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# Effect of Vesicular Arbuscular Mycorrhiza (VAM) and Tricho-compost Application on Chlorophyll Content and the Number of Stomata of Soybean Genotype.

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**ABSTRACT**

Water is the main limiting factor for soybean cultivation in dry land that is heavily dependent on rainfall. Characteristics of stomata and the amount of chlorophyll content are influenced by genetic and environmental factors. The objective of this study was to study the increase of chlorophyll content and stomatal characteristics of soybean genotype in the application of vesicular arbuscular mycorrhiza (VAM) and tricho-compost in dry land. The experiment was conducted in experimental form using Split Plot Design (SPD) with all treatments were given NPK fertilizer dose 50% from recommended dosage of fertilizer per plant and 5 g mycorrhiza (*Glomus* sp.+*Gigaspora* sp.+ *Acaulospora* sp.).Six genotypes of the 5$^{th}$ generation were set as main plot namely: g1 (gM50Gy); g2 (gO50Gy); g3 (gT50Gy); g4 (gM); g5 (gO); g6 (gT). As sub plot was application of Tricho-compost (t) ie. without tricho-compost (t0), tricho-compost 50g.plant$^{-1}$ (t1), and tricho-compost 100 plant$^{-1}$ (t2). Each treatment in the main plot, and sub plots were combined resulted in 18 treatment combinations with each treatment combination repeated 3 times resulted in 54 plots in total. The results show that the average increase of chlorophyll content and stomata amount on tricho-compost 100 g.plant$^{-1}$treatment was 2.09%, and 18.53% compared to tricho-compost control with mycorrhiza application and 50% NPK fertilizer reduction. An increase in mean chlorophyll content and stomata amount were positively correlated with seed weight per soybean genotype plot.

**Keywords:** Chlorophyll, mycorrhiza Stomata, Soybean, tricho-compost

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## INTRODUCTION

Dry land generally has water availability problems, marginal fertility with poor physical properties, nutrient deficiency, toxicity and high pest attack. Drought affects the low availability of macro nutrients, especially phosphorus, which causes the limited use of dry land for agricultural crops [1]. Most of the soils on dry land are react acid (pH 4.6-5.5) and have poor nutrients, which are generally formed from mineral soils [2].

The application of technology by utilizing Vesicular Arbuscular Mycorrhiza Fungi (VAM) is one of the efforts that can be done to overcome the problem of nutrient limitations, increase crop resistance to drought, and increase the carrying capacity of the land, and safe for the environment so that the development of soybean production is sustainable. According to [3], mycorrhiza is one of fungus that can improve the soil structure, mycorrhiza fungi through its external hyphae tissue can improve soil structure and able to maintain the condition of the plant. The working principle of the mycorrhiza is by infecting the root system of host plants and producing hyphae tissue intensively so that the plant containing mycorrhiza will be able to increase the capacity in nutrient absorption. VAM plays an important role in improving plant growth by increasing the uptake of plant nutrients through surface expansion of absorption areas, protecting plant roots from pathogen attack and increasing crop resistance to drought [4].

Stomata are present in all parts of plants above the soil, but most are found in leaves. Stomata is closely related to transpiration activity. The location of stomata to each other is mediated by a certain distance that affects the intensity of evaporation. The density of stomata and of the stomata and is closely related to the transpiration activity is because most of the transpiration is removed by the mouth of the leaf (stomata) [5]. Somaclon Gajahmungkur, Towuti, and IR 64 that are considered drought resistant generally have lower stomata density than their parent plants. Plants considered to be resistant are generally derived from callus induced mutations using gamma ray irradiation. The density of stomata can affect two important processes in planting, namely photosynthesis and transpiration [6]. The number of stomata and leaf stomata index is strongly influenced by the type of tree and the location where it grows [7].

The objectives of the study were to study the chlorophyll content and stomata characteristics of soybean genotype applied with Vesicular Arbuscular Mycorrhiza (VAM) and 50% NPK fertilizer reduction due to tricho-compost dose treatment in dry land

## RESEARCH METHODS

The experiment was conducted in the form of experiments using a Split Plot Design (SPD) with all treatments were given NPK fertilizer dose 50% from the recommended dosage of fertilizer per plant and 5 g mycorrhiza (*Glomus* sp. + *Gigaspora* sp. + *Acaulospora* sp.). Main plot (MP) was genotypes of 5$^{th}$ generation of Soybean (g): g1 (gM50Gy); g2 (gO50Gy); g3 (gT50Gy); g4 (gM); g5 (gO); g6 (gT). Sub Plot (SP) was Tricho-compost (t) ie. without tricho-compost (t0), and tricho-compost 50g.plant$^{-1}$ (t1), and tricho-compost 100g.plant$^{-1}$ (t2). Each treatment in the main plot, and sub plots were combined so that there were 18 treatment combinations. Each treatment combination was repeated 3 times resulted in 54 plots in total. In the cultivation of soybean genotype, plant was given with 50% of recommended dosage of NPK fertilizer per plant. The reduction of 50% of recommended NPK fertilizer with mycorrhiza application resulted inhigher dry crown weight, root dry weight, grain weight, nitrogen, phosphorus, and potassium uptake compared to 25% and 0% NPK fertilizer reduction [8]. Each treatment in the main plot, and sub plots were combined so that there were 18 treatment combinations. Each treatment combination was repeated 3 times so that there were 54 plots in total.

Morphophysiological characteristics of each M4 plant were identified to determine tolerant mutants in dry land. The observed variables were as follows: fresh weight of crown and root (g.plant$^{-1}$), stomata length (μm), stomata width (μm), number of stomata (stomata.mm$^{-2}$), chlorophyll content (unit), number of pods per plant (pod.plant$^{-1}$), number of grains per plant (grains.plant$^{-1}$), grain weight per plot (g.plot$^{-1}$).

## RESULTS AND DISCUSSION

### PLANT HEIGHT AND LEAF AMOUNT

The tricho-compost 100g per plant treatment was not significantly different from the 50g per plant tricho-compost on the plant height of soybean genotypes but significantly different from those without tricho-compost. Data on the number of leaves showed that the tricho-compost 100g per plant treatment was significantly different from the tricho-compost 50 g per plant and without tricho-compost (Fig. 1). Nutrients absorption can be increased due to the diameter of VAM external hyphae is smaller than the roots that can enter into the soil with smaller pores [9]. VAM functions in absorbing P as well as micro nutrients such as Zn and Cu. The increase of micro nutrients is expected to increase the number of leaves, although the plants needed these nutrients in small amounts [10]. Trichoderma compost can be combined with mycorrhiza because it can accelerate plant growth, root development and increase nutrients P [11]

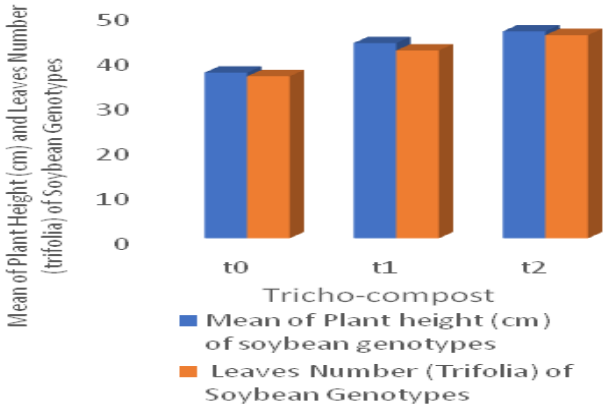



**Figure 1: Mean of Plant height (cm) and Leaves Number (Trifolia) of Soybean Genotypes on the Application of Tricho-compost.**

### FRESH WEIGHT OF PLANT CROWN AND ROOT

Soybean genotype of gT50Gy without tricho-compost treatment was not significantly different from the gO50Gy treatment; gM50Gy; and gT on the crown fresh weights but different from gM and gO (Fig. 2). The mean wet weight of soybean genotype roots in gM50Gy treatment was not significantly different from the gO50Gy and gT50Gy treatments in tricho-compost 100g.plant$^{-1}$ but different from the comparison treatments (Figure 3). Application of VAM and compost both separately and simultaneously is known capable to increase soil pH, available P as well as C-Organic on ultisol soil [12].

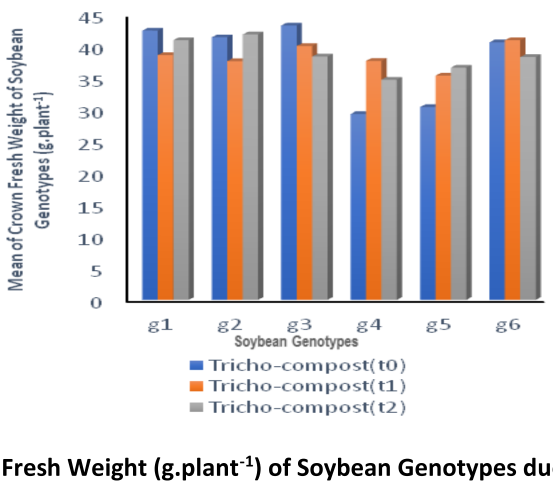



**Figure 2: Mean of Crown Fresh Weight (g.plant$^{-1}$) of Soybean Genotypes due to Application of Tricho-compost.**

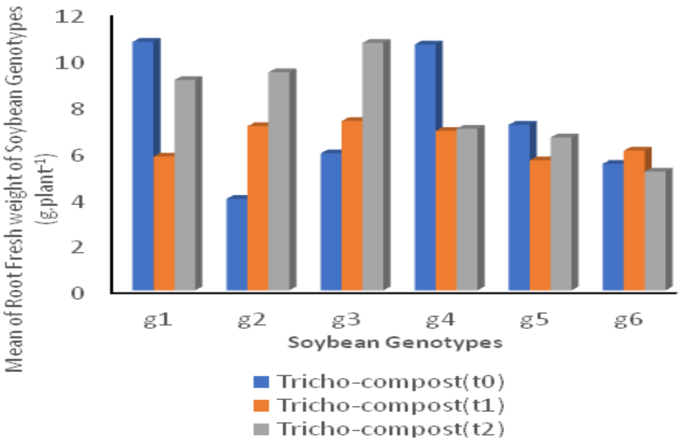



**Figure3: Mean of Roots Fresh Weight (g.plant$^{-1}$) of Soybean Genotypes due to Application of Tricho-compost**

**STOMATA**

Characteristics (length, width, and amount) of stomata in soybean genotypes increased with tricho-compost dosages applied. The tricho-compost dose of 100 g.plant$^{-1}$ resulted in stromata length that was significantly different from the treatment of trichoderma 50 g.plant$^{-1}$ and 0 g.plant$^{-1}$. The mean of stomatal width and stomata number of the genotypes in trichoderma 100 g.plant$^{-1}$ treatment were not significantly different from trichoderma 50 g.plant$^{-1}$, but differed from 0 g.plant$^{-1}$. The number of stomata increase in tricho-compost 100 g.plant$^{-1}$treatment (47.96 stomata mm$^{-2}$) was 18.52% compared with mycorrhiza treatment (40.46 stomata.mm$^{-2}$) (Figure 4). The application of VAM significantly influenced the increase of stomata, leaf thickness and palisade thickness, as well as chlorophyll content of sword broad bean leaf crop (*C.usiaormis* L) [13].

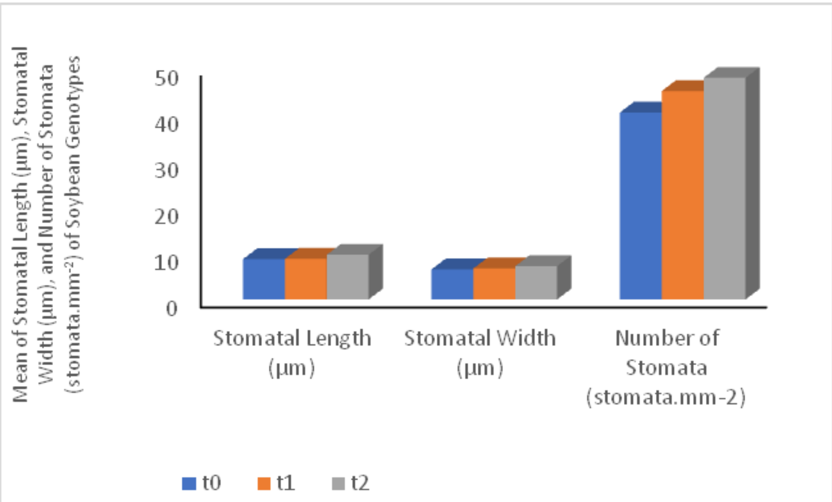
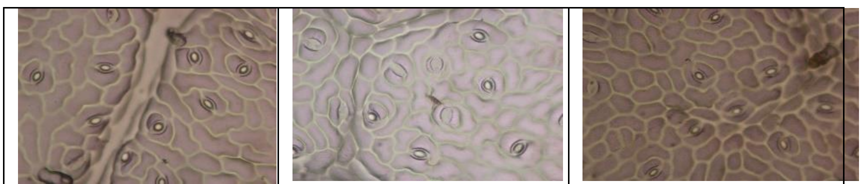
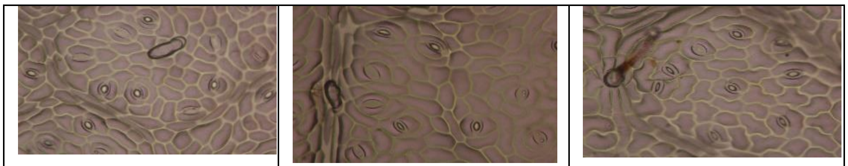



**Figure 4: Mean of Stomata Length (µm), Stomata Width (µm), and Number of Stomata (stomata.mm$^{-2}$) of Soybean Genotypes due to Application of Tricho-compost.**



| T0g3 | T1g1 | T2g2 |
|---|---|---|



| T0g6 | T1g5 | T2g4 |
|---|---|---|

**Figure 5: Stomata Characteristics of 50Gy, without Tricho-compost (t0g3); Menyapa, 50 Gy with Tricho-compost 50g.plant$^{-1}$ (t1g1); Orba, 50 Gy with Tricho-compost 100g.plant$^{-1}$ (t2g2); Tanggamus, without Tricho-compost (t0g6); Orba,with Tricho-compost 50g.plant$^{-1}$ (t1g5); and Menyapa, with Tricho-compost 100g.plant$^{-1}$ (t2g4).**

**CHLOROPHYLL**

Combination treatment of gM50Gy; gO50Gy; GT50Gy with tricho-compost treatments resulted in higher chlorophyll content than the other comparative genotypes. The mean increase of chlorophyll content in tricho-compost 100 g.plant$^{-1}$ (38.58 units) treatment was 2.09% compared with the control treatment (38.02 units) (Table 1). Increased mycorrhiza and rhizobium inoculation is effective in increasing chlorophyll content, leading to increased growth of nuts [14].

**Table 1: Mean of Chlorophyll content (unit) of Soybean Genotypes due to Application of Various doses of Tricho-composton addition of Mycorhizza and reduction of 50 % NPK Fertilizer.**

| Treatment | Mean of Chlorophyll content (unit) | | |
|---|---|---|---|
| Soybean Genotypes | Tricho-compost | | |
| | t0 | t1 | t2 |
| g1 | $42.60^{a}_{x}$ | $38.71^{ab}_{x}$ | $41.06^{a}_{x}$ |
| g2 | $41.53^{a}_{x}$ | $37.77^{ab}_{x}$ | $41.98^{a}_{x}$ |
| g3 | $43.39^{a}_{x}$ | $40.16^{ab}_{x}$ | $38.48^{ab}_{x}$ |
| g4 | $29.40^{b}_{y}$ | $37.81^{ab}_{x}$ | $34.82^{b}_{x}$ |
| g5 | $30.49^{b}_{y}$ | $35.47^{b}_{x}$ | $36.73^{ab}_{x}$ |
| g6 | $40.73^{a}_{x}$ | $41.06^{a}_{x}$ | $38.41^{ab}_{x}$ |
| Rerata | 38.02 | 38.49 | 38.58 |
| LSD(0.05)((MP) | 4.861 | | |
| LSD (0.05)(SP) | 4.806 | | |

**PRODUCTION**

Soybean genotype of gT50Gy was not significantly different from gO50Gy, gM50Gy, and gT on the average number of pods per plant, number of grains per plant and the grains weight per plot but different from gM and gO. The number of grains per plant and grain weight per plot increased with addition of tricho-compost doses of 50 g and 100 g per plant with mycorrhizal application and 50% NPK fertilizer reduction (Table 2). This is in line with the results of research [15] that found the highest number of pods per plant was recorded in mixed in the treatments of mycorrhizae + sterile + compost soil (22 pods/plants) and sterile soil + compost (20 pods / plants). The addition of compost can increase the porosity of the soil and lower the mechanical pressure of the soil against the growth of mycorrhiza.

**Table 2: Mean of number of pods per plant (pods.plant$^{-1}$), number of grains per plant (grains.plant$^{-1}$), and the weight of grains per plot (g.plot$^{-1}$) of Soybean Genotypes due to Application of Various doses of Tricho-compost with addition of Mycorhizza and reduction of 50 % NPK Fertilizer.**

| Treatment / Genotype | Number of pods per plant (pods.plant$^{-1}$) | Number of seeds per plant (grains.plant$^{-1}$) | Weight of grains per plot (g.plot$^{-1}$) |
|---|---|---|---|
| g1 | 88.30 *ab* | 209.04 *b* | 295.55 *ab* |
| g2 | 88.48 *ab* | 251.37 *a* | 322.54 *a* |
| g3 | 93.33 *a* | 214.93 *ab* | 312.92 *a* |
| g4 | 82.37 *b* | 205.63 *b* | 261.40 *bc* |
| g5 | 78.63 *b* | 175.70 *b* | 249.48 *c* |
| g6 | 92.93 *a* | 199.15 *b* | 306.40 *ab* |
| Notation | * | * | * |
| LSD(0.05) | 10.019 | 39.455 | 45.924 |
| Tricho-compost | | | |
| No Tricho-compost | 80.5637 *y* | 196.5237 *y* | 253.6237 *y* |
| Tricho-compost 50 g | 91.7237 *x* | 208.04 *xy* | 303.16 *x* |
| Tricho-compost 100 g | 89.74 *xy* | 223.35 *x* | 317.36 *x* |
| Notation | * | * | * |
| LSD(0.05) | 8.293 | 20.930 | 42.839 |

Correlation test was conducted to determine the correlation between the mean of the chlorophyll content, the number, length, and width of stomata and fresh weight of crown parameters (X) on the grains weight per plot parametersof the soybean genotypes (Y). A significant correlation was found between parameters of the number of chlorophyll and the number of stomata and the weight of the grains per plot, while the length and width of the stomata was not significantly correlated with the weight of the grains per plot (Table 3). The increase in the mean of chlorophyll content and stomata numbers were positively correlated with grain weight per soybean genotype plot (Figure 6). Stomata opening is related to plant metabolism process namely transpiration and photosynthesis. Stomata plays a role in the diffusion of $CO_2$ in the process of photosynthesis. In addition, stomata also serves as the exit of fluid from cells in the transpiration process [16] and plays a role in the exchange of gases coming from the atmosphere. This stomata density affects the rate of photosynthesis in plants [17]. Chlorophyll levels can be used as a sensitive indicator of the physiological condition of a plant because the chlorophyll content is positively correlated with leaf nitrogen content, which can be used as an indicator of photosynthetic rate [18].

**Table 3: Correlation between the mean of the grains weight per plot parameters of the soybean genotypes (Y) and the number of chlorophyll, the number, length, and width of stomata and fresh weight of crown parameters (X).**

| No. | Parameters | Regression Equation | r | 0.05 |
|---|---|---|---|---|
| 1 | Chlorophyll content | $y = -0.0353x^2 + 8.9525x$ | 0.538 | * |
| 2 | Length of Stomata | $y = -2.9185x^2 + 62.812x$ | 0.237 | ns |
| 3 | Width Stomata | $y = -2.9185x^2 + 62.812x$ | 0.237 | ns |
| 4 | Number of Stomata | $y = -0.0347x^2 + 8.1097x$ | 0.520 | * |

| | | | | |
|---|---|---|---|---|
| 5 | Crown Fresh Weight | $y = -0.0488x^2 + 7.6454x$ | 0.147 | ns |

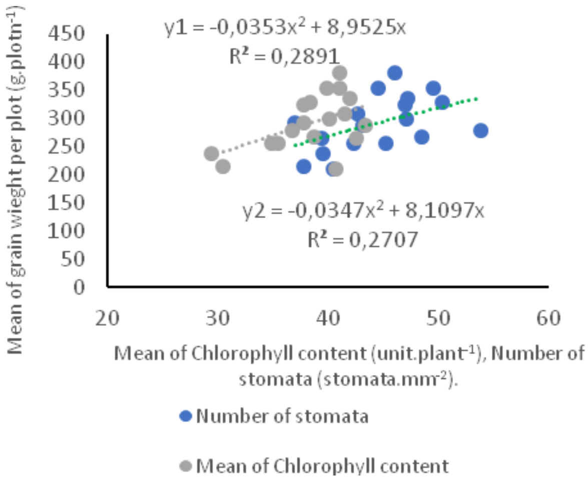



**Figure 6: Relation between chlorophyll content and number of stomata on grain weight per plot of Soybean genotypes.**

## CONCLUSION

The results show that the average increase of chlorophyll content and stomata number due to tricho-compost treatment of 100 g.plant$^{-1}$was 2.09%, and 18.53%, respectively, compared to no tricho-compost with mycorrhizal application and 50% NPK fertilizer reduction. An increase in the mean of chlorophyll content and stomata numbers were positively correlated with grain weight per plot of soybean genotypes.

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