Growth Response of Soybean Varieties to *Trichoderma* Application on Acid Soils

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Abstract. This study aims to determine the growth response of the vegetative stadia of three soybean varieties which are widely used in East Java on acid soils that have been given biological fertilizers from various *Trichoderma* isolates. The experiment was arranged factorially using a completely randomized design. The first factor was the variety consisting of Detam 3, Detam 4, Gema, Dering 1, and Burangrang. The second factor was *Trichoderma* isolates formulated as bio fertilizers, consisting of Tc-Jjr-02, Tc-Pjn-01, and Tc-Jro-01. Overall this experiment has 60 experimental units. Data were analyzed by variance analysis (ANOVA) followed by an honest significant difference test at the level of 5%. The results showed that there was no interaction between soybean varieties and *Trichoderma* isolates formulated in bio fertilizer. Soybean varieties significantly affected plant height, wet weight, and dry weight of roots and stover at 21 days after planting. The Burangrang variety shows the best growing ability in acid soils. There was no interaction between soybean varieties and *Trichoderma* bio fertilizers. *Trichoderma* isolate Tc-Jjr-02 increased the wet weight and dry weight of the roots and roots by 48.2 and 54.5%, respectively, and 38.9 and 48.2% compared to without *Trichoderma*. *Trichoderma* application maintains soil acidity between pH 4.50-4.67.

1 Introduction

Soybean as an important food industry ingredient in the world has a major impact on the adequacy of food and the health of the human body as well as the adequacy of animal feed [1]. The world demand for soybeans, which has increased rapidly in the last two decades, has encouraged crop expansion to marginal lands [2]. The decline in planting area and productivity of soybean in the past two decades has made Indonesia a constant importer of soybean because it is only able to produce no more than 40% of the national soybean needs [3]. The obstacles faced in order to utilize dry land as a measure for soybean expansion include low soil acidity and soil fertility [4]. Soil pH ≤5.5 or known as acid soil will produce high Al availability and is toxic to plants [5] and causes deficiency of macronutrients such as P, Ca, and Mg [6]. Application of lime and phosphate fertilization is another alternative to overcome soil acidity [7], but it requires an additional production cost which is not small and is often faced with the availability of materials. Research objectives

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are to find and utilize soybean germ plasma sources that are tolerant of soil acidity needs to be developed. Variety variability testing needs to be done considering that different varieties will show different levels of tolerance to soil acidity [8-9]. On the other hand, it is necessary to carry out more careful study and testing when various potential varieties of marginal land are juxtaposed with biological agents of the potential *rhizosphere* components to assist plant growth.

One of the important biological agents in the *rhizosphere* is the *Trichoderma* fungus. These fungi are cosmopolitan, easy to find from soil and organic matter [10]. Besides acting as *mycoparasites* against pathogenic fungi, it also produces metabolites that act as growth hormones for plants [11]. The results of the decomposition of organic matter by the *Trichoderma* fungus [12] are expected to support the growth of soybean plants. On the other hand, *Trichoderma* is able to live well on acid soils and even some of them are able to show optimal activity at a pH of less than 5.5 [13].

Testing the ability of various soybean varieties to overcome acid soil stress needs to be carried out. The growth of young plants, the speed at which they grow, and the success of seed germination are highly dependent on environmental conditions [14]. Meanwhile, the *Trichoderma* fungus which has the potential as a biological fertilizer agent also needs to be tested for its ability to survive and play a role in helping plants in acidic soil conditions. From the combination of testing between varieties and the application of *Trichoderma* fungi as biological fertilizers, it is expected to know how far the potential interactions can increase the performance of soybean plants in acid soils. The ability of plants and their life partners in the *rhizosphere* in facing acidic soil stress can be seen in the growth performance of soybean plants in the early vegetative phase. A good ability to live in this phase will guarantee optimal production success.

This study aims to determine the three-stage growth response of various soybean varieties that are widely used in East Java grown on acid soils that have been given biological fertilizers from various *Trichoderma* isolates.

2 Methods

2.1 Preparation and planting

Three *Trichoderma* isolates, namely Tc-Jjr-02, Tc-Pjn-01, and Tc-Jro-01 (collection of the Microbiology Laboratory of Muhammadiyah University, Sidoarjo) were cultured in PDA-m media [15]. 10 days the culture is harvested and formulated as biofertilizer with compost carrier that has been sterilized in autoclave. By adjusting the propagule suspension dilution, the conidiophores population of each *Trichoderma* in the compost was made the same, namely 10^8 CFU.g^{-1}. Compost that already contains *Trichoderma* isolates is ready to be applied as biological fertilizers. Meanwhile, alpha soil was prepared from former paddy fields planted with local varieties of soybean in Purwojati village, Ngoro sub-district, Mojokerto district, East Java with an average pH of 4.8; thus this land can be said to be acid soil.

In this study, indigenous nodule bacteria were used which were isolated from local soybean root nodules and reproduced in PDA media. The acid soil is to be used as a planting medium, mashed and sieved through a 100 mesh sieve, and sterilized in an autoclave. The soil is then put into the polybag with a capacity of 5 kg. Furthermore, the soil is composted at a dose of 200 g per polybag. For the biological fertilizer treatment, the compost was given with the same dosage as the treatment without *Trichoderma* but contained $2x10^{10}$ CFU.g^{-1} of *Trichoderma* conidiophores. Thus, one polybag contained an average of 4 $x10^6$ CFU.g^{-1} of planting medium. The soybean seeds tested in this study were

five varieties of soybean seeds obtained from Balitkabi - Department of Agriculture, Malang, East Java. The five varieties are Detam 3 and Detam 4, which are black soybeans that are commonly used to make soy sauce, while the other three varieties (Gema, Dering 1, and Burangrang) are types of soybean commonly used as the main ingredient for making tofu and tempeh. Soybean seeds from the five varieties were germinated on sterile paper towels soaked in aqua dest to maintain moisture and were inoculated with propagules/nodule bacterial cells taken from the culture with a population average of 10^9 CFU.ml^{-1} suspensions. Germination is uniformly regulated so that when transplanting into polybags they have the same germination size. The day before planting, the soil pH was measured and the soil chemical analysis was carried out, especially the content of N, P. K, Na, Ca, Mg, and soil cation exchange capacity. Fertilization is done once at the age of 14 days after planting, which is equivalent to 18.75 kg.ha^{-1} N, 50.00 kg.ha^{-1} P2O5, and 18.75 kg.ha^{-1} K2O [16]. Since mixing until the end of the savings, which is 21 days after planting and is considered the end of stage 3 vegetative soybean, no fertilizer is given except for compost which is a component of the growing media in this experiment.

2.2 Experimental design

This factorial experiment was arranged in a completely randomized design (CRD). The first factor is the types of varieties consisting of Detam 3 (V1), Detam 4 (V2), Echo (V3), Dering 1 (V4), and Burangrang (V5). The second factor was the type of *Trichoderma* isolate, the biological fertilizer agent consisting of: without *Trichoderma* (T0), isolate Tc-Clkt-01 (T1), isolate Tc-Jro-01 (T2). And isolate Tc-Jjr-02 (T3). With 3 repetitions, a total of 60 experimental units were obtained. The variables observed in this experiment were: plant height (cm), stem diameter (cm), number of leaves, wet and dry weight of roots (g), wet and dry weight of roots (g), wet and dry weight of stover (gr), and the pH of the planting medium.

2.3 Statistical analysis

All data were analyzed using analysis of variance (level 5%) to determine whether there was an effect of treatment on the response of plants in terms of vegetative growth until the end of the vegetative stage 3. With respect to the results of the analysis of the very significant variety of observational variables ($p < 0.01$), the Honest Significant Difference (HSD) test was performed at the 5% level.

3 Results and discussion

3.1 The plant growth

The results of the analysis of variance showed that soybean varieties had a significant effect ($p<0.05$) on plant height up to 21 days after planting (DAP), but had no significant effect on stem diameter and a number of leaves per plant ($p>0.05$). *Trichoderma* and its interaction with soybean varieties had no significant effect ($p>0.05$) on height, stem diameter, and number of leaves per plant from 7 to 21 DAP. The average plant heights of 7-21 DAP in all tested soybean varieties is shown in Figure 1.

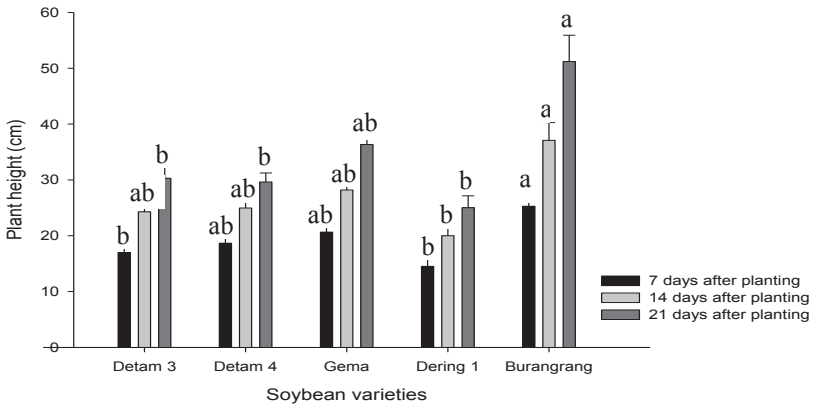

Fig. 1. The mean of plant height for various varieties of soybeans at 7-21 DAP (cm). Different letters on top of the same bars showed a significant difference between varieties according to the HSD test ($p < 0.05$).

3.2 Biomass production

3.2.1 Wet and dry weight of roots

The results of the analysis of variance showed that the *Trichoderma* variety and isolate of biological fertilizer had a significant effect ($P<0.05$) on root wet weight; however, in terms of dry weight, only varieties had a significant effect. The interaction between varieties and *Trichoderma* isolates did not significantly affect both wet weight and root dry weight. The mean wet and dry weight for all varieties is shown in Figure 2, meanwhile, the mean wet and dry weight of the roots in the *Trichoderma* isolate treatment can be seen in Figure 3.

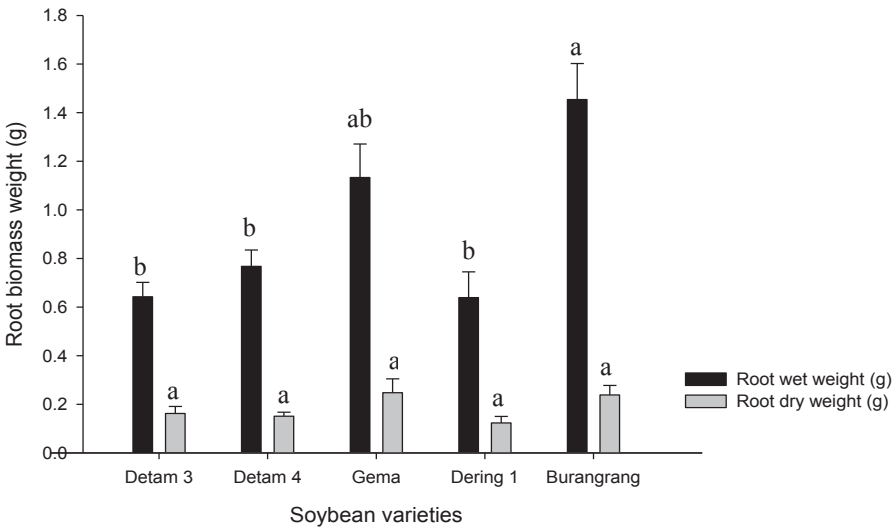

Fig. 2. The mean of wet weight and dry weight of roots in varieties of soybeans at 21 DAP various *Trichoderma* isolates (gr). Different letters on top of the same bars showed a significant difference between varieties according to the HSD test ($p < 0.05$)

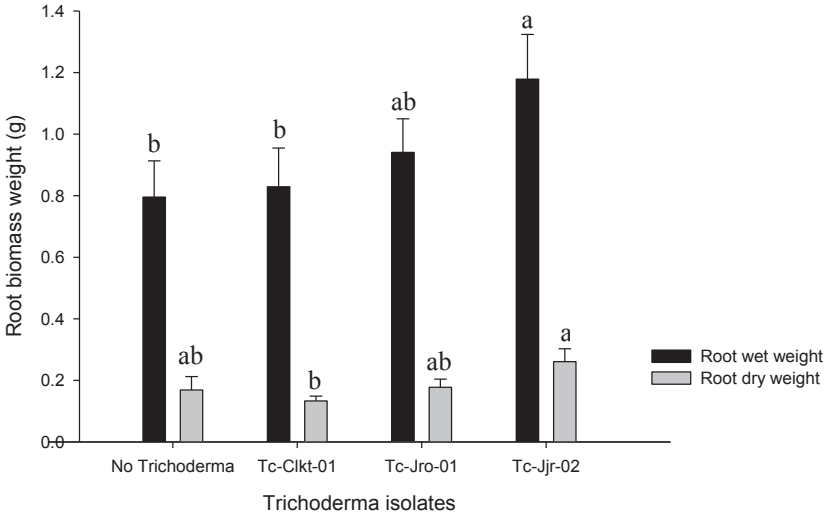

Fig. 3. The mean of wet weight and dry weight of roots in various *Trichoderma* isolates at 21 DAP (gr). Different letters on top of the same bars showed a significant difference between the applications of *Trichoderma* isolates according to the HSD test ($p < 0.05$)

3.2.2 Wet and dry weight of stover

The results of the analysis of variance showed that the variety only had a significant effect ($p<0.05$) on the wet weight of stover. *Trichoderma* isolates and their interactions with varieties had no significant effect on the wet weight and dry weight of stover at the end of the vegetative stage 3. The mean wet and dry weights in all treatment varieties are presented in Figure 4, while all *Trichoderma* isolates are presented in Figure 5.

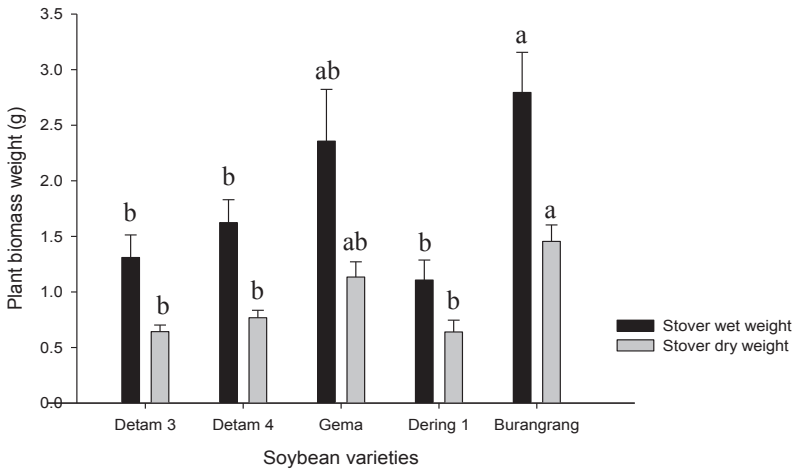

Fig. 4. The mean of wet weight and dry weight of stover in varieties of soybeans at 21 DAP various *Trichoderma* isolates (gr). Different letters on top of the same bars showed a significant difference between varieties according to the HSD test ($p < 0.05$)

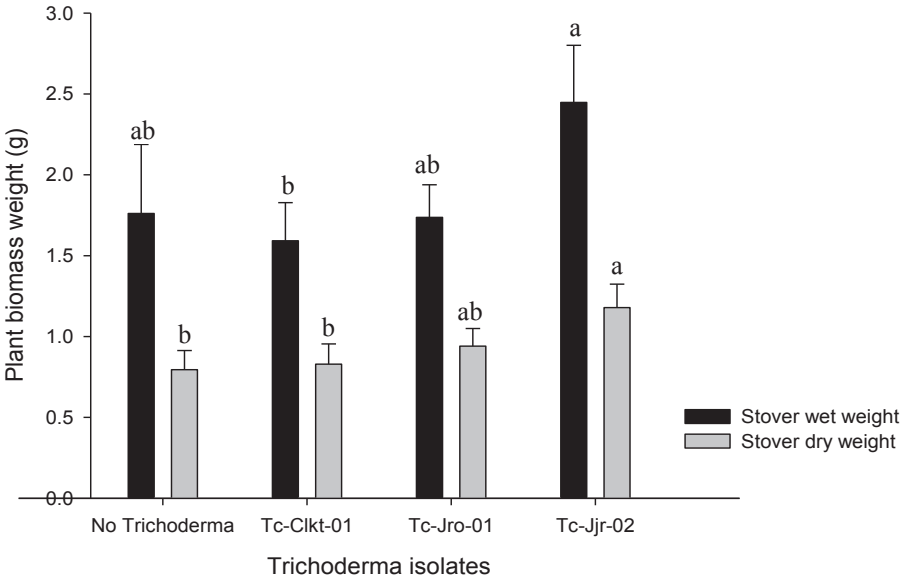

Fig. 5. The mean of wet weight and dry weight of stover in various *Trichoderma* isolates at 21 DAP (gr). Different letters on top of the same bars showed a significant difference between the applications of *Trichoderma* isolates according to the HSD test ($p < 0.05$)

The Detam 3, Detam 4, Gema, and Dering 1 varieties showed suboptimal vegetative growth. The total growth biomass represented by root wet weight and stover wet weight (Figures 2 and 4) appeared to be lower than Burangrang. This shows that the four varieties are less tolerant of acidic stress. Low pH soils will increase Al solubility [17], low P availability [18], N deficiency, and other nutrients [19] and inhibit plant root symbiosis with Rhizobium [20]; thus Al is the cause of the decline in all agronomic parameters [21].

Until the end of the third stage of vegetative growth, the Burangrang variety showed the highest values in almost all parameters, namely plant height (Figure 1), wet weight and dry weight of roots (Figure 2), and wet weight and dry weight of stover (Figure 4). This indicates that Burangrang is more tolerant than other varieties in dealing with soil acidity stress during the early vegetative growth period.

The differences in the response of soybean plants in different varieties reflect differences in genetic potential in responding to environmental influences including acid soil stress. However, these five varieties are not acid-tolerant varieties [22]. Therefore, the appearance of vegetative growth in stage three is likely influenced by *Trichoderma* activity in the rhizosphere.

Of the three isolates tested and compared with the treatment without *Trichoderma*, it was seen that the isolate Tc-Jjr-02 gave a higher plant response in terms of wet weight and dry weight of roots (Figure 3), as well as wet weight and dry weight of stover (Figure 5). It appears that Tc-Jjr-02 is able to play its role in producing plant growth hormone [23-24], various enzymes that degrade organic matter [25-26] that produce nutrients for plants, producing various important metabolites [27] that can support growth and plant health [28].

Auxins affect stem growth [29] and their synergy with cytokines is closely related to root growth.

3.3 Planting media pH and *Trichoderma* population

Based on the analysis of variance, it was found that soybean varieties and *Trichoderma* isolates and the interaction between them did not significantly affect the pH of the growing media ($p>0.05$). The mean soil pH of growing media on soybean varieties and *Trichoderma* isolates is presented in Table 1.

Table 1. The mean soil pH of growing media on several soybean varieties and isolates of *Trichoderma* 21 DAP

Variety	soil pH[*)]	*Trichoderma* isolates of biofertilizer	soil pH [*)]
Detam 3	4.63±0.11	Without *Trichoderma*	4.67±0.12
Detam 4	4.58±0.14	*Trichoderma* Tc-Clkt-01	4.60±0.10
Gema	4.54±0.13	*Trichoderma* Tc-Jro-01	4.57±0.12
Dering 1	4.54±0.13	*Trichoderma* Tc-Jjr-02	4.50±0.12
Burangrang	4.54±0.13		

[*)] The pH of the soil at the beginning of planting is 4.80±0.12

Both the varieties and *Trichoderma* isolates and their interactions did not significantly affect the *Trichoderma* population at the end of the observation ($p>0.05$). The mean population of *Trichoderma* in various soybean varieties and some *Trichoderma* isolates is shown in Table 2.

The highest living population of *Trichoderma* conidiospores was found in the soil of the Detam 3 variety soybean planting medium, namely 2.31 x 10^7 CFU.g^{-1}, followed by Gema, Dering 1, and Detam 4 at 21 DAP. On the other hand, at the end of the observation (21 DAP), it appears that the conidiospores of the Tc-Jjr-02 isolate showed the highest population.

Table 2. The mean populations of Trichoderma conidiophores on soybean varieties and Trichoderma isolates at 21 DAP

Variety	Population of *Trichoderma* conidiospores (CFU.gr^{-1} of soil)	*Trihcoderma* isolates of biofertilizer	Population of *Trichoderma* conidiospores (CFU.gr^{-1} of soil)
Detam 3	2.31 x 10^7	Without *Trichoderma*	0
Detam 4	1.17 x 10^7	*Trichoderma* Tc-Clkt-01	2.06 x 10^7
Gema	1.61 x 10^7	*Trichoderma* Tc-Jro-01	0.96 x 10^7
Dering 1	1.45 x 10^7	*Trichoderma* Tc-Jjr-02	2.84 x 10^7
Burangrang	0.77 x 10^7		

All tested *Trichoderma* isolates were unable to increase the soil pH of the growing media, although it has been reported that this fungus is able to cope with copper stress [30] in acid soils. In this experiment, it appears that this fungus is unable to cope with acid soil stress which increases the availability of metals, especially Al [17]. Soil with a pH of ≤ 5.5 will affect the biotic activity and growth of fungal cells and various other microflora in the rhizosphere [31]. Many species of fungi in the genus *Trichoderma* have the ability to establish special relationships with their hosts [32-33] as bio control agents. Fungi like this

in their activity in the rhizosphere will produce specific molecules that encourage the emergence of plant responses in compounds that are important in defense mechanisms against pathogens such as H_2O_2, anthocyanins, camalexin, and various induced proteins [34-35]. In this case, soybean plants use more energy and metabolites to produce defense compounds for pathogens. Therefore, it appears that all plant growth parameters treated with the Tc-Jro-01 bio-fertilizer showed the lowest values. On acid growing medium soil (pH 4.5) (Table 2), the population of Tc-Jjr-01 isolate showed the lowest population of 0.96 x 10^7CFU.g^{-1} compared to other isolates (Table 1,2). This is in line with the fact that the biomass of *Trichoderma* fungi in Czapeks-dox liquid medium decreased from an average of 1.35-1.40 g at pH 6.5 to 1.20-1.36 at pH 4.5 [13]. The results of this experiment indicated that there was a difference in the sporulation response among the *Trichoderma* isolates tested (Table 2). Some *Trichoderma* isolates were reported to show no difference in sporulation levels at pH 4 compared to pH 6, while several other *Trichoderma* isolates showed a higher total spore at pH 6 than at pH 4 [36].

4 Conclusion

Soybean varieties had a significant effect on plant height, wet weight, and dry weight of roots and stover at the age of 21 days after planting (DAP). The Burangrang variety showed better growth ability on acid soils than the Detam 3, Detam 4, Gema, and Dering 1 varieties. This variety showed an average plant height of 51.20±4.71 cm, wet weight and dry weight of roots 1.45±0.15 and 0.24±0.04 g, wet weight and dry weight of stover 2.80±0.36 and 1, 45±0.14 g per plant at 21 DAP. There was no interaction between soybean varieties and *Trichoderma* isolates formulated in biofertilizers. The isolate of *Trichoderma* Tc-Jjr-02 increased the wet weight and dry weight of roots and increased the wet weight and dry weight of the stover of soybean plants by 48.2 and 54.5% and 38.9 and 48.2%, respectively, compared to without *Trichoderma*. *Trichoderma* biofertilizer application cannot reduce soil acidity.

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