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Effects of mycorrhiza and phosphate fertilizers on the growth and yield of foxtail millet (*Setaria italica* L.) under drought stress conditions

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Abstract

Millet is a cereal plant that's potential for rice substitution. This plant is adaptive to be cultivated in dry land but has a tolerance limit to drought stress. Mycorrhiza and phospate (P) fertilizer treatments help plants adapt to this condition. This study aimed to determine the effects of phosphate fertilizer and mycorrhiza as well as their interaction effects on the growth and yield of foxtail millet (*Setaria italica* L.) under drought stress. The experiment was carried out from January to June 2020 in the experimental farm, Faculty of Agriculture, University of Jenderal Soedirman. The research was arranged in a factorial randomized complete block design consisting of two factors. The first factor was the dose of SP-36 fertilizer per polybag, namely P0 = 0 g, P1 = 37,5 kg.ha^{-1} (25%), P2 = 75 kg.ha^{-1} = 0.88 g/polybag (50%), and P3 = 150 kg.ha^{-1}. The second factor was the dose of mycorrhiza biofertilizer, namely M0 = 0 g.polibag^{-1}, M1 = 33.3 g. polibag^{-1} and M2 = 66.6 g.polybag^{-1}. The treatment was replicated three times. The data observed were analyzed using the F test, continued with DMRT test at p=0.05. The results showed that SP-36 fertilizer application at half of the recommended dose (0.88 g/polybag) could increase growth variables, such as leaf area, panicle length, and seed weight. Mycorrhizae application 33.3 g/polybag could improve variables such as plant height, leaf area, panicle length, and seed weight. It also accelerated the initiation of panicle emergence compared to control.

INTRODUCTION

In Indonesia, most people consume rice. Based on data of BPS (2018), the total population of Indonesia reaches 291,934 million people with an average consumption of rice per year of 52.143 kg/capita/year. National rice production in 2018 amounted to 33,323,544 million kg, not comparable to the need for rice consumption of 35,520,638 million kg. The dependency of the Indonesian population on rice is high, thereby causing food insecurity if there is no balancing between the increasing production or diversification of other staple foods (Ridwan et al., 2018). Indonesia has a very high biodiversity, including cereals that contain carbohydrates and protein as the main food ingredients. One of the potential cereals with good prospects to cultivate is millets, which are widely grown around the world as cereal crops or grains for human food and fodder (Rao, 2021). In Indonesia, millet is widely grown in West Sulawesi.

Globally, foxtail millet is one of high grain crops (FAOSTAT, 2014), contain good nutrition, and provide income for small-scale farmers (Patel et al., 2015). However, foxtail millet has not been considered as an important crop to support food security (Saleh et al., 2013; Newmaster et al., 2013). This plant has good nutrition, carbohydrates, protein, fat, and lots of fiber (Bandyopadhyay et al., 2017). The nutritional content

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of millet includes 72–84.2% carbohydrates, 1.4–10% crude fiber, 2.5–3.3% fat, 9.9–12.7% protein, and several important minerals, which are higher in corn (Tirajoh, 2015). Tail millet seeds also contain gluten, which is elastic and airtight, so it is not easy to break if millet flour is made into noodles (Faesal, 2013).

Millet has much benefit for human health due to low glycemic index (Thathola et al., 2011), high anti-oxidant content (Sharma et al., 2015), and anti-cancer (Saleh et al., 2013). The other benefits of millet include improvement of the muscular system, protection against diabetes, the prevention of heart disease, an increase in digestive system well-being, the lowering of cancer risks, and a reduction in cholesterol (Sobana et al., 2009; Amadou et al., 2013; Hassan et al., 2021).

Millet is well adapted to wide temperature ranges from 10°C to 35°C. Even though millet has a drought-tolerant characteristic, both intermittent and terminal droughts have an impact on the growth (Mwangoe et al., 2022). Drought-tolerant characteristic of millet is better than that of cereal crops, such as maize. In addition, millet is relatively tolerant to drought or salinity stress (Widyawan et al., 2018), and it grows well in marginal land, especially in dry land. However, foxtail millet has a tolerance limit to drought conditions. Low soil water content will inhibit root growth or make it harder for roots to penetrate the soil, thereby inhibiting the rate of diffusion of nutrients, especially phosphate (Riwandi et al., 2014). Phosphate is an important nutrient for plants that plays a role in the process of photosynthesis, respiration, energy transfer and storage, cell division and enlargement, and other plant processes (Nainggolan et al., 2020). Munawar (2011) states that the most essential function of phosphate is involvement in energy storage and transfer in plants. Phosphate also plays a role in the process of photosynthesis and carbohydrate metabolism, formation of the nucleus, and division and multiplication of cells. The absorption of phosphate nutrients can run optimally even under drought stress conditions. According to Nuryani et al. (2019), the supply of nutrients that are not suitable will also cause a deficiency or excess of nutrients.

One of the efforts for plants to survive from drought stress is to promote symbiosis of plant roots with Arbuscular Mycorrhiza Fungi (AMF), such as *Glomus etunicatum*. Some of the roles of AMF include helping roots in increasing the uptake of phosphorus (P) and other nutrients such as N, K, Zn, Co, S and Mo from the soil, increasing plant resistance to drought, and improving soil aggregates. One alternative to overcome nutrient deficiencies, especially facilitating the availability of phosphate, is using mycorrhizae (Pangaribuan, 2014). Healthy AMF will grow and infect the roots of host plants, which then develop a network of hyphae that develop not only in the root rhizosphere, but also in plant roots (Suharno et al., 2015). Based on the description of the potential development of millet cultivation on dry land, it is necessary to study the role of AMF in increasing P uptake in foxtail millet growth and yield under drought stress conditions.

MATERIALS AND METHODS

The experiment pot was conducted from January to June 2020 in the Experimental Field, Faculty of Agriculture, Universitas Jenderal Soedirman.

Tools and materials

The tools used in the research were hoes, buckets, polybags, ruler/meter, lux meter, markers, label paper, trays, stationery, camera, thermohygrometer, spectrophotometer, microscope, incubator oven, blender, scissors, tweezers, analytical balance, electric stove, and spatula. The materials used in this study consisted of the seeds of foxtail millet (Polman Kuning accession), mycorrhiza fertilizer, inceptisol soil, Urea, and KCl fertilizer of 50% recommended dose, Phosphate fertilizer (SP-36), water, acetone, paper bags, silk plastic, paper, 10% KOH solution, 2% HCl, H_2SO_4 solution, H_2O_2, glass object, brown envelope, alcohol, filter paper, and syringe.

Experimental design

This study was arranged in a factorial Randomized Complete Block Design (RCBD) with two treatments. The first factor was the dose of P fertilizer per polybag, namely P0 = 0 g, P1 = 37.5 kg.ha^{-1} (25%), P2 = 75 kg.ha^{-1} = 0.88 g/polybag (50%) and P3 = 150 kg.ha^{-1} (in accordance to recommendation dose of SP-36 fertilizer ha^{-1}). The second factor was the dose of mycorrhiza biofertilizer, namely M0 = 0 g, M1 = 33.3 g and M2 = 66.6 g polybag^{-1}. Each treatment consisted of three replications, resulting in 36 experimental units, and each unit consisted of three polybags.

Preparation of planting media in drought stress conditions

The planting medium used was 10 kg of Inceptisol. Based on the analysis, Incepticol has pH of 5.7, organic

C of 1.98% Total N of 0.07 %, available P of 70.05 ppm, and K of 0.36 me/100 g, containing an initial soil moisture content of 25%. The planting medium was conditioned at a soil moisture content of 28%, similar to 60% field capacity, by adding 0.5 liters of water to 10 kg of soil, so that the weight of the planting medium in the polybag was 10.5 kg. The amount of water loss can be determined by weighing each polybag after soil preparation under field capacity treatment conditions. The amount of water required to restore the field capacity conditions according to the treatment is the difference between the weight of the wet soil in the original polybag and the weight of the soil and polybag when watering (Siregar et al., 2017). Field capacity was determined by gravimetric method, then the soil water content was calculated using the following formula:

$$\text{Dry matter content (\%)} = \frac{W - w}{T} \times 100\% \quad \text{.....(1)}$$

Water content (%) = 100% - % dry matter
W = dry soil wight + bottle (g)
w = weight of bottle (g)
T = weight of wet soil taken to oven (g)

Mycorrhizal root infection

Analysis of mycorrhizal root infection was carried out when the plant was 5 WAP (last vegetative phase). Marwani et al. (2013) explained that root infection was observed at the tip of the root (±1 cm from the tip). Isolation and preparation of roots to observe the degree of infection were based on the method of Kormanik and Mc. Grow (1982), followed by staining of lactofenol cottonblue, which were then then observed under a microscope. The calculation of the degree of infection was based on the following formula:

$$\text{Root Infection (\%)} = \frac{\text{Number of infected roots}}{\text{Total number of roots}} \times 100\% \quad \text{....(2)}$$

Variable observed

Variable observed in this research were plant height, number of leaves, total of leaf area, dry crop weight, dry root weight, chlorophyll content, percentage root infection by AMF (%), panicle length, dry crop weight in harvest time, dry root weight in harvest time, panicle weight, panicle initiation time, and P uptake.

P uptake

P uptake by plants was measured based on P of all plant tissues, at the time of the last vegetative phase. Measurement of P levels was carried out by wet destruction method using 97% concentrated H_2SO_4 and H_2O_2 30%. The absorbance value of the solution was measured using a spectrophotometer at a wavelength of 693 nm.

P uptake = P content (%) × dry weight of crop (g) (Mayang et al., 2012).

Data analysis

The research data were analyzed by F test using DSTAT software, and if there were significantly differences, further tests were carried out using DMRT (Duncan Multiple Range Test) with p=0.05.

RESULTS AND DISCUSSION

Foxtail millet is one of adaptive crops to be cultivated in dry land but has a tolerance limit to drought stress. Mycorrhiza and P fertilizer treatments help plants adapt to this condition. The results of the analysis of variance on the influence of P fertilizer and mycorrhizae on the growth and yield of foxtail millet (*Setaria italica* L.) under drought stress condition are presented in Table 1. Each plant showed a different response to the dose of P fertilizer and AMF.

Effects of Phosphate fertilizer on the growth and yield of foxtail millet (*Setaria italica* L.) under drought stress conditions

The variables measured showed different responses to morphological characters and plant yields. Besides being influenced by treatment, the differences were also influenced by temperature, humidity, light intensity and other environmental factors around the planting area. The results of the further test analysis (DMRT) based on the observed variables are presented in Table 2.

The application of phosphorus fertilizers at various doses has not been able to increase the P uptake of plant tissues. According to Rahman et al. (2019), the main goals of P fertilization are to prevent the development of P depletion zones surrounding plant roots and to enhance the amount of P nutrients available for plant growth and formation. According to the soil analysis, Inceptisol had a P availability of 70.05 ppm, and the P absorbed by plants varied between 0.47 and 0.65 ppm without significant differences between treatments. However, the increase in P doses is not always directly proportional to the level of P nutrient uptake by plants. The availability

of P nutrients in the soil can be influenced by soil acidity, time, temperature, amount of organic matter, and rainfall (Onwuka and Mang, 2018). According to Nuryani et al. (2019), the excessive level of P will disturb the absorption of other elements in the soil, thereby inhibiting plant growth.

Application of SP-36 fertilizer at 1.75 g/polybag or half the recommended dose was able to increase the total leaf area by 21.98% compared to the control, but the addition to 100% of the recommended dose was not able to increase the total leaf area significantly. Half the recommended dose of SP-36 fertilizer can increase root infection by 20.57% compared to control. Panicle length was increased by 16.5% in SP-36 fertilizer treatment 0.88g/polybag, and seed weight was increased by 26.34% compared to control at 25% recommended dose of P fertilization.

Leaf is the main place for photosynthesis process in plant. Giving 1.75 g SP-36/polybag was able to increase the total leaf area yield. This was presumably because 1.75 g SP-36/polybag was the best dose for the growth of barley. According to Ajmera et al. (2019), major role in physiological processes, such as energy storage and transfer, meristematic tissue

Table 1. Matrix of F test on the effects of phosphate fertilizer and AMF on the growth and yield of foxtail millet (*Setaria italica* L.)

No	Variable observed	F test		
		P	M	P×M
1	Plant height (cm)	ns	s	ns
2	Number of leaves	ns	ns	ns
3	Total of leaf area (cm^2)	s	s	ns
4	Dry crop wight (g)	ns	ns	ns
5	Dry root weight (g)	ns	ns	ns
6	Chlorophyll content	ns	ns	ns
7	Percentage root infection by AMF (%)	s	ns	ns
8	Panicle length (cm)	s	s	ns
9	Fill grain weight per panicle (g)	s	s	ns
10	Dry crop weight on harvest time (g)	ns	ns	ns
11	Dry root weight on harvest time (g)	ns	ns	ns
12	Panicle weight (g)	ns	ns	ns
13	Panicle initiation time (day after planting)	ns	s	ns
14	P uptake	ns	ns	ns

Remarks: P = Phosphorus fertilizer doses; M = Mycorrhiza fertilizer doses; P×M = Combination of phosphorus and mycorrhiza fertilizer doses; ns = non-significant, n=significant on F test at *P* = 0.05.

Table 2. Effects of phosphate fertilizer doses on growth and yield of foxtail millet (*Setaria italica* L.) under drought stress

SP-36 fertilizer dose	PH (cm)	NL	LA	DWCV (g)	DWRV (g)	CC (mg/L)	RI (%)	PL (cm)	GW	CWH (g)	RWH (g)	PW (g)	PI (dap)	PU (ppm)
0 g	131.52	10.65	650.64 a	4.32	1.07	15.2	75.56 a	44.88 a	8.39 a	11.91	2.66	16.28	39.78	0.47
0.88 g	135.57	10.72	713.42 b	5.47	1.22	1.36	81.11 b	53.70 b	10.60 b	11.20	2.56	14.87	39.85	0.58
1.75 g	146.69	10.54	793.65 c	5.64	1.23	1.51	91.11 c	54.91 b	10.73 b	11.36	2.13	14.38	39.50	0.63
2.63 g	140.5	10.93	820.94 c	5.09	1.15	1.38	92.22 c	55.14 b	10.89 b	13.12	2.65	14.91	39.65	0.52
CV (%)	9.2	10.64	14.04	18.25	19.47	25.81	10.19	8.6	13.91	18.25	14.71	24.49	4.02	14.32

Remarks: PH= Plant height (cm), NL= Number of Leaves, LA = Leaf Area, DWCV= Dry wight of crop at vegetative phase, DWRV= Dry weight of root at vegetative phase, CC= Chlorophyll content, RI=Percentage of root infection, PL=Panicle length, GW=Grain wight, CWH= Crop weight at harvest, RWH= Root weight at harvest time, PW= Panicle weight, PI= Panicle initiation, PU=P uptake; Means followed by different letters show significant difference.

cell division and enlargement, photosynthesis, and respiration, are performed by macro nutrient of phosphate. Rahman et al. (2019) stated that increasing the dose of phosphate fertilizer would potentially increase the availability of P nutrients and P uptake for plants. These compounds are then used by plants in the process of photosynthesis. Phosphate is known to function as a constituent component of ATP, PEP, NADPH and other components that have important functions in the photosynthesis process (Lakitan, 2010). The energy obtained from photosynthesis and carbohydrate metabolism is then used for plant growth processes such as leaf formation (Rahman et al., 2019).

Based on Table 2, phosphate fertilizer application of 1.75 g SP-36/polybag was able to increase mycorrhizal root infection. Mycorrhizal root infection can be increased by adding more phosphate fertilizers. It is suspected that phosphate fertilizer is a nutrient that is needed by plants to carry out plant metabolic processes. The results of metabolism will be translocated to all parts of the plant, including roots. Photosynthate in roots is a food source for AMF. Therefore, mycorrhizae will infect plant roots to take advantage of food sources that will be used to sustain the growth and development of mycorrhizae. This is in accordance with Ginting et al. (2018), stating that AMF had symbiotic relationship with plant roots and are obligate symbionts, so they require host plants to grow and develop. According to Muzakkir (2010), AMF can receive carbohydrates and growth factors from host plants as an energy source for growth and development, while plants can increase the uptake of P nutrients and other nutrients by the presence of root colonies with mycorrhizae.

Panicle length is a plant yield variable whose growth and development are influenced by the Phosphate addition. The application of phosphate fertilizer at 0.88 g SP-36/polybag was the able to increase panicle length and seed weight per panicle. The longer the panicle of a plant, the heavier the seed weight per panicle. Munawar (2011) stated that the most essential function of P was involvement in energy storage and transfer in plants. P also plays a role in the process of photosynthesis and carbohydrate metabolism, formation of the nucleus, and division and multiplication of cells. Suyono and Citraresmini (2010) stated that the availability of soil P was also able to increase the accumulation of photosynthate in the stem, in which the assimilated results produced in the leaves would be translocated into grain so as to increase crop production at harvest.

Effects of mycorrhiza fertilizer on the growth and yield of barley (*Setaria italica* L.) under drought stress conditions

Mycorrhizae are soil fungi that actively work on soils stressed by nutrients and drought. On dry land, these fungi also reach source of water and nutrition needed by plant. This research was set on 60% water field capacity. Effects of mycorrhizae on the growth and yield of foxtail millet are presented on Table 3.

Application of mycorrhiza at 33.3 g could increase plant height by 11.59%, total leaf area by 28.21%, panicle length by 12.21%, seed weight by 16.93% and panicle initiation time of millet plants to about 4 days faster than the treatment without mycorrhizae.

Giving AMF can increase plant height and total leaf area of foxtail millet. According to Hamida and Dewi (2014), mycorrhizae can increase the content

Table 3. Effects of mycorrhiza fertilizer doses on the growth and yield of foxtail millet (*Setaria italica* L.) under drought stress

Mycorrhiza fertilizer doses	PH (cm)	NL	LA	DWCV (g)	DWRV (g)	CC (mg/L)	RI (%)	PL (cm)	GW	CWH (g)	RWH (g)	PW (g)	PS (dap)	PU (ppm)
AMF 0 g	125.83 a	9.94	625.66 a	4.40	1.09	1.39	81.67	47.30 a	8.80 a	12.09	2.28	15.23	39.40 a	0.48
AMF 33.3 g	140.42 b	10.74	802.18 b	5.43	1.13	1.41	83.33	53.36 b	10.29 b	11.75	2.55	14.80	40.67 b	0.56
AMF 66.66 g	149.35 b	10.96	806.15 b	5.56	1.28	1.51	90.00	55.82 b	11.37 b	13.35	2.67	15.30	39.01 a	0.61
CV (%)	9.2	10.64	14.04	18.25	19.47	25.81	10.19	8.6	13.91	18.25	14.71	24.49	4.02	14.32

Remarks: AMF=Arbuscular mychoeeiza fungi. PH= Plant heigt (cm), NL= Number of Leaf, DWCV= Dry wight of crop at vegetative phase, DWRV= Drf weight of root at vegetative phase, CC= Chlorophyll content, RI=Percentage of root infection, PL=Panicle length, GW=Grain wight, CWH=Crop weight at harvest, RWH= Root weight at harvest time, PW= Panicle weight, PI= Panicle initiation, PU=P uptake; Means followed by different letters show significant difference.

of gibberellins, which stimulates stem elongation by increasing cell division. The increase in the number of cells caused faster stem growth, so that the posture of the mycorrhizal inoculated plants was higher than the control. Nurbaity et al. (2017) explained that giving mycorrhizae to sorghum plants was able to increase growth in general. Giving AMF is also known to increase the number of leaves, plant height, diameter of plant stems and dry weight of the plant canopy (Wicaksono et al., 2014). Plants that were treated with AMF experienced an increase in their ability to absorb the nutrients needed, so that the growth process could run well and did not experience obstacles (Rivana et al., 2016), one of which was leaf area. Addition of mycorrhiza fertilizer has not been able to increase root infection. This is presumably due to the lack of sterility of the planting media used. This is in accordance with the research of Permanasari et al. (2016), reporting that plants that were not given AMF had a higher root infection rate than plants that were given AMF treatment because the spore of AMF was inactive. According to Sagala et al. (2013), the soil as a planting medium had been sterilized twice, allowing mycorrhizae that exist naturally in Andisols to be inactive, in other words, the possibility of mycorrhizae infecting plant roots is very small. It is also suspected that the growing media used in this study had a high fertility rate, so that the mycorrhizae applied did not work actively. Kowalska et al. (2015) stated that high P content will inhibit the growth of hyphae propagule, spore germination, and initiation of root colonization. The available phosphate elements in the plant will be absorbed by the root hairs of the plant so that the plant has a better metabolism which is characterized by an increase in the growth of the plant shoot (Fuady, 2013; Rillig and Mummey, 2006).

Nainggolan et al. (2020) state that AMF has the ability to decompose P bound in the soil so that it can be absorbed by plant roots. Hyphae that secrete the phosphatase enzyme so that P in the soil will be dissolved and available to plants so as to stimulate growth and fruit formation in long bean plants. Plants infected by Mycorrhiza are able to absorb higher P elements than uninfected plants (Musfal, 2010). El-Mesbahi et al. (2012) showed that giving mycorrhizae at a dose of 5 g was able to increase stem diameter, cob length, and the percentage of mycorrhizal infected roots.

Effects of combination of P fertilizer and mycorrhizae on the growth and yield of barley (*Setaria italica* L.) under drought stress conditions

Application of phosphate and mycorrhiza fertilizers did not show any interaction effect on all variables. It is suspected that the treatments have an equal role in increasing plant growth and yield. According to Hariyadi (2015), no interaction between treatments indicates that the two factors are unable to influence each other because their mechanism of action is different or one of the factors does not play an optimal role. According to Herliana et al. (2018), the reason why the two treatments given to the plants did not have significant effects might be because they provided the plants' requirements separately and did not work synergizing with each other.

Soil media used in this experiment was Incepticol. Based on Barokah (2020), Inceptisol soil has a medium–high level of fertility; pH of inceptisol soil ranges from 6.24 to 7.19; soil available P is 74.12 ppm (very high); available K is 68.13 ppm (very high); and total N is classified as 1.63% (very high). According to Nurbaity et al. (2017), organic matter interacts positively with mycorrhiza on critical lands, like those with low nutrients. In addition, the percentage of root infections by mycorrhiza that were not given organic matter resulted in the highest yield (61.10%) (Ginting et al., 2018). This proves that mycorrhiza is more effective in infecting plant roots in nutrient-poor soils its existence is more utilized by plants (Rahman et al., 2019).

Drought stress conditions reduce the percentage of colonization of arbuscular mycorrhizal fungi so that the capacity of AMF is reduced as reported by Gong et al. (2015) on foxtail millet plants. Drought stress conditions inhibited spore germination and dispersal of the AMF mycelium. According to Setiadi (2000), not all types of arbuscular mycorrhizae can have a significant effect on the growth of a plant. This effect is largely determined by the effectiveness of the isolate, the nutrient status of the media, and the level of dependence of the plant on mycorrhizae. Phosphate is a nutrient that is not easily soluble and available to plants so that it can be absorbed directly by plant roots. Phosphate is a nutrient that is not easily soluble and is available in the soil so it needs the help of mycorrhizae in the absorption process (Widiastuti et al., 2002). Mycorrhizal hyphae help roots reach water, but under drought stress conditions at a constant

wilting point, the P dissolution process is not optimal. Environmental factors, such as soil pH, soil moisture, rainfall, organic C content, and NPK nutrient content, also affected mycorrhizal activity (Saputra et al., 2015).

CONCLUSIONS

Foxtail millet is one of cereal crops adaptive to be cultivated on upland. A half dose of P fertilization (75 $kg.ha^{-1}$) can improve millet plants physiological characteristics and produced millet seeds 20% higher than without P fertilizer application. Mycorrhizae fertilizer treatment of 66.6 g/polybag can improve physiological characteristics and produced millet seeds 14% higher compared to without mycorrhizae fertilizer treatment. The combination of P fertilizer and mycorrhizae has not been able to increase the growth and yield of foxtail millet under drought stress conditions. It is necessary to study the application of mycorrhizae and P fertilizer under drought stress conditions in the field.

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