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The Effects of Dose Rhizoctonia Binucleat (BNR) and Phosphorus to Nitrate Reductase Activity (NRA) and Chlorophyll of Vanilla Seedling (*Vanilla planifolia Andrews*)

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Abstract

Vanilla (*Vanilla planifolia Andrews*) is one of the important exported commodities in Indonesia. Indonesia is one of top five major vanilla exporters in the world, that produce the high quality of Indonesian vanilla with high vanillin content (2.75%). The aims of this research were to determine the effects of dose binukleat Rhizoctonia (BNR) and phosphorus as well as the interaction of the nitrate reductase activity (NRA) and chlorophyll of the vanilla seedling (*Vanilla planifolia Andrew*). Method in this research used completely randomized factorial design, by involving two factors (dose of BNR inoculation and Phosphor). The first factor is without inoculation and inoculation BNR (M_0, M_1, M_2, M_3) wich consists of (0,5, 10, 15) g/polybag, the second factor is the dose of phosphorus fertilizer (P_0, P_1, P_2, P_3) which consists of (0, 3, 6, 9) g/polibag. The results showed that the inoculation dose of BNR and doses of phosphorus not significant and lower levels of NRA and chlorophyll while the interaction dose of BNR and phosphorus significantly and increase levels of NRA and chlorophyll of vanilla seedling. Nitrate Reductase Activity and chlorophyll has important role in metabolism process as a plant growth indicator

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INTRODUCTION

Vanilla (*Vanilla planifolia* Andrews) is a tropical and subtropical plants with high economic value, are able to live at an altitude of 1.500 m above sea level, between the optimum temperature (21- 32) ^{0}C (Rismunandar & Sukma, 2002). Vanilla belongs to the orchid group, cultivated to produce the vanilla (hydroxy3-methoxy benzaldehyde) are used in the food and beverage industry worldwide (Zhao et al., 2015). Lack of knowledge and culture system resulted in a decrease in the quality and price of vanilla in the market. Climate change is experienced in Indonesia, lack of care, and lack of water is an important issue for vanilla. Even the vanilla experience a state of the long dry season and fertilization are low so that decreases the ability of the soil as nutrients and water providers (Rismunandar & Sukma, 2002; Haryuni, 2012).

Phosphorus/P is an essential nutrient required by plants in their growth and development. Phosphate is actually present in abundant amounts in the soil, but about 95-99% are in the form of insoluble phosphate and so can not be used by plants (Sanjotha et al., 2011). In acid soils, phosphate will be compounded in the forms of Al-P, Fe-P, and occluded-P, while the alkaline earths, phosphate will combine with calcium (Ca) as the Ca-P to form complex compounds soluble (Ginting et al., 2006). Supported by the opinions Damanik et al., (2011) *cit*. Hapiza et al., (2014) that the lack of supply P plant inhibits the metabolism of the plants used in the process of photosynthesis to produce carbohydrates, proteins, nucleic acids, energy and new cell formation.

Given the availability of phosphate slow for plants and the ability to stimulate the germination of spores of mycorrhiza, the availability of P for plants and mycorrhizal spores germinated more optimal for the growth of plants (Novi & Rizki, 2014). Previous research from Nikolaou et al., (2002) states that the phosphate treatment stimulates the germination of spores of mycorrhizal.

Microorganism-phosphate solvent have the ability to dissolve the phosphate through the secretion of organic acids produced for the release of phosphorus (P) of complex sorption (Hanafi et al., 2009). Research of Haryuni (2012) states that the fungus *Rhizoctonia* binukleat (BNR) isolated from the roots of vanilla acts as mycorrhizae. Infection mycorrhizal symbiosis with plants able to utilize P soils used in metabolism. Hapiza et al., (2014) stated that there was strong evidence that the arbuscularmycorrhiza contribute to an increased ability to fix N_2 from legumes.

This study aims to determine the effect of inoculation BNR and phosphorus fertilization on the formation of chlorophyll leaves NRA and vanilla seeds.

METHODS

This study was held on January 25, 2015 until June 10, 2015 in Green House Faculty of Agriculture Development Branch Surakarta, with the altitude of 300 meters above sea level. The materials used in this study include: vanilla plant seeds, inoculants BNR, phosphorus fertilizer, and soil. The tools used in the study include: poly bag size (12x15) cm, weights, paranet 70% (8X15) m, pens, notebooks, rulers, calipers, rope, stakes bamboo, label, small knife, waste paper, and staples. This study uses factorial archetype completely randomized design (CRD), which consists of 2 treatments 3 repetitions with the following weights. The first factor is dose of *Rhizoctonia* binukleat /BNR (M) consists of four levels ie M_0: 0 g = without BNR, M_1: 5 g/plant, M_2: 10 g/plant, M_3: 15 g/plant, while the factor of the second dose of Phosphorus (P) consists of four levels ie P_0: 0 g/plant, P_1: 3 g/plant, P_2: 6 g/plant, P_3: 9 g/plant, There are 16 combinations of treatment, respectively - each treatment repeated 3 times.

Implementation Research

Phosphorus is mixed into the soil sterile (vanilla planting medium) 500 g/polybag according to treatment. *Rhizoctonia* isolates rejuvenation binukleat (BNR) through a sterile medium broken milled corn inoculated into the soil that has been added P according to treatment. Then the vanilla cuttings are planted with the number 6 leaves for 24 weeks. Parameter observations consist of nitrate reductase activity and *chlorophyll*.

Levels of Analysis of Nitrate Reductase Activity (NRA)

According McCahsin (2000) nitrate reductase activity was measured by the roots washed with distilled water until clean, then cut into small pieces of approximately 1 mm with a small sharp knife. The root sections 500 mg inserted into the tube dark film contains Na-phosphate buffer solution 0.1M 5 ml (mixture NaH_2 $PO_4 2H_2O$ and Na_2 HPO_4 $2H_2O$) with a certain ratio. After soaking for 24 hours is replaced with a new buffer solution and added 0.1 ml of 5M $NaNO_3$ as the substrate with a pipette and recorded his time as the start of incubation. Prepared dye reagents consisting of

0.2 ml of 0.02% solution of N-Naphthyle thylene diamine and 0.2 ml of 1% sulfanil amide in HCl 3N. After 1 hour incubation 0.1 ml of incubation taken and put into a test tube containing the dye reagents. Then wait 1 minute until a pink color as a sign there has been a reduction of nitrate to nitrite by the enzyme nitrate reductase. One test tube is not given the filtrate and used as a blank. On the tube that has been added to measure the absorbance in a spectrophotometer cuvette is measured at λ 540 nm. Nitrate reductase activity expressed in micromoles nitrate/g tissue material per hour using the following formula:

$$NRA = \frac{\text{Absorbansi sampel}}{\text{Absorbansi standart}} \times 50 \times \frac{1000}{BB} \times \frac{1}{W_1} \times \frac{1}{1000}$$

Information:
The absorbance of the standard: 0.0142;
BB: Wet weight (mg)
W_1: incubation time (hours)

Levels of Total Chlorophyll Analysis

Total chlorophyll content is measured by the way: the sixth leaf has been stretched perfectly taken 0.1 g. Pieces of leaves crushed in a mortar and then added 10 ml of acetone 80%. The solution was allowed to stand for a moment. The solution was filtered with Whatman filter paper no. 42. The filtrate was put in a 3 ml cuvette is then inserted into the spectrophotometer. The solution was measured absorbance at a wavelength of 645 nm and 663 nm. Chlorophyll content calculated using the formula: Chlorophyll Total = 8.02 (A.663) + 20.2 (A.645) mg / L (Peni et al., 2004).

Analysis of Data

Data obtained from the results were analyzed with ANOVA statistical analysis if significantly different test followed by Duncan Multiple Range Test (DMRT) on the diversity level of 5%.

RESULTS AND DISCUSSION

Levels of Nitrate Reductase Activity

The enzyme activity affect the metabolism, especially nitrate reductase enzyme used in the chain of elements nitrate reduction. (Lea & Leegood, 1993), this enzyme plays a role in chemical reactions formation of amino acids and is widely used as a selection criteria are high yielding crops in crop breeding programs (Alnopri, 2004).

Figure 1 shows that inoculation BNR and P doses were not significantly different. BNR plants NRA lower levels, while the P dose increase levels of NRA. Dose of BNR escalation into 15g **cholesterol** 0g NRA. P at P1 P0 be decreased while P1 into P2 and P3 NRA levels tend to rise. Nitrate reductase (NRA) is an enzyme present in the outer membrane of protoplasts at an early stage role in the assimilation of nitrogen (N) in the metabolic process. The compound acts as secondary metabolites (Raharjo et al., 2000) and is one of the limiting factors of plant enzymes as nitrate assimilation process (Alnopri, 2004). Increased P increase the availability of plant nutrients that help in the process of photosynthesis to produce carbohydrates. (Haryuni, 2012). Nitrate reductase activity is affected by the formation of carbohydrates is further used in the process of respiration. Reduction of NAD + to NADH occurs in respiration and NADP + to NADPH occurs during photosynthesis. NADH or NADPH is an effort to reduce nitrate (NO_3) to nitrite (NO_2). NADH and NADPH serve as electron donors to be transferred to the coenzyme FAD as prosthetic groups or electron carriers (Hess, 1995 *cit*. Latifa & Anggarwulan, 2009).

Interaction BNR and P were significantly different, M_0 (P_0, P_1, P_2, P_3) NRA levels increased and were not significantly different, M_1P_0, M_1P_3 not significantly different but significantly different with M_1P_2 and M_1P_4. M_2P_0 not significant-

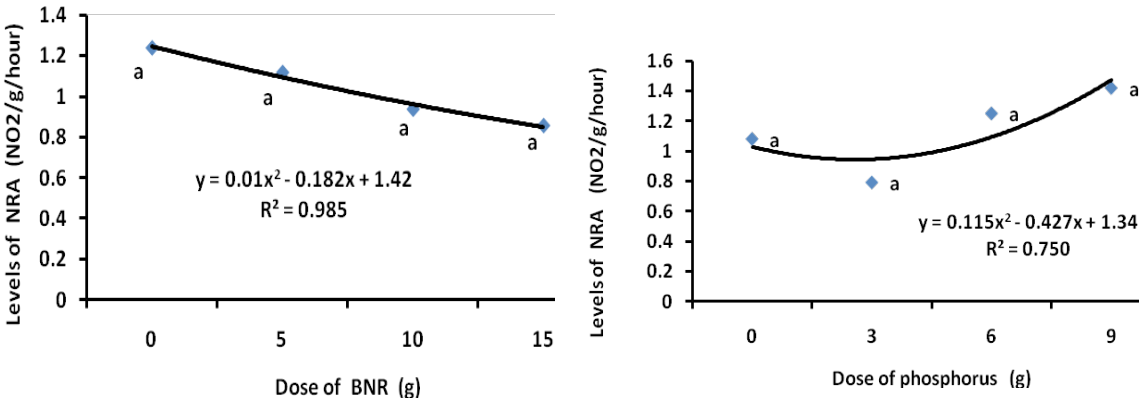

Figure 1. Levels of Nitrate Reductase Activity (NRA) of vanilla seedlings inoculated after BNR.

ly different with M_2P_3 but significantly different with M_2P_2 and M_2P_3. M_3 (P_0, P_1, P_2, P_3) NRA levels increased and did not differ significantly (Fig. 2). This is in accordance revelation Novi & Rizki (2014), that infection mycorrhizal fungi on plant roots can establish colonization of hyphae in the root zone and reaching a maximum when inoculated up to a certain dose limits. Tests on banana 10g dose inoculated mycorrhizal colonization of hyphae on rooting 53.33% while a dose of 15g and 20g of hyphae in root colonization decreased to 46.3%.

Nitrates are absorbed by plants is reduced by the enzyme nitrate reductase is converted to nitrite and then reduced to form ammonium together through biosinteis amino acid glutamine and glutamate (Fitriana 2009). The relationship between the rate of reaction with the enzyme activity is directly proportional (Fitriana 2009). The increase in nitrate reductase activity causing the resulting product increases with the increase in the rate of reaction (Fitriana et al., 2009). Nitarreductase enzyme activity in the metabolism of plants used in the formation of amino acids, proteins, chlorophyll and other compounds that contain nitrogen. Such compounds act as a factor limiting the assimilation of nitrate is very important in the process of vegetative growth and generative plant (Lea & Leegood, 1993; Alnopri, 2004).

NRA gives an affect in plant metabolism process (Fitriana et al., 2012), especially on forming an amino acid, protein and nitrogen molecule for plant's sel synthesis (Indradewa et al., 2004; Latifa & Anggarwulan, 2009; Miswar, 2013).

Chlorophyll

Figure 3 shows that the BNR inoculation significantly different to the levels of chlorophyll, whereas P doses were not significantly different. BNR at doses significantly different from the M_0 M_1, Inoculation BNR increased the M_1 then tended to decline in M_2 and M_3, while the dose of P decreased in P_1 and then tends to increase after P_2. External hyphae formed BNR help plant roots absorb nutrients and soil nutrients to be stored at the plant tissue through internal hyphae BNR (Haryuni, 2012). The growth and development of plants affected by nutrient availability (Purwandani, 2014), nutritional deficiencies resulting in interference pigment chlorophyll biosynthesis and degradation of proteins (Purwandani, 2014).

Provision of P in P1 lower levels of chlorophyll but the increase P tends to increase levels of chlorophyll (P_3). Decreased levels of kholorofil caused by protein degradation, resulting in inhibition of photosynthesis and nitrate reductase activity (Fig. 3) (Vaajpayee et al., 2000). Chlorophyll plays a role in the process of photosynthesis to form carbohydrates through solar energy and CO2 fixation. Carbohydrates are converted into proteins, lipids, nucleic acids and other organic molecules (Ai & Banyo, 2011).

Synthesis khorofil do in the leaves, influenced by light, sugar or carbohydrates, water, temperature, genetic factors, nutrients like N, Mg, Fe, Mn, Cu, Zn, S and O (Dwidjoseputro, 1994; Hendriyani & Setiari, 2009). The main component of chlorophyll is positively correlated to the chloroplast photosynthetic rate (Li et al., 2006). Increased BNR of 5g be 10g/plant reduce levels chorophyll

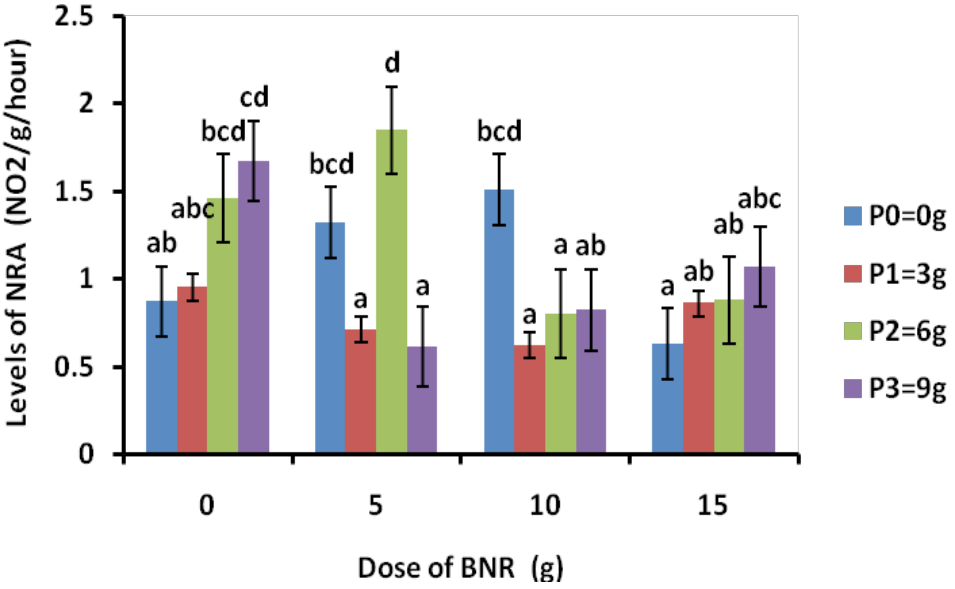

Figure 2. Levels of Nitrate Reductase Activity (NRA) of vanilla seedlings inoculated after BNR to BNR and Interaction of Phosphorus.

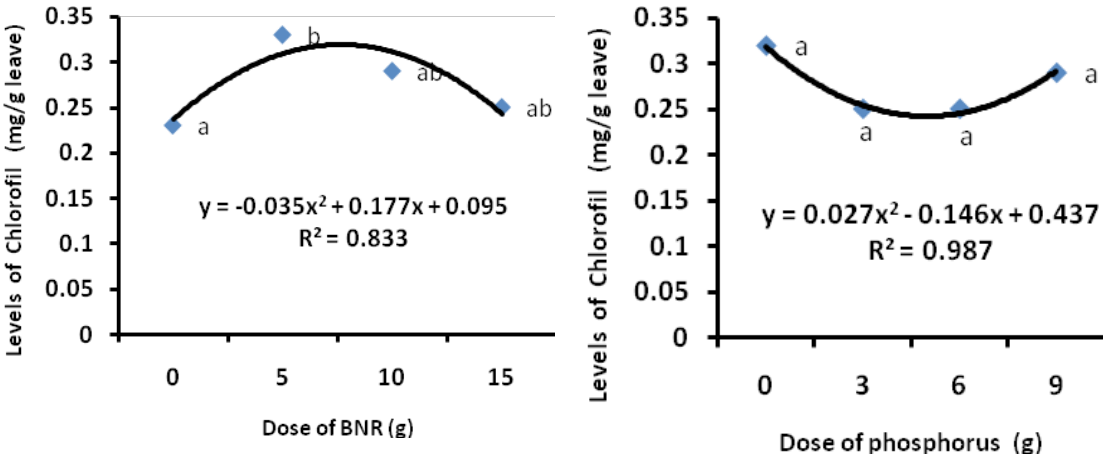

Figure 3. Levels of Chlorophyll of vanilla seedlings inoculated after BNR,

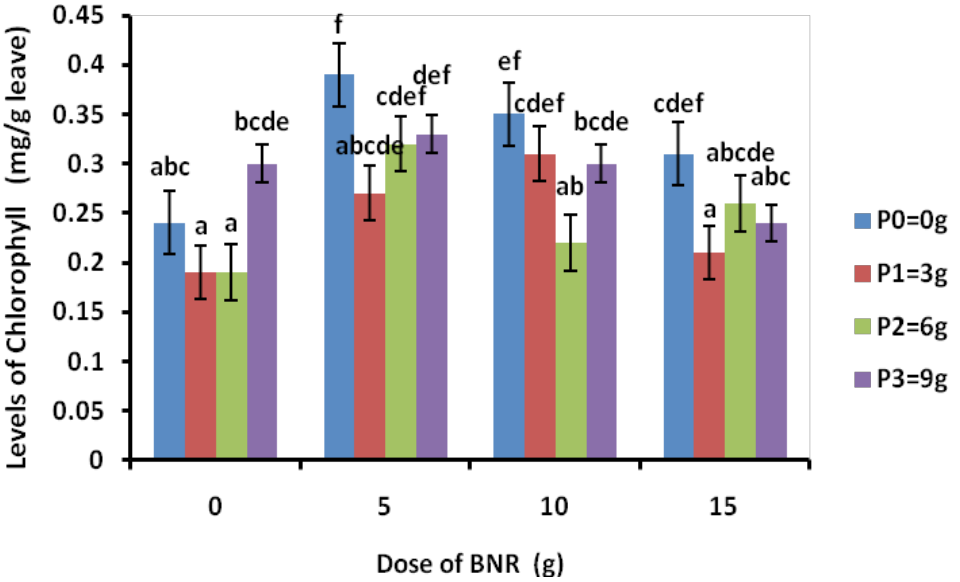

Figure 4. Levels of Chlorophyll of vanilla seedlings inoculated after BNR to BNR and Interaction of Phosphorus.

plants, inhibition of the formation of chlorophyll decrease of photosynthetic activity, giving P had a positive influence increase chlorophyll formation. Radiation light received by plants in photosynthesis is absorbed by the pigment chlorophyll and additional a chlorophyll protein complex. Furthermore, the radiation energy is transferred to the reaction center of photosystem as the occurrence of a change of light energy into chemical energy (Li et al., 2006). Chlorophyll protein complex is an important component of photosynthesis (Van der Mescht et al., 1999).

Interaction BNR and P significantly different to the levels of chlorophyll. M_0 (P_0, P_1, P_2) were not significantly different, M_1P_0 highest chlorophyll content of 0.39 mg/g of leaves significantly different with all the treatment and the lowest was 0.19 mg / g leaf on M_0P_1 and M_0P_2. Not significantly different with M_0P_0, M_1P_1, M_2P_3, and M_3 (P_1, P_2, P_3). Chlorophyll is the main pigment green chloroplasts found in plants algae and photosynthetic bacteria that play a role in the process of photosynthesis in plants, has the ability to absorb and convert light energy into chemical energy. Chloroplasts are organelles of plant cells that have outer membrane, the inner membrane, the space between the membrane and the stroma. The internal membrane surface chlorophyll chain fitil (C_20H_39O) turned into fitol (C_20H_39OH) when exposed to water with a catalyst klorofilase. Fitol is saturated primary alcohols that have a strong affinity towards O_2 in the reduction process of chlorophyll (Li et al., 2006; Ai & Banyo, 2011). Inhibition of the formation of chlorophyll causing decreased photosynthetic activity and primary metabolism depressed. (Lakitan *cit*. Ha-

ryuni, 2012).

BNR as mycorrhizal allegedly able to absorb P from sources of mineral P soluble because it produces organic acids and enzymes fosfotase. These compounds are able to release the bonds P soluble, such as Al-P and Fe-P so that the availability of P increased, but based on these trials efficacy has not shown a significant increase due to the strong affinity of P uptake by allophane (Sufardi et al., 2013), Mycorrhizal fungi infect part of the root tip and then hyphae multiply within the network and increasing the formation and growth of roots (Brundrett, 2004; Krishna, 2005). Mycorrhizae produce a compound glykoprotein which served to increase the ability of soil aggregates, the presence of hyphae external expanding field of uptake to the size of hyphae are smooth and small can infiltrate into the pores of the soil and an increase in the synthesis of organic compounds that fat, protein and carbohydrates (Brundrett, 2004; Krishna, 2005, Song, 2005).

Levels of the chlorophyll in photosynthesis process, chlorophyll which has been synthesized on leaf to get sun light that the total is different in every species depends on the environment factor and it's genetic (Hendriyani & Setiarti, 2009). Chlorophyll synthesis is an metabolism indicator between plant's photosynthesis and biomass (Li et al., 2006).

CONCLUSION

BNR Inoculation and Phosphorus significantly different with NRA and chlorophyll levels vanilla seeds, BNR which acts as mycorrhizae help rooting vanilla absorb water and nutrients (especially P) utilized in the metabolic process to form NRA and chlorophyll.

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