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Amendment of mycorrhizae and its residual effect on growth and yield of maize (*Zea mays*) hybrids in coastal land

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Abstract. *Rustikawati, Suprijono E, Murcitro BG, Herison C, Sitohang SJ. 2022. Amendment of mycorrhizae and its residual effect on growth and yield of maize (*Zea mays*) hybrids in coastal land. Biodiversitas 23: 5600-5605.* Maize (*Zea mays* L.) is known sensitive to less optimal environmental conditions. Maize cultivation in coastal land has low productivity due to the availability of low nutrients and the ability to hold ground water. The addition of mycorrhizal fungi (MF) to the coastal land is expected to improve the root environment suitable for plants. This study aimed to evaluate growth and yield of maize hybrids grown in coastal areas amended with MF and examining its residual effect. The newly developed hybrids, CT34 and CT17, and a commercial check cultivar, BISI-18, were grown on a coastal land with the application of mycorrhizae at the rate of 0, 5, and 10 g.plant^{-1} in a factorial experiment with three replications. Observations were made on plant height, stem diameter, number of leaves, leaf length, leaf width, leaf greenness, root fresh weight, ear length, ear diameter, number of grain rows, number of grain.ear^{-1}, and grain weight.plant^{-1}. Data were analyzed for ANOVA, and the mean comparisons were conducted with DMRT at α=5%. The results showed that there was no interaction effect between the maize hybrids and the rate of mycorrhizae on any observed variables. The new hybrid of CT34 showed almost similar growth to that of the check cultivar, BISI 18, but the yield was significantly lower. CT34 showed better performance than CT17 on all variables. The application of MF significantly increased stem diameter and root fresh weight, which in turn increased maize yields in the coastal land. Increasing the mycorrhizal rate up to 10 g.plant-1 in coastal lands was ineffective in increasing maize productivity compared to 5 g.plant-1. In a bioassay study on the mycorrhizal residues in the second season, the treatment of 10 g.plant^{-1} significantly increased maize growth by more than 100% compared to without mycorrhizae.

Keywords: Coastal land, hybrid maize, salinity

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereals in Indonesia as it is a raw material for animal feed and industry (Ranum et al. 2014; Soetedjo 2017). It contains approximately 72%, 10%, and 4%, respectively, containing starch, protein, and fat, supplying 365 Kcal of each 100 g diet (Ranum et al. 2014). The plant grows well in various environmental conditions, so it is widely cultivated in many farming areas of Indonesia. In 2017, the maize planting area of Indonesia was about 5.5 million ha, with the production of 28.9 million tons of dry grain (Kementerian Pertanian Republik Indonesia 2018). However, to fulfill the national demand for maize grain, mainly for the animal feed industry, Indonesia still imported as much as 1.33 million tons in 2016 (Sulaiman et al. 2018).

The expansion of maize planting area to marginal lands, one of which is coastal areas, has to be carried out to elevate the national maize production. Indonesia is an archipelagic country with a huge coastal area, which is most of the time influenced by high salinity (Yu et al. 2014) and low nutrient content (Edman et al. 2018). Therefore, improving maize productivity in this type of land, it requires specific treatment in cultivation. Maize is known sensitive to environmental stresses. Salt content with electrical conductivity (EC) of more than 4.0 dS.m^{-1} seriously interferes with maize growth (Rustikawati et al. 2022). Moreover, coastal land with sandy soil contains low nitrogen (N), phosphorus (P), and potassium (K) nutrients (Naher et al. 2011) which limits plant growth and production (Carpenter and Bennett 2011). N, P, and K fertilizers are often necessary to increase crop production (Macdonald et al. 2011), besides manipulating crop growing environment.

Environmental engineering by biological fertilizers is currently becoming more popular approaches to maintaining good soil health. One of potential biological fertilizer is the one containing mycorrhizal fungi. Mycorrhizal fungi (MF) improves plant nutrient absorption and help plants adapt to changing environments. These fungi, in particular, express proteins that transport inorganic phosphate (P_i) from the soil to colonized roots *via* symbiotic interfaces (Plassard et al. 2019). Mycorrhizae are fungi that live on plant roots and aid in the absorption of nutrients such as N, P, K, Ca, Mg, Zn, and Mn (Shao et al. 2018). The use of mycorrhizal fungi has been shown to improve roots, increase nutrient absorption, and maintain plant conditions under salinity stress (Palupi 2021). It is commonly known that arbuscular mycorrhizal (AM) fungi provide the direct link between soil and roots.

Applying mycorrhizae at a rate of 5 g.plant-1 increased the diameter of the stem, the length of the ear, and the percentage of mycorrhizae-infected roots (Yoseva et al.

2014). Mycorrhizal treatment of 20 g.plant^{-1} improved plant height, the number of ears per plot, dry weight of ears per plot, and P uptake of maize plants (Supriyadi and Jaenudin 2013). Besides the amendment of mycorrhizae, the use of suitable varieties may increase maize production in coastal areas. Hybrid varieties are superior varieties developed by many breeding programs. In this study, the evaluated maize hybrid was developed from across between inbred lines generated from gamma irradiated mutants with an inbred line of local accession of Bengkulu. This study aimed to evaluate the growth and yield of maize hybrids grown in coastal areas amended with mycorrhizal fungi and examine its residual effect.

MATERIALS AND METHODS

Study area

The experiment was situated in a coastal land of Bengkulu, Indonesia, at 1 m above sea level and about 100 m from the seashore.

Growth of maize hybrids in coastal land amended with mycorrhizal fungi

Prior to study, the soil was analyzed for its physical and chemical properties. The experiment was arranged in a factorial randomized complete block design with two factors and three replications. The first factor was the maize hybrid consisting of three genotypes, i.e. CT34, CT17, and BISI-18 as the check. The second factor was the rate of mycorrhizae, i.e. 0, 5, and 10 g.plant^{-1}. The combination of the two factors resulted in 9 treatments, which accounted for the total of 27 experimental units, each of which was a twin row of 10 plants. The plant distance between rows was 75 cm and in rows was 25 cm apart.

The evaluated hybrids were developed from a crossed between inbred line of local Bengkulu and inbred lines generated from Gamma-ray irradiation mutant. The seeds were sown singly in planting holes previously applied with Furadan 3G to prevent from insect interference. Mycorrhizae were applied simultaneously in planting holes, accordingly with the designated rate. Fertilizer of urea, triple super phosphate (TSP), and KCl was side-dressed at a rate of 150, 50, and 100 kg.ha^{-1}, respectively. At the age of 4 weeks after seeding, the plants were row mounted covering the roots to strengthen the stems. Plant maintenance, such as watering, weed controlling, and pests and diseases controlling, was conducted following the methods of commercial maize growers.

Harvesting was carried out at full maturity stage indicated, with yellowish maize husk detected in more than 75% of the plant population. The ear were husk-stripped and sun-dried in the field. After the seeds were sufficiently dry, the ears were harvested and the grains in each ear were then loosened and measured for number of grain per ear and per plant. The observation was carried out on 5 sample plants selected by a simple random sampling method. Measurement was taken on plant height, stem diameter, number of leaves, leaf length, leaf width, leaf greenness, root fresh weight, ear length, ear diameter, number of grain rows, number of grain.ear^{-1} and grain weight.plant^{-1}.

Mycorrhizal residue bioassay

A bioassay was carried out in the greenhouse to examine the effects of mycorrhizal residues. The experiment was arranged in a completely randomized design with 5 replications. The treatment was the residue of the field application of mycorrhizae consisted of 0, 5, and 10 g.plant^{-1}. The bioassay was conducted for 8 weeks. The hybrid used as the test plant was CT34. The media was the soil taken from the field experiment ameliorated with 0, 5, and 10 g.plant^{-1} in the first season. The soil media was put into polybags of 35 cm wide × 35 cm high filled with as much as 10 kg media. The assay was conducted without inorganic fertilization to determine the effectiveness of mycorrhizae in supporting the growth of maize plants. Each polybag was singly seeded in a planting hole previously dressed with Furadan 3G at the rate of 20 kg.ha^{1} to prevent insect disturbance and the plants were maintained until 8 weeks after seeding (WAS). Measurements were carried out on plant height, number of leaves, leaf length, leaf width, and stem diameter. Observations were made twice, at 4 and 8 WAS.

Data analysis

The data was analyzed by ANOVA at α=5%. The mean comparison was performed with the DMRT α=5%.

RESULTS AND DISCUSSION

Growth of maize hybrids in coastal land ameliorated with mycorrhizal fungi

The experiment was conducted in a coastal area which was strongly influenced by coastal agro-climates. The coastal soil samples were collected for soil physical and chemical analysis at the Soil Science Laboratory, Faculty of Agriculture, the University of Bengkulu. The results of the analysis showed that the texture of the soil was a sandy loam (80% sand) with a neutral pH level (6.8). The C-organic content was low (1.32%), $K_{ex}$was relatively low (0.37 me.100g^{-1}) and the CEC value was also low (14.28 me.100g^{-1}). The total N was low (0.24%) and P-Bray was very low (3.19 ppm). Land cultivation was not carried out intensively with these physical characteristics to prevent further nutrient leaching. The land preparation was performed merely to remove weeds and create experimental plots.

The results of ANOVA on all variables showed that all genotypes responded similarly to the increasing rate of mycorrhizae. The genotype significantly affected on the number of leaves, leaf width, leaf length, root fresh weight ear diameter, number of grain.ear^{-1}, grain weight.plant^{-1}, and ear length. Meanwhile, mycorrhizae rate significantly affected stem diameter, number of kernel rows, root fresh weight and grain weight.plant^{-1} (Table 1). The coefficient of variance ranged from 6.76% to 29.97%, indicating a moderate data distribution for field research.

Further examination of the growth phase showed that the new hybrid CT34 was better than CT17. Although in regard of plant height, stem diameter, leaf greenness, and number of grain rows, no significant difference between the two hybrids were observed, but there was a tendency that CT34 was higher than CT17. The new hybrid CT34 was not significantly different from the commercial hybrid BISI18 in the number of leaves and root fresh weight. In term of plant height, stem diameter, and leaf greenness, CT34 tended to be higher than BISI18 (Table 2).

The two newly developed hybrids were significantly different in all yield component variables. The CT34 had a markedly larger ear diameter. The other variables were not significantly different, but the number of grain.ear^{-1} and grain weight.plant^{-1} tended to be larger on CT34. In line with the vegetative variables, the performance of BISI18 yield components was also better than CT34 (Table 3).

The application of mycorrhizae on coastal land improved the performance of maize in both vegetative growth and yield components. The significant increase in the vegetative growth was noticeable in stem diameter and roots fresh weight. However, increasing the rate of mycorrhizae from 5 to 10 g.plant^{-1} did not significantly increase these two variables (Table 4). Although the increase in each the yield component was not substantial, there was a significant increase in yield at 5 g .plant^{-1} mycorrhizal treatment, which was up to about 50%. However, doubling the rate slightly reduced the increase in grain weight.plant^{-1} (Table 5).

Table 1. Summary of the F values and coefficient of variance of analysis of variance on the observed variables

Variables	F value			Coefficient of variance (%)
	Genotype	Mycorrhizae	Interaction	
Plant height	1.23 ns	1.84 ns	0.05 ns	14.79
Stem diameter	2.85 ns	13.30 *	1.52 ns	13.47
Number of leaves	15.03 *	1.53 ns	1.53 ns	8.59
Leaf length	4.81 *	0.55 ns	0.04 ns	14.76
Leaf width	7.32 *	0.77 ns	0.36 ns	8.50
Leaf greenness	1.70 ns	1.16 ns	2.73 ns	11.62
Root fresh weight	4.24 *	4.49 *	0.61 ns	16.12
Ear length	5.73 *	1.24 ns	1.01 ns	10.46
Ear diameter	7.04 *	1.64 ns	0.64 ns	6.76
Number of grain rows	0.33 ns	5.61 *	1.11 ns	5.12
Number of grain.ear^{-1}	18.72 *	3.24 ns	0.13 ns	19.87
Grain weight.plant^{-1}	13.25 *	4.44 *	0.14 ns	29.97

Note: * = significantly difference at α=5%, ns = not significantly difference at α=5%.

Table 2. Average growth of three hybrid maize genotypes in coastal

Treatment	Plant height (cm)	Stem diameter (mm)	Number of leave	Leaf length (cm)	Leaf width (cm)	Leaf greenness	Root fresh weight (g)
CT17	162.55	19.74	10.33 b	75.5 b	7.6 b	50.98	43.24 b
CT34	173.88	21.62	12.55 a	81.3 ab	8.6 a	51.14	51.66 a
BISI18	156.11	18.62	12.66 a	88.0 a	8.4 a	46.62	54.00 a

Note: Numbers in the same column followed by different letters are significantly difference according to DMRT at α=5%.

Table 3. Yield components of three hybrid maize genotypes in coastal

Treatment	Ear length (cm)	Ear diameter (mm)	Number of grain rows	Number of grain.ear^{-1}	Grain weight.plant^{-1} (g)
CT17	15.45 b	40.59 b	12.4 b	260.1 b	71.81 b
CT34	15.72 b	44.27 a	12.6 b	329.1 b	104.33 b
BISI18	17.95 a	45.62 a	13.8 a	457.1 a	150.66 a

Note: Numbers followed by different letters in the same column are significantly difference according to DMRT at α=5%.

Table 4. Effect of mycorrhizae on growth of maize hybrid

Treatment	Plant height (cm)	Stem diameter (mm)	Number of leaf	Leaf length (cm)	Leaf width (cm)	Leaf greenness	Root fresh weight (g)
Without mycorrhizae	151.66	16.21b	11.5	80.6	7.9	51.84	43.30b
Mycorrhizae 5 g.plant^{-1}	168.66	21.90a	12.3	84.0	8.5	49.26	52.46a
Mycorrhizae 10 g.plant^{-1}	172.22	21.87a	11.7	80.3	8.1	47.64	53.13a

Note: Numbers followed by different letters in the same column are significantly difference according to DMRT at α=5%.

Table 5. Effect of mycorrhizae on the yield of maize hybrid

Treatment	Ear length (cm)	Ear diameter (mm)	Number of grain rows	Number of grain.ear^{-1}	Grain weight.plant^{-1} (g)
Without mycorrhizae	15.64	42.05	12.7	302.1	86.88 ^{b}
Mycorrhizae 5 g.plant^{-1}	16.67	44.09	13.0	381.9	132.68 ^{a}
Mycorrhizae 10 g.plant^{-1}	16.81	44.35	13.1	362.3	107.23 ^{ab}

Note: the numbers followed by different letters in the same column are significantly difference in the DMRT at α=5%.

Table 6. Results of ANOVA on maize growth in mycorrhizal residue treatment at 4 weeks and 8 weeks after seeding

Variables	4 weeks after planting		8 weeks after planting	
	$F_{calculated}$	Probability	$F_{calculated}$	Probability
Plant height	4.95	0.0354 *	6.99	0.0147 *
Stem diameter	3.39	0.0796 ^{ns}	2.55	0.1324 ^{ns}
Number of leaves	4.12	9.9537 ^{ns}	6.18	0.0205 *
Leaf length	5.71	0.0521 *	5.45	0.0282 *
Leaf width	6.66	0.0168 *	1.47	0.2801 ^{ns}

Note: the numbers followed by the asterisk symbol (*) are significantly difference at α=5% and 'ns' are not significantly difference at α=5%.

Table 7. Growth of maize at week 8 on media treated with mycorrhizal residues

Treatment	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaves
Without mycorrhizae	62.12 ^{b}	44.13 ^{b}	2.53^{b}	6.50^{b}
Mycorrhizae 5 g.plant^{-1}	103.00 ^{ab}	65.25 ^{ab}	3.53^{b}	8.75^{a}
Mycorrhizae 10 g.plant^{-1}	135.75 ^{a}	80.50 ^{a}	6.73^{a}	9.20^{a}

Note: numbers followed by different letters in the same column are significantly difference in the DMRT at α=5%.

Mycorrhizal residue bioassay

Based on the results of ANOVA at the age of 4 WAS, mycorrhizal treatment affected plant height, leaf length, and leaf width. The number of leaves and stem diameter were not affected by the rate of mycorrhizae. Meanwhile, based on observations at 8 WAS, in addition to plant height, leaf length and the number of leaves were also affected by the addition of mycorrhizae (Table 6).

Further examination on the variables affected by the residue of mycorrhizal treatment indicated that the higher the rate of mycorrhizae, the greater the increase in plant height, leaf length, and leaf width of maize. In the rate of 10 g.plant^{-1}, plant height, leaf length, and number of leaves were significantly higher than in other treatments. The average increase of all growth variables, but the leaf width, was not significantly different between 5 g.plant^{-1} and 10 g.plant^{-1} (Table 7).

Discussion

Coastal land becomes marginal land for agriculture due to various constraints. The main problem of coastal areas is low nutrient content due to leaching of sandy type of soil and high salinity (Edman et al. 2018). The results of the analysis of the chemical properties of coastal land at the experimental site also showed low macronutrients (N, P, K, Ca), so they were not suitable for maize growth (Solaimalai et al. 2020). The soil acidity was 6.8, and this is the ideal pH for maize growth. The soil pH level most suitable for maize was in the range of 5.6 to 7.5 (Tripathi et al. 2011). Although the pH level was in the ideal range, low nutrient status of the experimental site was also noticed by the poor growth of maize in the control treatment.

The study was conducted at the beginning of the dry season. Based on agro-climatic data at the research site, rainfall in the first month of the study was quite high, 459 mm.month^{-1}. This condition illustrates that water needed for plants can be fulfilled, but it can also increase nutrient leaching on the sandy land as well. Meanwhile, the average rainfall in the following 3 months was only 93 mm.month-1. Maize plants showed drought symptoms so that manual irrigation had to be carried out every day. Maize plant requires rainfall at least in a range of 135 to 225 mm.month^{-1} (Solaimalai et al. 2020). The changes in environmental condition determine the stability of growth and yield of maize (Rustikawati et al. 2020).

The use of adaptive cultivars and soil manipulation are approaches can be taken to improve coastal land productivity. Although evaluation of new elite hybrids in this study did not reveal an astonishing outcome, a new hybrid CT34 was more adaptive in coastal areas than CT17. However, both of them were still lower than the commercial maize hybrid BISI18. Genetic makeup of a genotype determines its performance in the field. Genotype and environment play important roles in the adaptability of genotypes to their new environment (Anley et al. 2013; Solaimalai et al. 2020).

Amelioration is a manner of soil manipulation to improve soil condition to be more suitable for the desired crop plant. One of this is the application of mycorrhizae. It is widely recognized that mycorrhizae have mutual relationship with many plants. The fungi facilitate absorbility of many nutrients readily, and the plant provides carbohydrate for the fungi to grow (Varma et al. 2017). Mycorrhizae also increases P nutrient uptake by facilitating P transport to roots (Püschel et al. 2021). Application of mycorrhizae revealed positif effect on growth and yield of maize. Mycorrhizal fungi improve maize salt tolerance by increasing plant nutrient availability (Farooq et al. 2015). In the present study, the responses of the three maize hybrids to the addition of mycorrhizal rate were the same. Although, the effect of mycorrhizal treatment of 5g and 10g.plant^{-1} on maize was significantly noticeable only on root fresh weight and grain weight per plant, there was a tendency of increasing the value of all variables in relation to mycorrhizal treatment. The result of present investigation was in harmony with the finding of Mobasser and Moradgholi (2012), that the application of mycorrhiza increased number of grain per ear of maize.

The weak effect of mycorrhizae on the growth and yield of maize in the coastal land in the present study was probably due to the influence of soil salinity of the land. The soil of our experimental site is classified as saline soil as its EC value of 5.84 dS.m^{-1}. High concentration of salt in the soil adversely influenced both plant growth and the colony of mycorrhizae (Elhindi et al. 2017). Significant differences in mycorrhizal diversity indices between non-saline (EC_{se} 2 dS.m^{-1}) and extremely saline (EC_{se} > 16 dS.m^{-1}) soils, with *Glomus caledonium* dominating only in soils with EC_{se} 8 dS.m^{-1} (Krishnamoorthy et al. 2014).

Mycorrhizae have a variety of propagules, including spores, hyphae, and rhizomorphs, which are structures that survive in environments like the rhizosphere (Santoyo et al. 2021). Therefore, mycorrhizae improve soil fertility in the long term. In our study, the effects of mycorrhizal residues were evaluated on maize plants up to 8 WAS without any addition of fertilizer. Although, based on previous research, it was known that the application of mycorrhizal fertilizers alone could not substitute for the need for nitrogen nutrients (Shokri and Maadi 2012), plants growing in nitrogen-limited soils may have their mycorrhization processes stimulated (Hodge and Storer 2014). The results of present study showed that there was a significant increase of plant growth in soil media previously ameliorated with mycorrhizae. The higher the mycorrhizal rate, the higher increase of plant height, number of leaves, leaf length, or leaf width. In the treatment of mycorrhizal residues of 10 g.plant^{-1}, plant grew more vigorously and the plant height could increase by more than 100% compared to without mycorrhizae. Maize roots secrete organic compounds such as sugars, organic acids, nucleosides, mucus and amino acids so that the concentration of the solution around the roots increases which can attract microorganisms to the rhizosphere (Edwards et al. 2015; Porcel et al. 2012). There were significant differences in microbial population and rhizospheric biodiversity associated with the hybrid genotypes, due to differences in the composition of the root exudates (Yu and Hochholdinger 2018). AM fungi trade P and N with plant roots that provide the most Carbon (Williams et al. 2017).

In conclusion, the application mycorrhizae took a relatively long time to enhance the plant growth and yield. The application on food crops with a short life cycle, only about 3 months, showed a less measurable effect, although it has been confirmed to increase yield of maize. The rate of 5 g.plant^{-1} mycorrhizae on coastal land increased stem diameter and root fresh weight which in turn increased the grain weight.plant^{-1} of maize. However, increasing the rate to 10 g.plant^{-1} did not improve growth and yield of maize. More pronounced effects occur after 3 months. In our bioassay study, the effect of mycorrhizal residues on coastal land significantly increased maize growth even without inorganic fertilizer. The residual effect of mycorrhizal application in the following season confirmed that the higher the mycorrhizal rate up to 10 g.plant^{-1}, the higher the plant height, leaf length, or the number of leaves. The new hybrid CT34 is known to be more adaptive than CT17 although their performance was less than that of the commercial hybrid BISI18.

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