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Proline Reduction and Increasing Growth of Vanilla Plants Induced by BNR Fungus with Dose of Goat Manure and Husk Charcoal

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Abstract. Goat manure and husk charcoal are agricultural wastes that have not been used optimally, contain soil microorganisms that are effective in converting nutrients into more perfect forms, play a role in improving soil physical properties and increase the ability of soil to bind nutrients. The purpose of this study is to determine the effect of goat manure and husk charcoal to proline reduction and increase growth of vanilla plant induced by Binucleate Rhizoctonia (BNR). The study was designed with a factorial completely randomized block design. The first factor is the dose of manure (0,5,10,15) g/plant, the second factor is the dose of husk charcoal (0,5, 10, 15) g/plant. Data were analyzed by analysis of variance ANOVA with Duncan's Multiple Distance Test (DMRT) with a significant difference of 5%. The effect of doses of goat manure and husk charcoal induced by BNR can reduce proline contents and increase growth of vanilla plant. The benefit of this research is to provide treatment information to reduce proline levels and increase plant growth with the application of goat fertilizer and husk charcoal.

Key words: binucleate-Rhizoctonia, husk, manure, vanilla

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INTRODUCTION

Vanilla is one of the plantation crops which is the country's foreign exchange reserves from exports. Indonesian vanilla comes from Mexico, the first use of vanilla was done by the Aztecs as a preserved chocolate fragrance that enhances the taste. Vanillin (C₈H₈O₃) content which causes vanilla (Nurcholis, 2017) is a distinctive fragrance used in the food, beverage, cosmetic, and pharmaceutical industries. Vanilla plants are characterized by growth that propagates up to 30 meters, epiphytes and vanilla fruit producing pods (Frenkel & Belanger, 2018). The initial growth of vanilla requires a medium that has the ability to hold water, loose soil structure, smooth aeration and drainage, does not experience drought and contains sufficient nutrients needed for growth (Fileccia et al., 2014)

Fertilization treatments, especially organic fertilizers, accelerate soil decomposition so that soil microorganisms can help provide nutrients needed by plants. Organic fertilizer derived from goat manure has the advantage of increasing soil humus content, stimulating soil microorganisms, and improving soil structure so as to increase the

availability of nutrients for plants (Singh, 2012); (Assefa & Tadesse, 2019); (Pratomo & Prasetyo, 2018). Application of organic matter derived from goat manure enriches microorganisms, produces nutrients (macro and micro), increases soil aggregates so as to increase water infiltration and acts as a soil enhancer. Goat manure has a long-term effect on Potassium content in goat manure is 2.88%, other nutrients nitrogen is 2.77%, phosphorus is 1.78% (Handajningsih et al., 2018).

In addition to goat manure, soil organic matter can also be added by giving husk charcoal. Treatment with husk charcoal increased growth and reduced the percentage of seedling mortality caused by the fungus *Fusarium oxysporum* f. sp. *Vanilla* (Haryuni et al., 2018). The results of the decomposition of organic matter from goat manure and husk charcoal (N, P, K, Ca, S and Mg) which were eliminated directly were able to increase the pH and increase the ability of the soil as a cation buffer because the decomposition of organic matter produced a complex compound (humus) (Fileccia et al., 2014). Organic fertilizer

derived from goat manure has the advantage of increasing soil humus content, stimulating soil microorganisms and improving soil structure so as to increase the availability of nutrients for plants. Continuous use of land without being followed by nutrient supply, it is difficult to obtain maximum production considering the very limited capacity of the soil, the provision of goat manure with an appropriate dose is expected to help the availability of soil nutrients, goat manure and husk charcoal ash serve as biofertilizers (Harahap & Hidayat, 2021).

Plant growth is influenced by the availability of nutrients that can be utilized by roots and available to plants, this role can be carried out by Binucleate Rhizoctonia (BNR). The peloton that is in the root tissue plays a role in storing nutrients that are absorbed by external hyphae and sent into plant cells (Haryuni, et al., 2020). The characteristic preserved mycorrhizal fungi associated with orchids is the formation of a symbiotic structure called the peloton, which is shaped like a coil of hyphae in which metabolic exchange between symbionts occurs in both protochome and mycorrhizal roots (Valadares et al., 2014). The material in the peloton that is in the network is utilized by plants during the metabolic process. In conditions of water shortage, the material contributes to the availability of water for plants (Begum et al., 2019). BNR-mediated mechanisms regulate tolerance and promote plant improvement in drought stress conditions (Boutasknit et al., 2020).

Proline is a compatible osmolyte commonly found in plants experiencing drought stress. Proline accumulation is a typical physiological response to drought stress in some species. (Kumar et al., 2021). Proline is an indicator of a plant's ability to maintain turgor through a decrease in osmotic potential so that an increase in proline content indicates an increase in plant tolerance to water shortages, which results in plant growth and the quantity and quality of crop yields. Proline affects the air potential, opening and closing of leaf stomata further down regulates photosynthesis and CO₂ deficiency causes plant stress (Chun et al., 2018); (Osakabe et al., 2014).

The purpose of this study was to determine the effect of goat manure and husk charcoal on proline reduction and increased growth of vanilla plants induced by Binucleate Rhizoctonia (BNR). The benefit of this research is to provide information about the application of the right dose of goat manure and vanilla husk charcoal by inducing BNR and its effect on proline levels and plant

growth.

METHODS

Preparation of goat manure

The goat manure used is derived from wind-dried goat manure until the moisture content reaches 60%, put in a wooden box then added with molasses/Javanese sugar and coconut water in a ratio (10:1:3) mixed evenly and stored closed, every 7 days once inverted and stirred for 5 times. If the fertilizer has changed color and comes off when held by hand, the fertilizer is ready to use (Pratomo & Prasetyo, 2018).

Preparation of husk charcoal

The husk charcoal is cleaned of other impurities and collected together and then a chimney/tube is installed in the middle which is made of perforated wire. wire is shaped like a tube as a means of combustion. This means that the flames will stick out. For burning, it is better to use a base made of zinc plate placed on a flat, hard and heat-resistant floor. The hole in the chimney is filled with charcoal and doused with kerosene, added with paper, so that a fire arises, when it is black the unburned part is piled up and moved near the chimney. After everything is burned, the husks turn black, allow to cool and are ready to be used for planting media mixtures (Surdianto et al., 2015).

Preparation of Binucleate Rhizoctonia (BNR)

The BNR used is the same as in the (Haryuni et al., 2015 b) procedure, namely the corn is washed, drained and then steamed for 45 minutes until half, it is half cooked, cooled, then sprayed with water that has been mixed with chlorine, broken corn and then put into a heat-resistant plastic bag with a size of 10 x 20 cm until it reaches 1/3 part . After being put in a small plastic bag, stored in a large plastic bag to be sterilized for 60 minutes by steaming, cooling at room temperature. Pure isolates of BNR were propagated on PDA media, inoculated to 100g sterile corn medium and stored at room temperature for 10-15 days in a separate room not used for other activities. After the corn medium was filled with BNR growth, it was inoculated into the vanilla growing medium. Each polybag was filled with 1 vanilla seed and 10 g of BNR.

Research Design

The research was conducted at Rejosari village, Jambu district, Semarang Regency,

Central of Java from March to September 2020 the altitude is around 800-900 m above sea level, soil type andosol soil. Vanilla plants that are 2 years old in the field, sprinkled with BNR 10 g/plant around the plant roots by covering the surface soil, after 2 weeks of giving BNR goat manure is given at a dose of 0.5,10,15 g/plant as (P0 , P1, P2, P3) every 2 weeks up to 4 times. The second factor was the dose of husk charcoal 0,5,10,15 g/plant as (S0, S1, S2, S3) added every 2 weeks up to 4 times. each treatment was repeated 5 times used samples.

Observation

Growth Observation of vanilla plant

Vanilla plant was carried out 4 weeks after the first treatment of goat manure and husk charcoal

Statistical analysis

Data from analysis of proline and vanilla plant growth (height, fresh weight and dry weight of plants, analyzed by variance (Anova). If significantly different, Duncan's Multiple Range Test (DMRT) test at 5% level was carried out, to distinguish between treatments. To find out the direct and indirect relationship, regression and correlation analysis were carried out.

RESULT AND DISCUSSION

The average effect of goat manure on proline content, height, fresh weight and dry weight of vanilla plants induced by BNR fungus analyze the duncan multiple range test (DMRT) 5% level in activities in experiment and control is presented in

Table 1. Effect of goat manure to proline content, height, fresh weight and dry weight of vanilla plants induced by BNR fungus

Treatment	Observation of parameters			
	Proline content (μmol g ⁻¹)	height of plant (cm)	fresh weight of plant (g)	dry weight of plant (g)
P0	2.12 ^b	50.17 ^a	6.05 ^b	1.27 ^c
P1	2.06 ^b	58.71 ^{ab}	6.14 ^b	1.31 ^b
P2	1.91 ^{ab}	64.88 ^{bc}	6.19 ^{ab}	1.34 ^a
P3	1.77 ^a	71.08 ^c	6.37 ^a	1.36 ^a

Note: Numbers followed by the same letter show no significant difference in the duncan multiple range test (DMRT) 5% level.

including, plant height (measured from the base of the stem to the top of the stem), number of leaves (counted for all the leaves that grew for each sample plant), leaf fresh weight (taken 10 leaves. leaf starting with 5 leaves from the stem surface and then weighed), leaf dry weight all leaves were observed on leaf fresh weight by drying the leaves and continued in an oven at 105°C for 2 h and continuously at 80°C until the weight was constant (Huang et al., 2019).

Measurement of Proline contents

Analysis of proline contents in leaf of vanilla was determined following (Bates et al., 1973). Extract: 20 to 50 times diluted fresh weight of leaf (w/v), typically in a 70:30 ethanol:water mixture (v/v). Standards: proline solutions ranging from 0.04 to 1 mM, in the same medium as the one used for the extraction. Reaction mix: ninhydrin 1% (w/v) in acetic acid 60% (v/v), ethanol 20% (v/v). Protect from light. Proline analysis on Laboratory of fisiology at Universitas Gadjah Mada Yogyakarta.

Table 1.

Goat manure has a significant effect on proline content, height, fresh weight, and dry weight of vanilla plant (Tabel 1). It caused by goat manure contains macro and micro nutrients rich in phosphorus, nitrogen and potassium which are needed for plant growth and development. Increasing the dose of goat manure decreased and significantly different on plant proline content (Yenni et al., 2021), are (P0) 2.21 μmol g⁻¹, (P3) 1.77 μmol g⁻¹. But it tends to increase and significantly different on height of vanilla plant (P0) 50.17cm, (P3) 71.08cm. the fresh weight of plant (P0) was 6.05 g, (P3) 6.37g, and the dry weight of plant (P0) was 1.27 g, (P3) 1.36 g. There are caused by BNR fungi on plants acts as mycorrhizae capable of forming internal and external hyphae on roots and peloton in plant tissues (Haryuni. et al., 2020). Table 1 shows that increasing the dose of goat manure fertilizer decreased plant proline content and increased plant height, plant fresh weight, plant dry weight.

Glutamate is an important amino acid and acts as a precursor for biosynthesis -aminobutyric acid, arginine, glutamine, and proline in plants and other eukaryotes, proline as an osmoprotectant in stress tolerance. Proline participates in

plants will affect physiological processes and even cause plants to die (Pardosi, 2021).

The average effect of husk charcoal to proline content, height, fresh weight and dry weight of vanilla plants induced by BNR fungus analyze the

Table 2. Effect of husk charcoal dose to proline content, height, fresh weight and dry weight on vanilla plants induced by BNR fungus

Treatment	Observation of parameters			
	Proline content ($\mu\text{mol g}^{-1}$)	height of plant (cm)	fresh weight of plant (g)	dry weight of plant (g)
S0	3.59 ^d	25.04 ^a	5.41 ^d	1.13 ^d
S1	2.14 ^c	37.83 ^b	6.13 ^c	1.28 ^c
S2	1.27 ^b	73.21 ^c	6.50 ^b	1.40 ^b
S3	0.85 ^a	108.75 ^d	6.70 ^a	1.47 ^a

Note. Numbers followed by the same letter show no significant difference in the DMRT test at 5% level.

biosynthesis primary metabolism, but also has a special function, a metabolite during growth and development, proline not only accumulates during abiotic stress, but also in various plant tissues under non-stress condition too (Kavi Kishor et al., 2015). Proline is an indicator of plant stress against drought tolerance of peanut plants (Furlan et al., 2020) with the addition of goat manure to help soil microorganisms more readily utilized by plants and then stored in plant roots and stems in pelotons produced from BNR fungus inoculation. Fungal hyphae penetrate cortical cells, branch and form peloton (cells in this phase are called trophocytes), as an anatomical feature of orchid mycorrhizae

This is the same as research carried out by (Zhang et al., 2016) that BNR application as mycorrhizal can increase total root long on the black grasshopper plan. BNR fungus forms external and internal hyphae on roots and forms peloton in plant transport bundles, microorganisms contained in goat manure are able to improve soil structure so that water in the soil is more easily absorbed by plant roots. Lack of water of vanilla plant tissues is indicated by high contents of proline (Haryuni et al., 2020).

Accumulation of free proline as a form of general response of a plant to stress factors in drought, salinity, cold air and pathogen. Height, fresh weight and dry weight are indicators of plant growth and development of the plant, groundwater that enters through the roots is stored in plant tissue, used in metabolic processes and used for plant growth and development. Lack of water in

Duncan multiple range test (DMRT) 5% level in activities in experiment and control is presented in Table 2.

Table 2 shows the significant effect between the application treatment of husk charcoal and without husk charcoal to proline content, plant height, weight, and dry weight of plant. Proline contents were significantly different and decreased with the application of husk charcoal at a dose of 15 g/plant (S3) by 0.85 $\mu\text{mol g}^{-1}$ all (S0) 3.59 $\mu\text{mol g}^{-1}$ but tended to increase and significantly different in plant height from 25.04 cm (S0) to 108.75 (S3). the weight of the wet stove from 5.41 g (S0) to 6.70 g (S3) and the dry weight of 1.13 g (S0) to 1.47 g (S0).

The treatment of increasing doses of husk charcoal charcoal, proline has increased, in accordance with the opinion of (Hendrati et al., 2016), proline will increase in tolerant plants not in sensitive plants, so that proline can be used as an indicator of drought tolerant from *Acacia auriculiformis* Cunn., *Tectona grandis* L., *Alstonia spectabilis* Br., and *Cedrela odorata* L. that are susceptible to drought. resistant to drought does not produce proline. Supported by the opinion of (Ichsan et al., 2016), The role of husk charcoal as soil organic matter stimulates soil granulation, cohesion and plasticity, improves soil structure and increases soil resistance to water and increases activity and number of soil microorganisms. The increase in soil organisms was supported by the induction of the BNR which was inoculated at the time of *Shorea robusta* seedlings (Tapwal et al., 2015).

The increase in the fresh weight of plant was

Table 3. The interaction effect of goat manure and husk charcoal dose on proline content, height, fresh weight and dry weight to vanilla plants induced by BNR fungus

Treatment	Observation of parameters			
	Proline content ($\mu\text{mol g}^{-1}$)	height of plant (cm)	fresh weight of plant (g)	dry weight of plant (g)
P0S0	3.94 ^j	22.33 ^a	5.20 ^f	1.05 ^h
P1S0	3.45 ^{hi}	25.00 ^a	5.23 ^f	1.10 ^h
P2S0	3.65 ⁱ	25.00 ^a	5.33 ^f	1.16 ^g
P3S0	3.33 ^h	27.83 ^a	5.90 ^e	1.20 ^{fg}
P0S1	2.35 ^g	30.00 ^{ab}	6.00 ^{ed}	1.23 ^{ef}
P1S1	2.34 ^g	33.67 ^{ab}	6.13 ^{cde}	1.26 ^{de}
P2S1	2.37 ^g	40.33 ^{bc}	6.17 ^{cde}	1.30 ^{cd}
P3S1	1.51 ^f	47.33 ^{cd}	6.23 ^{bcd}	1.32 ^{cd}
P0S2	1.09 ^{bc}	54.00 ^d	6.40 ^{abcd}	1.34 ^c
P1S2	1.21 ^{cde}	73.50 ^e	6.50 ^{abc}	1.40 ^b
P2S2	1.42 ^{ef}	80.33 ^e	6.53 ^{abc}	1.42 ^b
P3S2	1.35 ^{def}	85.00 ^{ef}	6.60 ^{ab}	1.43 ^{ab}
P0S3	1.11 ^{bcd}	94.33 ^{fg}	6.63 ^{ab}	1.47 ^a
P1S3	0.64 ^a	102.67 ^g	6.70 ^a	1.47 ^a
P2S3	0.77 ^a	113.83 ^h	6.73 ^a	1.47 ^a
P3S3	0.89 ^{ab}	124.17 ^h	6.77 ^a	1.47 ^a

Note: Numbers followed by the same letter show no significant difference in the DMRT test at 5% level.

indicated by an increase in the dose of husk charcoal, the fresh weight was related to the photosynthate of the plant that was stored in the tissues and the absorption of water through the roots, the availability of nutrients and water in the soil affected the growth and fresh weight. Dry weight is an indicator of photosynthetic accumulation of plants stored in soybean plant tissue used for growth and development (Widiastuti & Latifah, 2016). However, used leaf dry weight as the representative of leaf biomass when studying the scaling relationship between leaf biomass and area. The scaling of leaf mass and leaf area could directly influence the amount of light-capturing surface area, which gives an investment of dry biomass (Huang et al., 2019).

Determine leaf biomass related to leaf moisture content, by measuring leaf dry weight and leaf fresh weight so that it can determine water loss, to make it easier to determine gas exchange (CO_2 , O_2 , and H_2O) leaf dry weight must be constant. (Hughes et al., 1970) reported that leaf area of several dicot genotypes had a linear relationship with absolute leaf water content (Lin et al., 2018) described the comparison between fresh leaf weight and leaf area of 11 bamboo species.

Table 3 shows the interaction effect of goat manure and husk charcoal dosage charcoal induced by BNR fungus on decreasing proline content, but increase to height, fresh weight and dry weight. We can see without goat manure and husk charcoal dosage charcoal (P0S0). the highest proline content but height, fresh and weight of plant lowest, while in the treatment interaction (P2S3) proline content 0.77 molg^{-1} was not significantly different from (P1S3) and (P3S3), height (113.83cm), dry weight (6.73 g) and fresh weight (1.47 g) were not significantly different from (P1S3) on vanilla, while the observed levels of proline decreased without manure treatment and husk charcoal (P0S0).

The increase in proline indicates the plant is able to survive in times of drought (Daryanti et al., 2014). Drought in vanilla has an effect on physiology and anatomy, because drought causes a decrease in the rate of photosynthesis and leaf area, and even closure of leaf stomata, at the cellular level there is an increase in the concentration of dissolved matter including plant proline (Prihastati, 2012) (Subantoro, 2014) (Siregar, 2015) (Pardosi, 2021). BNR which acts as mycorrhizae forms external hyphae that absorb

nutrients around the roots and are stored in peloton in plant tissues through internal hyphae. The peloton will be destroyed in the plant after 2 weeks, because hypha-infected tissue becomes lysed so that the plant tissue contains nutrients (Rasmussen & Rasmussen, 2009). This is supported by research conducted by (Haryuni, et al., 2020) which states that the application of biopesticides and *Fusarium* inoculation after induction of BNR can increase nitrogen, phosphorus, and potassium content of vanilla plants. Another study by (Muslim, 2019) showed that the success of biological control of fusarium wilt in tomato plants was by using hypovirulent binucleate rhizoctonia (HBNR). Another study using antagonistic fungi was carried out on *Trichoderma* sp inoculated treatment 25g/plant and water heating for 60 minutes on sugarcane bud chips seedling growth (*Saccharum officinarum*) showed the lowest proline content of 1.422 mol g⁻¹, this caused the seeds to be drought-resistant and not easy to die (Haryuni, 2015 a).

The novelty in this research is the use of husk

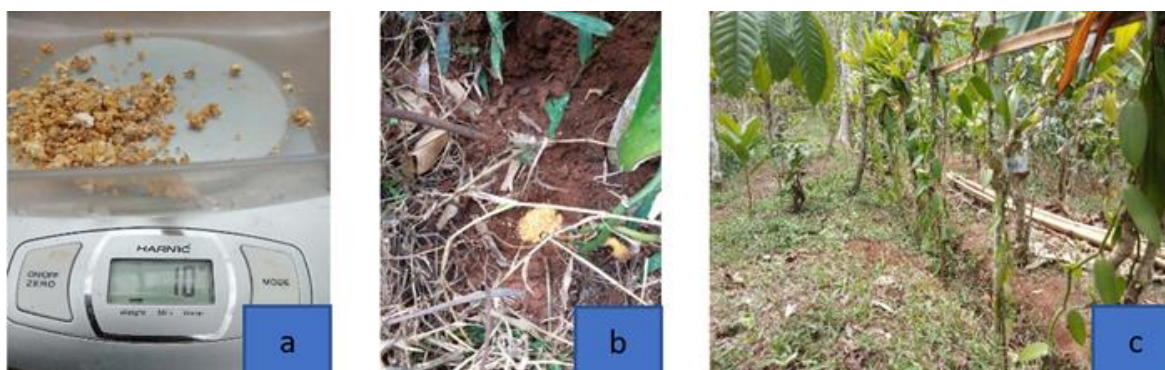


Figure 1. Application BNR to vanilla plants at Rejosari village, Jambu district, Semarang Regency, Central of Java (a. BNR isolate on corn medium; b. BNR inoculation on growing medium; c. Vanilla plant in the planting field).

charcoal and goat manure which is induced by BNR to control plant proline, the decrease in proline indicates that the plant is in sufficient water conditions. They really help the decomposition process that produces plant nutrients. The results of this study helped vanilla agronomists adjust the dose of husk charcoal and goat manure by influencing the BNR fungus so that plant growth was better and resistant to drought stress conditions.

CONCLUSION

The effect of doses of goat manure and husk charcoal induced by BNR can reduce to proline and increase vanilla plant growth. The decrease in

proline affects the resistance of vanilla plants to drought. The results showed that the combination of treatments (P0S0), namely the dose of goat manure 0g/plant (P0) and husk choarcoal 0g/plant (S0) the plant proline was 3.94 $\mu\text{mol g}^{-1}$, plant height 22.33 cm, fresh weight 5.20 g/plant. plant and dry weight 1.05g/plant, while the treatment (P2S3), namely the dose of goat manure 10g/plant (P2) and husk charcoal 15 g/plant (S3), the plant proline was 0.77 $\mu\text{mol g}^{-1}$, plant height 113.83 cm, fresh weight 6.73 g/plant. plant and dry weight 1.47g/plant.

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