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Research Article
Effect of Arbuscular Mycorrhizal Fungi and NPK Fertilizer on Roots Growth and Nitrate Reductase Activity of Coconut

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Abstract

Background and Objective: A good seeds characteristics of coconut are expected to have optimal root and shoot growth which can be affected by mycorrhizal inoculation. This study aimed to determine the effect of arbuscular mycorrhizal fungi (AMF) and NPK fertilizer doses to colonization, roots and shoots growth, physiological parameters (Nitrate Reductase Activity) (NRA) and chlorophyll on early growth of coconut. **Materials and Methods:** Coconut seedlings were transplanted in the field in polybags and treated with a dose of treatment. The first treatment was the application of NPK fertilizer doses (4 and 6 g/seeds and without NPK). The second treatment was the application of AMF inoculum doses (4 and 2 g/seeds and without AMF). The parameters of colonization, chlorophyll contents, roots dry weight, total root length, root surface area, root diameter, number of primary roots, fresh weight of root, stem and leaf were evaluated using analysis of variance based on 3×3 factorials completely randomized design followed by Duncan's multiple range test with $p<0.01$ as the *post hoc*. **Results:** The combination of AMF application doses of 2/seeds with NPK fertilizer application doses of 4 g/seeds significantly increased ($p<0.01$) the highest colonization by reaching 41.60% compared with control. The application of AMF doses without NPK fertilizer treatment significantly increased ($p<0.01$) NRA and root surface area. The AMF application (2-4 g/seeds) combined with NPK fertilizer treatment (6 g/seeds) significantly increased ($p<0.05$) the root surface area compared with control. **Conclusion:** The AMF application combined with NPK fertilizer increased colonization, NRA and root surface area.

Key words: Arbuscular mycorrhizal fungi, NPK fertilizer, NRA, chlorophyll, roots-shoots-parameters

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The early growth of coconut seedlings after transplanting is a critical phase of the plant to constraint with environmental stress in dry land such as limited nutrients and low soil moisture[1,2]. The vigorous root growth which is depicted by roots surface area, roots diameter, roots dry weight: Shoot dry weight ratio is determinant of yield[3]. Vigorous root growth is also essential to support vigorous shoot growth and the improvement of healthy crop[4]. This is because vigorous roots growth plays a role in supporting the anchorage of plants[5], water and nutrient uptake[6,7] and improving soil physical condition[8]. All of these root functions are very essential for the early growth stages of transplanting coconut seedlings in the face up to the constraints of environmental factors or climate change for example drought stress.

To increase the vigorous root growth of coconut transplanting seedlings in the dry land, it is needed the treatments to increase root growth capability. Mycorrhizal inoculation is known to increase root growth in coconut nurseries in the form of root volumes and root dry weight[9]. Likewise, mycorrhizae in coconut nurseries increase seed tolerance to nematodes *R. similis* and increase shoot growth[10]. However, the treatment of mycorrhizae application in the field when transplanting seedlings and their effects on root growth has not been widely informed. This is important because it will increase plant vigor. According to Senarathne *et al.*[2] the coconut seedlings that have good vigor, are characterized by optimal development of roots and shoots to suppress seedling mortality after transplanting in the field.

The application of mycorrhizae is important because the role of mycorrhizae increases the hyphae of fungi which causes an increase in surface area for absorption of nutrients, especially P[11]. The P element and other nutrients are absorbed through the mycelium into the roots of the host plant so that it increases the availability of the P element and other nutrients[11,12]. The role of mycorrhiza also increases water absorption, plant resistance to soil pathogens, adaptability to drought stresses such as heat, salinity and heavy metals[13].

The absorption of nutrients especially N by the ability of roots is also determined by the activity of the enzyme namely nitrate reductase. Nitrate reductase activity (NRA) is a nitrogen cycle enzyme activity that plays an important role in the absorption of nitrogen in plants for the subsequent photosynthate formation process[14]. The NRA is determined by the substrate in the form of nitrate (nitrogen source). However, very little information on the NR activity is related to increased root growth by mycorrhizal inoculation. Information that can be learned at this time that the leaves NRA was also reported to increase affected the application of mycorrhiza[15]. Observation of leaves NRA in coconut seedlings is important as an early identifier of the ability of high-yielding of coconut seedlings[16]. Therefore, to improve the quality and early selection of coconut seedlings, the application of mycorrhiza and fertilization to improve roots growth and leaf NRA was treated

The aim of this study was to gain insight of the influence of doses application of AMF inoculum and NPK fertilizer into coconut transplanted seedlings on dry land from the viewpoints of (1) Development of mycorrhizal infection in rooting system, (2) Effect of combination treatments (AMF inoculum and NPK fertilizer) on roots growth, NRA, chlorophyll and early shoots growth parameters of coconut, (3) To determine the optimal dose of mycorrhizae and NPK fertilizer application for the early growth of transplanted coconuts seedlings. Therefore, the optimal dose of mycorrhiza and NPK fertilizer is essential to support the vigorous plant growth so that they are adaptive in the face up to environmental stresses such as drought, heat, salinity during early growth after transplantation.

MATERIALS AND METHODS

Site description and field management: The experiments were conducted in Kusu-Sofifi- (48 m a.s.l., S: 3° and E: 3°) the city of Tidore Island, North Maluku, Indonesia and according to Koppen's classification, belongs to a tropical monsoon climate. The soil in the experimental station is classified as latosol. This study was conducted from January to December, 2018 in the research land of Assessment Institute for Agricultural Technology (IAIT) of Sofifi, North Maluku. The highest and the lowest temperature on the research site were 33 and 25°C. The study of chlorophyll content and ANR, root parameters and colonization were carried out at Agronomy Department, Crop Production Management Laboratory, Crop Production Laboratory and Microbiology Laboratory of Agriculture Faculty of Universitas Gadjah Mada, Yogyakarta Indonesia from July, 2018 to January, 2019.

Plant material, treatments and experimental design: This study used coconut seedlings from Susupu West Halmahera. The seedlings of coconut were transplanted in the polybags (50×50 cm). The experimental had a factorial completely randomized design (CRD) with three replicates. The first factor consisted of three of mycorrhizal inoculum application doses (0 g/(M1), 2 g (M2), 4 g/seedlings (M3). While the second factor consisted of the three treatments of NPK doses (0 g/seeds (F1), 2 g/seeds (F2), 4 g/seeds (F3), 6 g/seedlings (F4). There were set up using 9 combination treatments. Each combination treatment consisted of 7 seedlings and it was replicated

3 times according to randomized completely scheme. Inoculation of AMF and NPK fertilizer application in the field begin after the coconut seedlings were transplanted in the field (polybags), 3 days after transplanted. Each coconut seedlings were subjected to one of the combinations treatments. The experiment was conducted by raising the coconut seedlings in polybags of 50×50 cm. The space between seedlings polybags was 2.5×2.5 m.

Strains of arbuscular mycorrhizal fungi (AMF) and NPK doses: The AMF inoculum was in the form of zeolite granular media. The doses of AMF inoculum was 4, 2 and 0 g/seeds (3.6/g of inoculum mycorrhizae) spore). The AMF was obtained by collecting the fungi from various regions in Java Island of Indonesia. Some genera of AMF obtained from isolation by different experimental team were *Glomus* sp., *Funneliformis* sp., *Acaulospora* sp., *Gigaspora* sp. and *Scutellospora* sp. Fertilization was conducted in the polybags according to each treatment. The fertilizer used NPK Phonska with 15% of nitrogen content for N, 15% of phosphate content for P and 15% of potassium content for K. The doses of NPK Phonska fertilizer was 6, 4 and 0 g/seedling.

Measurements: The parameters observed were: Colonization, chlorophyll content, ANR, roots dry weight, total roots length, roots surface area, roots diameter, a total of primer roots, fresh weight of roots, fresh weight steam, fresh weight leaf. The observation was done 9 months after transplanting coconut seedlings.

The colonization was measured by using modified clearing and staining[17]. Thirty root cuttings (1 cm) per combination treatment were randomly chosen from 3 sample plants. The observation was done from 8 months after transplantation. Total root length and root surface area were measured using area meter. Measurement of the total root length, root surface area and root diameter was done using the method of line intersection perfected by Indradewa[18]. The result was compared with the calculation of length according to Tennant[19]. To obtain the root surface area, roots were assumed to be cylindrical so that the root projection area = 2 RP with R was the radius, P was the root length. The root surface area was the area of the cylinder bark without cover at both the root edges, i.e., circumference multiplied with root length = 2πRP. The root diameter was obtained from the formula of root surface area, i.e., 2R. Measurement of the root dry weight was used by weighing from all roots after they were constantly dried. The measurement of the root, shoot and stem wet weight were carried out by weighing in the fresh conditions of each of these parameters. Measurement of a total of primer roots was obtained by using a calculated number of roots that emergence from radicle. Leaf chlorophyll content was measured using the Wintermans and de Mots method[20].

Statistical analysis: Data were submitted to analysis of variance of CRD using SAS 9 program for Windows. If there was an interaction between treatment, a comparison of the interaction effects was made. Treatment mean effects were compared based on Duncan's multiple range test at $p<0.05$. To find out the correlation between the parameters of colonization and root and shoot growth, a correlation analysis test was performed.

RESULTS

Colonization of mycorrhizal: The doses of AMF inoculum application that interacted with the doses of NPK fertilizer application significantly increased ($p<0.01$) the colonization of mycorrhizal at the age of 9 months after transplanting coconut seedlings. The application dose of 2 g mycorrhizal inoculum combined with 4 g of NPK fertlizer/seed caused the highest mycorrhizal colonization (97.67%) and was significantly different ($p<0.01$) among all treatment combinations (Table 1). These results indicated that the importance of optimal doses of AMF inoculation combination with NPK fertilization to improve mycorrhizal colonization during the transplanting seedlings in the dry land (Fig. 1).

Table 1: Effects of treatment combination on mycorrhizal colonization, NRA and chlorophyll

Doses of NPK fertilizer (g/seeds)	Doses of AMF inoculum (g/seeds)	Colonization (%)	NRA (μmol NO_2 g^{-1} h^{-1})	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total of chlorophyll (mg g^{-1})
6	4	85.000^{b}	2.034^{a-c}	0.4757^{a}	0.5292^{a}	1.0047^{a}
6	2	77.675^{b}	2.662^{a}	0.4557^{a}	0.5052^{a}	0.9607^{a}
6	0	60.000^{cd}	2.007^{a-c}	0.4300^{a}	0.4717^{a}	0.9012^{a}
4	4	50.000^{e}	1.318^{c}	0.4472^{a}	0.4930^{a}	0.9400^{a}
4	2	97.675^{a}	1.646^{b-c}	0.4210^{a}	0.4520^{a}	0.8722^{a}
4	0	77.675^{b}	2.3740^{ab}	0.4780^{a}	0.5237^{a}	0.9927^{a}
0	4	57.675^{de}	2.78^{a}	0.4432^{a}	0.4827^{a}	0.9250^{a}
0	2	67.675^{c}	1.544^{b-c}	0.4110^{a}	0.4337^{a}	0.8440^{a}
0	0	77.325^{b}	1.484^{b-c}	0.4467^{a}	0.4840^{a}	0.9292^{a}

Numbers followed by same letters in the same columns did not differ significantly at $p<0.05$ according to Duncan's multiple range test

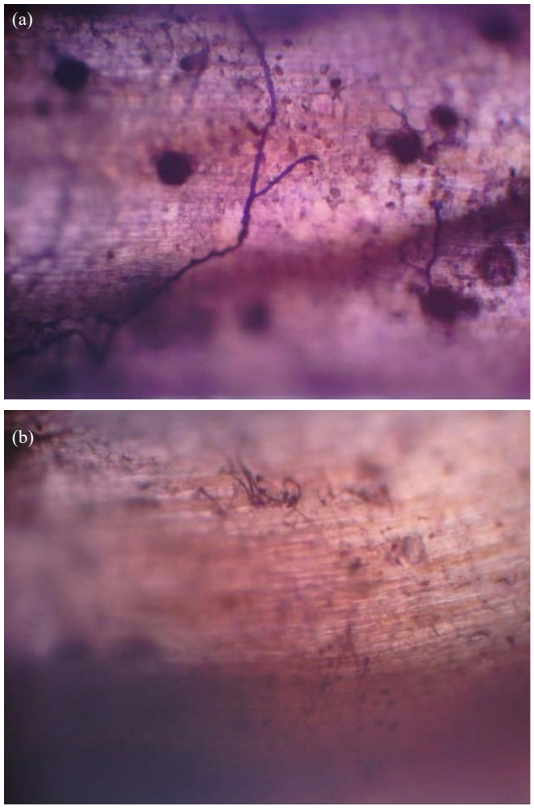

Fig. 1(a-b): Mycorrhizal colonization on roots in the early growth of coconut seedlings transplanting effected of (a) 4 g NPK+4 g inoculum AMF/seedlings treatments and (b) 6 g NPK+0 g inoculum AMF/seedlings treatments at the age of 9 months after inoculation
Scala bar: 10 µm, objective 10x

Nitrate reductase activity: The application doses of mycorrhizal inoculum that interacted with the NPK fertilizer significantly increased ($p<0.01$) the nitrate reductase activity (NRA) at the age of 9 months after transplanting. There are variations between application doses of mycorrhizal inoculum on the difference of NPK fertilizer doses application to the NRA. The treatments combination of doses application of 6 g AMF inoculum combined with 2g NPK fertilizer/seedlings caused higher NRA among all treatments combination and was significantly different ($p<0.01$) from doses application of 4 g NPK combined with 4 g AMF and 2 g AMF/seedlings also compared to 0 g NPK combined with 2 g AMF and 0 g AMF (Table 1). However, application of AMF inoculum dose (0 g/seedlings) still produces a high NRA at a high NPK fertilizer dose (6 g/seed). These results indicated that the high NRA is more determined by the availability of N substrate, i.e., NPK fertilizer. The NPK fertilizer that was not application caused a decrease in NRA except AMF was applied in high doses (4 g/seedlings). These results showed that the role of AMF in providing nutrients including nitrogen as the nitrate reductase enzyme substrate resulting in an increase in NRA.

Chlorophyll content: The doses application of AMF and NPK fertilizer did not significantly effect the leaf chlorophyll content compared with control (without doses application of AMF and NPK fertilizer). These results indicate that the leaf chlorophyll content at 9 months after application was not significantly determined by the AMF inoculum and NPK fertilizer in the dosage range of 1-4 g for AMF and 1-6 g for NPK fertilizer (Table 1).

Root dry weight: The doses application of AMF inoculum interacted with the NPK fertilizer significantly increased ($p<0.01$) the root dry weight at the age of 9 months after transplanting. The doses inoculum application of AMF 2 g/seedlings combined with 4 g NPK fertilizer caused highest root dry weight than all treatments combinations and was significantly different ($p<0.01$) from the without doses application of NPK fertilizer and AMF inoculum (control) (Table 2). These results showed that the optimal of doses application of AMF inoculum and NPK fertilizer was needed to increased root dry weight.

Root surface area: The root surface area of coconut at the age of 9 months after transplanting was significantly increased ($p<0.01$) by the interaction of doses application of AMF inoculum and NPK fertilizer. The doses application treatments of 4 g AMF combined with 4 g NPK fertilizer application resulted in the highest root surface area. It significantly differs ($p<0.01$) from without AMF inoculum application doses combined with without NPK fertilizer application doses and 6 g NPK fertilizer application doses or combination treatments of NPK fertilizer doses application of 6 g/seedlings with without AMF inoculum application (Table 2). The results showed that mycorrhizal inoculation was needed in fertilizing transplanted coconut seedlings to increase root surface area. In addition, mycorrhizal inoculation reduces the need for fertilizer in producing root surface area. The optimal doses application of AMF at the fertilization of 4 g NPK fertilizer doses application to improve of root surface area was 4 g/seedlings.

Root length: The root length of coconut at the age of 9 months after transplanting was significantly increased ($p<0.01$) by the interaction doses application of AMF inoculum and NPK fertilizer. The doses application treatments of 2 g AMF inoculum combined with 6 g NPK fertilizer resulted in the

Table 2: Effect of combination treatment of mycorrhizae and NPK doses on the root parameters of coconut plants

Doses of NPK fertilizer (g/seeds)	Doses of AMF inoculum (g/seeds)	Root dry weight (g)	Root surface area (cm^2)	Root length (m)	Root diameter (cm)	Number of primary roots
6	4	60.74^{a}	3693.9^{ab}	19.15^{ab}	193.30^{a}	13.50^{a}
6	2	62.60^{a}	3582.5^{ab}	19.81^{a}	184.38^{a}	14.50^{a}
6	0	53.25^{ab}	2936.4^{b-d}	14.99^{a-c}	200.20^{a}	13.50^{a}
4	4	56.70^{ab}	3853.8^{a}	18.96^{ab}	211.69^{a}	12.250^{a}
4	2	58.68^{ab}	3108.6^{a-d}	15.66^{a-c}	198.17^{a}	16.000^{a}
4	0	58.74^{ab}	3440.0^{a-c}	18.11^{a-c}	194.86^{a}	11.500^{a}
0	4	48.30^{ab}	2647.7^{c-d}	14.59^{b-c}	181.30^{a}	12.75^{a}
0	2	58.06^{ab}	3747.2^{ab}	17.73^{a-c}	212.26^{a}	14.00^{a}
0	0	41.92^{b}	2423.7^{d}	13.62^{c}	180.00^{a}	11.750^{a}

Numbers followed by same letters in the same columns did not differ significantly at $p<0.05$ according to Duncan's multiple range test

Table 3: Effect of combination treatment of mycorrhiza and NPK doses on the wet weight of shoot and root parameters

Doses of NPK fertilizer (g/seeds)	Doses of AMF inoculum (g/seeds)	Wet leaf weight (g)	Wet stem weight (g)	Root wet weight (g)
6	4	301.25^{ab}	583.75^{a}	199.50^{ab}
6	2	357.00^{a}	586.00^{a}	198.25^{ab}
6	0	298.25^{ab}	589.00^{a}	178.75^{ab}
4	4	334.00^{ab}	522.50^{a}	177.50^{ab}
4	2	268.00^{b}	439.00^{a}	219.50^{a}
4	0	323.50^{ab}	512.00^{a}	169.50 ^{ab}
0	4	278.25^{ab}	444.50^{a}	141.50^{ab}
0	2	314.00^{ab}	632.00^{a}	183.00^{ab}
0	0	274.75^{ab}	444.50^{a}	126.25^{b}

Numbers followed by same letters in the same columns did not differ significantly at $p<0.05$ according to Duncan's multiple range test

highest root length and it significantly differs ($p<0.01$) from control and combination treatments of doses application of 4 g AMF inoculum/seedlings and 0 g NPK fertilizer/seedlings (Table 2). These results indicate that the importance of mycorrhizal inoculation and application of NPK fertilizer at optimal dosages to increase root length.

Root diameter: The doses application of AMF inoculum and NPK fertilizer did not significantly effect the root diameter compered with control (Table 2). These results indicated that root diameter was not responsive effect of doses application of AMF inoculum and NPK fertilizer among root parameters ie. root length, root surface area, root dry weight and root wet weight. These results also showed that coconut roots tended to have relatively uniform root diameters.

A number of primary roots: The doses application of AMF inoculum and NPK fertilizer did not significantly effect the number of primary roots compered with control (Table 2). These results indicate that the number of primary roots is not determined by the NPK dose and mycorrhizal application.

Wet leaf weight: The leaf weight was influenced by the interaction ($p<0.01$) of AMF inoculum doses application and NPK fertilizer application. Wet leaf weight was significantly higher ($p<0.01$) in application doses of 6 g NPK fertilizer/seedlings combined with 2 g AMF inoculum/seedlings than the combination treatments of 4 g NPK fertilizer/seedlings and 2 g AMF inoculum/seedlings (Table 3). These results indicate that the optimal application dose of NPK fertilizer and AMF inoculum causes an increase in the weight of fresh leaves of the plant at the age of 9 months after transplanting.

Wet stem weight: The doses application of AMF inoculum and NPK fertilizer did not significantly effect the wet stem weight compered with control (Table 3). These results indicated that wet stem weight of coconut transplanted seedlings at the age of 8 months after transplanting has not effected by NPK and mycorrhizal application at this dose. These results indicate that the wet stem weight of coconut at the age of 9 months after transplanting is not determined by the range of these doses application of NPK fertilizer and AMF inoculum.

Root wet weight: The application doses of mycorrhizal inoculum that interacted with the NPK fertilizer significantly increased ($p<0.01$) the root wet weight at the age of 9 months after transplanting. The doses application treatments of 2 g AMF inoculum/seedlings combined with 4 g NPK fertilizer/seedlings resulted in the highest root wet weight and it significantly differs ($p<0.01$) from control (Table 3). These results indicate that root growth was more responsive in the form for increased root wet weight than shoot growth.

Table 4: Correlation of colonization, NRA, chlorophyll, rooting and growth properties

	Colonization (%)	NRA	Chlorophyll a	Chlorophyll b	Chlorophyll total	Root dry weight	Root surface area	Root length	Wet leaf weight	Wet stem weight
Colonization (%)	1.000									
NRA	0.875^{ns}	1.000								
Chlorophyll a	0.448^{ns}	0.819^{ns}	1.000							
Chlorophyll b	0.456^{ns}	0.732^{ns}	<0.0001**	1.000						
Chlorophyll total	0.649^{ns}	0.687^{ns}	<0.0001**	<0.0001**	1.000					
Root dry weight	0.502^{ns}	0.530^{ns}	0.765^{ns}	0.658^{ns}	0.916^{ns}	1.000				
Root surface area	0.868^{ns}	0.470^{ns}	0.460^{ns}	0.475^{ns}	0.248^{ns}	0.0768^{ns}	1.000			
Root length	0.987^{ns}	0.686^{ns}	0.843^{ns}	0.580^{ns}	0.891^{ns}	0.0035**	<0.0001**	1.000		
Wet leaf weight	0.401^{ns}	0.641^{ns}	0.971^{ns}	0.768^{ns}	0.624^{ns}	0.662ns	0.516^{ns}	0.256^{ns}	1.000	
Wet stem weight	0.518^{ns}	0.422^{ns}	0.383^{ns}	0.424^{ns}	0.250^{ns}	0.521ns	0.296^{ns}	0.345^{ns}	<0.0001**	1

**Significant difference, ns: Non significant

Correlation of colonization with physiological, roots and shoots parameters: There was no positive correlation of mycorrhizal colonization with NRA, leaf chlorophyll content, root and shoot parameters (Table 4). These results indicate that the higher colonization at 9 months after inoculation does not cause the higher growth or activity of these parameters.

DISCUSSION

The results of this study indicate that the application of optimal doses of NPK fertilizer and mycorrhizal inoculum for the early growth of coconuts seedlings in a dry land was 6 g NPK fertilizer and 4 g AMF /seedlings (Table 1). These results are consistent with the report of Wang *et al.*[21] that mycorrhizal colonization of forest plant seedlings was determined by environmental factors such as P and nitrate. According to Kumar *et al.*[22] on the determinant of root colonization is the concentration of soil P nutrient. The interaction effect of AMF doses and NPK fertilizer doses application was variation among combination treatments of NRA. This finding is in line with Moche *et al.*[23] that the relationship between NRA and mycorrhizal colonization is parabolic. Caravaca *et al.*[24] reported that root NRA increased by 137% on plants inoculated with *G. deserticola* in good irrigation.

The leaf chlorophyll content was not significantly affected by the mycorrhizal inoculum doses and NPK fertilization doses application (Table 1). This result is in line with the report of El Kinany *et al.*[25]. Increased chlorophyll content occurs by the influence of vermicompost application[26]. The presence of compost will strongly control the development and abundance of the soil microbial community, thus there is a linear correlation between them[27].

The results of Table 3 indicate that mycorrhizal treatment determines root growth and development more than leaves. The ability of mycorrhizae to increase root growth was also reported by Sulistiono *et al.*[28] that AMF inoculation of sugarcane seeds in the nursery significantly accelerates the growth of shoot roots in sugarcane seedlings. These results are in line with previous research reports that AMF inoculation improves root parameters in yellow oranges[29] and tea[30].

In general, the correlation of mycorrhizal colonization with physiological and roots-shoots growth parameters shows did not a positive correlation (Table 4). According to Wang *et al.*[21] mycorrhizal inoculation was positively correlated with leaf P and K content while mycorrhizae play a lot in the absorption of P elements in plant[11,31].

The NRA which was not positively correlated with colonization shows that the effect of colonization is not linear with leaf NRA. This is consistent with the opinion of Moche *et al.*[23]. According to Carelli and Fahl[32], NRA is positively correlated with the assimilation of CO_2 and nitrates. Colonization was not positively correlated with the growth of root parameters. Based on the report Sulistiono *et al.*[28] showed that the sugarcane root colonization changed according to the length of time after inoculum application time.

The implications of the findings of this study are the application of AMF and NPK fertilization at optimal doses (6 g of first NPK fertilizer and 4 g of AMF inoculum) increasing surface area, length, wet and dry weight of roots and nitrate reductase activity. Seedlings that have more high root growth parameters and NRA are expected to have higher growth capacity in the constrain of drought stress and limited nutrients to reduce seedling mortality during early growth after transplanting. The application method is sowing in a circle around the stem of coconut seedlings after transplantation. Thus the limitations of this study are the application of doses optimal of NPK first fertilizing and mycorrhizal inoculation for root-shoots growth and plant physiological traits such as ANR on the early growth of coconut in the field.

SIGNIFICANCE STATEMENTS

This study discovers the optimum doses of AMF inoculum and NPK fertilizer combination that can improve the roots growth and leaf NRA at the early growth of coconut transplanting seedlings. This study will help the researcher to uncover the critical area of the improve on roots-shoot growth and physiological traits of the early growth of coconut transplanted seedlings on the field that many researchers were not able to explore. Thus, a new theory on these doses application of AMF inoculum and NPK fertilizer affected the growth of the roots and leaf nitrate reductase activity, may be arrived at.

CONCLUSION

The AMF application combined with NPK fertilizer increased colonization and root surface area. The combination of AMF application doses of 2/seeds with NPK fertilizer application doses of 4 g/seeds significantly increased the highest colonization by reaching 41.60% compared with control. The application of 4 g of mycorrhizal inoculum has the highest leaf NRA with nonapplication of NPK fertilizer doses. The AMF application (2-4 g/seeds) combined with NPK fertilizer treatment (6 g/seeds) significantly increased the root surface area compared with control.

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