et al. (2020), Purwaningsih et al. (2012), and Winara et al. (2018). *B. subtilis* may stimulate plant growth by producing amino acids, vitamins, and growth-inducing substances (Djaenuddin & Muis, 2015). Rhizobium sp. application increases nitrogen content and the number of seeds per plant (Purwaningsih et al., 2012). Hence, the increasing of nitrogen advantageous in the photosynthesis of plants and increase the solubility of phosphorus, that in the end support in seed formation (Lakitan, 2007; Jamilli et al., 2017).

Table 4: effect of biological seed coatings on yellow and black soybean on seed weight per plant.

Perlakuan	Seed weight (g)	Yield per ha (ton)
Anjasmoro		
Control	8.61	1.1
B. subtilis	13.17*	1.69
Rhizobium sp.	11.18*	1.43
Trichoderma sp.	11.57*	1.48
Detam 4		
Control	11.57	1.48
B. subtilis	13.62**	1.74
Rhizobium sp.	13.25**	1.7
Trichoderma sp.	10.89	1.39

Note: The sign (*) on yellow soybean and (**) on black soybean shows that the treatment results are significantly different according to the 5% LSD test when compared to the control

Seed weight. The results of statistical analysis showed that the application of biological seed coatings can increase seed weight per plant (Table 4). This is indicated by significant differences in the three treatments of biological seed coating for yellow soybean and *B. subtilis* and Rhizobium sp for black soybean. This increase in seed weight occurs along with the increase in the number of seeds. *B. subtilis* can increase seed weight per plant due to *B. subtilis* can increase the growth of soybean plant roots (Hungria et al., 2013). The increase in nutrient content along with the production of photosynthates that will be support for seed formation. The nitrogen fixation with the presence of Rhizobium sp. Bacteria, also may increase seed weight (Meirina et al., 2007). Sumadi et al. (2015) reported that the application of Trichoderma sp. can increase seed weight up to 10% when compared to the control. This is because the Trichoderma sp. can stimulate plant growth by increasing the absorption of nutrients, especially phosphorus and nitrogen, resulting in an increase in seed weight that improve the yield (Marra et al., 2019).

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Weight of 100 grains. The results of further tests showed no effect due to the application of biological seed coatings on the weight of 100 grains. The difference in 100-grain weight in yellow and black soybean is due to genetic.

Conclusion

The biological seed coating application, particularly *B. subtilis,* can be used for enhancing soybean yield as indicated by the increase of seed number and seed weight per plant in yellow soybean cv. Anjasmoro and black soybean cv. Detam. Although, the effect of biological seed coating was not showed in physiological seed traits due to high quality of seed use in this study.

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