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A Study of Mycorrhizal Inoculation on Some Genotypes Of Wheat (Triticum Aestivum L.)

Rizki Ramadhani[1], Damanhuri[2], Nur Basuki[2]

[1] *Student of Postgraduate, Study Program of Plant Science, Faculty of Agriculture, University of Brawijaya, Malang*

[2] *Lecturer on the Department of Agricultural Breeding, Faculty of Agriculture, University of Brawijaya, Malang*

Abstract: *Objective of the research was to study the effect of mycorrhiza on growth and yield of 8 (eight) genotypes of wheat has been done at the field in the District of Dadaprejo, Subdistrict of Junrejo, Batu and Laboratory of Plant's Disease and Pest, Faculty of Agriculture, University of Brawijaya, from February to September 2013. The experimental treatment comprised of 3 (three) factors and they were done in Spit-Split Plot Design by 3 (three) replications. The first factor is the method of mycorrhizal inoculation that includes 2 levels, such as seedling inoculation and field inoculation, which are placed as the Main Plots, the second factor is the amount of inoculated mycorrhizal spores that includes 2 levels, such as 75 spores and 150 spores that are placed as the Sub Plot, and the third factor is the wheat genotypes that include M4, M6, M7, M9, SO3, SO8, Nias and Selayar, which are placed as Sub Plots. Result of the research showed that both seedling inoculation and field inoculation were effective in increasing mycorrhizal colonization and high colonization could not increase growth and production of wheat. Single treatment on the amount of inoculated mycorrhizal spores has significant effect on characters of plant height, weight of harvested seeds per plot, the amount of spores when the plant is under the vegetative phase and harvest, as well as percentage of mycorrhizal colonization. Single treatment of wheat genotype always has significant effect on characters of growth and production. Response for development on the amount of spores during the vegetative phase is more positive by the increasing amount of inoculated spores and the method of field inoculation in comparison with seedling inoculation.*

Keywords: *method of mycorrhizal inoculation, amount of spores, wheat genotype*

I. Introduction

Wheat (Triticum aestivum L.) is cerealia that belongs to Gramineae (Poaceae) family, which is originated from subtropical area and it contains 11% glutein, 13% protein, and 69% carbohydrate. It has high nutrient content and varied products made of wheat as staple for a third of population in the world (Porter, 2005). In Indonesia, consumption of wheat and wheat flour keeps increasing annually and has reached 21 kg/capita/year, the second top-ranked after rice. Indonesia is the third-biggest wheat importer in the world following Egypt and Brazil. It is presumed that the national imported wheat keeps increasing year-by-year by mean value of 8% (APTINDO, 2013).

Efforts to reduce the imported wheat in Indonesia can be done by producing the domestic wheat. Wheat is suitable to be cultivated at the upland, but it must compete with horticulture. As a result, the farming area of wheat has become limited. Therefore, it requires extending area for wheat cultivation, both at medium land and lowland, in order to increase production of local wheat at the national level. However, the problems are high temperature and drought stress. One of efforts to solve such drought stress on wheat, both at the medium land and lowland is through mycorrhizal inoculation.

Mycorrhiza is a mutualistic symbiosis between fungus and the plant's roots. The mycorrhizal fungus could increase the plant's growth by increasing the nutrient adsorption, particularly P, increasing plant's resistant to drought, controlling root infection by pathogene, producing the growth stimulant compounds, stimulating activities of some advantageous organisms and assisting to improve structure and soil aggregation, as well as mineral nutrient distribution. Effectiveness of mycorrhizal inoculation is affected by some factors, such as inoculum placement, placement time (synchronization), inoculum potency, inoculum density, inoculums type, and environmental condition (Sastrahidayat, 2011), as well as the host due to the plant's dependency on mycorrhiza, among species or even among varieties (cultivars) in a species, is relatively different. Objectives of the research were to study the existence or nonexistence of the interaction between the method of inoculation, the amount of spores and genotypes as a result of mycorrhizal inoculation on wheat, and to find out the ideal combination of mycorrhizal inoculation methods, the amount of inoculated mycorrhizal spores and wheat genotypes in increasing growth and production of wheat.

II. Material And Methods

The research comprised of VMA (Vesicular Arbuscular Mycorrhizal) inoculums propagation dominated Glomus sp, which was conducted at Laboratory of Plant's Disease and Pest and at the greenhouse in Faculty of Agriculture, University of Brawijaya, Malang, as well as at the field experiment, which was done at the District of Dadaprejo, Subdistrict of Junrejo, Batu, at the altitude ± 560 m asl (above sea level), rainfalls ± 1700 mm/y, daily mean temperature 24°C and in Inceptisol type. The research was conducted from February to September 2013.

The experimental treatment comprises of 3 (three) factors and it was done in Spit-Split Plot Design by 3 (three) replications. The first factor is the method of mycorrhizal inoculation that comprises of 2 levels, such as seedling inoculation and field inoculation, which are placed as the Main Plots, the second factor is the amount of inoculated mycorrhizal spores that includes 2 levels, such as 75 spores and 150 spores that are placed as the Sub Plots, and the third factor is the wheat genotypes that include M4, M6, M7, M9, SO3, SO8, Nias and Selayar, which are placed as Sub Plots. The experimental plot of each replication comprises of 32 plots plus 8 plots without mycorrhizal inoculation as control. As a whole, there are 120 units of treatment in the plot form, 1 x 1 m.

Soil cultivation was done before planting by adding stable manure 20 $ton.ha^{-1}$ and dolomite lime 4 $ton.ha^{-1}$. Planting the wheat seeds is divided into 2 (two) ways in accordance with the treatment, in which the seeds are firstly raised in seedlings and then planted directly at the field. On treatment by mycorrhizal inoculation through seedbed, the seeds are disseminated in seedling plastics using inoculums as a result of mycorrhizal spore propagation. It is presumed that inoculums of 75 spores are equivalent to 8 g inoculums and inoculations of 150 spores are equivalent to 16 g inoculums. Such calculation is derived from result of observation on the amount of spores during the harvest in cultured pots that contain 939 spores/100 g soil. After 7 DAP (days after planting), the seedlings are transferred to the field in accordance with the experimental plots. For direct inoculation in the field, the seedlings are planted into the planting holes immediately, which were previously layered by tissue papers that are made in funnel shape. The fertilizers apply urea 150 $kg.ha^{-1}$, SP-36 100 $kg.ha^{-1}$, and KCl 100 $kg.ha^{-1}$. The maintenance processes include irrigation, weeding, as well as pest and disease controlling, which are adjusted to the real condition in the field. Harvest time will be done if the straws, stems, and leaves have dried and turning yellow.

The observation included observation on wheat, VMA, as well as climate and the soil supports. For observation on wheat, 10 plants were taken as samples that include days of flowering, days of harvest, plant height, numbers of leaf, diameter of stem, number of plantlets per tribus, number of productive plantlets per tribus, panicle length, number of spikelets per panicle, number of seeds per panicle, weight of 1000 seeds, and weight of harvested seeds per plot. Observation on VMA includes the amount of mycorrhizal spores per 100 g soil before planting (preliminary observation on endogenous spores), number of spores when the plant is in vegetative phase and during the harvest time, as well as percentage of root colonization by mycorrhiza.

Observation on the amount of mycorrhizal spores was done using the sieving and decanting method (Brundrett, 1996). Observation on percentage of mycorrhizal colonization used method Kormanik dan Mc Graw (1982). Then, criteria of mycorrhizal colonization percentage on wheat's roots are being analyzed O'Connor et al., (2001).

III. Result

3.1 General Condition of the Research

Pest infection on wheat has started at the beginning of vegetative phase, since the emergence of shoots. The pests include Exopholis hypoleuca, which damage the rooting system and stem of the wheat plant, grasshopper, caterpillar, and aphids. Before planting, preliminary observation has been conducted at the field of the research in order to find out the existence and density of the endogenous spores (natural) per 100 g soil. Samples of the soil were taken from five diagonal spots of the field and repeated three times (3 replications). The amount of spores per 100 g soil on five spots, where the samples were taken, ranged 215 - 344 spores by 285 spores/100 g soil on average. It found natural mycorrhizal spores, but during early planting, no sterilization applied on the field to distinguish any organism in the soil, including the endogenous mycorrhiza.

3.2 Variance of the Agronomic Character on Wheat

Based on result of the variance analisis, it showed that single treatment by mycorrhizal inoculation has significant effect on characters of plant height, number of productive plantlets per tribus and the amount of spores during the plant in vegetative phase. Single treatment on the amount of the inoculated-mycorrhizal spores has significant effect on characters of plant height, weight of harvested seeds per plot, the amount of spores during the plant in vegetative phase and harvest time, as well as percentage of mycorrhizal colonization. Single treatment of wheat genotype always has significant effect on characters of growth and production. Interactions between mycorrhizal inoculation and wheat genotype, as well as the amount of mycorrhizal spores and wheat genotype have occurred on weight of harvested seeds per plot. Interactions between mycorrhizal inoculation, the amount of mycorrhizal spores and wheat genotype have occurred on weight of harvested seeds per plot. Interactions between mycorrhizal inoculation, the amount of mycorrhizal spores and wheat genotype have occurred on the amount of spores during the plants are in vegetative phase (Table 1).

Based on Table 2, it shows that single treatment of genotype always has significant effect on the growth of wheat. However, the mycorrhizal inoculation has only significant effect on plant height and numbers of the productive plantlet per tribus. The amount of mycorrhizal spores has only significant effect on character of plant height.

Genotype M7 and Nias have shorter days of flowering and have significant differences with other genotypes. For character days of harvest, genotype M7 has the fastest days of harvest and it has significant difference with other genotypes. Genotype that has high variance, M4, has insignificant difference with M6, SO3, and SO8. Genotype M6 has insignificant difference with genotypes of Selayar, M4, SO3, and SO8 has more leaves. Genotypes M6 and SO3 have bigger diameter than other genotypes. Genotype SO8 has the greatest numbers of plantlet per tribus and numbers of productive plantlet per tribus in comparison with other genotypes. Genotypes M6 and SO3 have significant difference of long panicles in comparison with other genotypes. Genotype M4 has the greatest numbers of spikelet per panicle. Genotype M6 has the greatest numbers of seed per panicle in comparison with other genotypes. Genotype M4, M6, and SO3 have heavier weight of 1000 seeds than other genotypes (Table 2).

Character on weight of the harvested seeds per plot is affected by interaction between mycorrhizal inoculation and wheat genotypes, as well as amount of mycorrhizal spores and wheat genotypes. Table 3 shows that the seedling inoculation has significant effect on the increasing wheat's genotype M6 and SO8, while direct inoculation in the field could only increase weight of the harvested seeds per plot on genotype SO3. The mycorrhizal inoculation has no significant effect on genotypes M4, M7, M9, Nias and Selayar so that, as a whole, it can be said that the seedling inoculation and field inoculation have equivalent effect on weight of harvested seeds per plot.

Interaction between the amount of mycorrhizal spores and wheat genotype has significant effect on the increasing weight of harvested seeds per plot on genotypes M4, M7, M9, SO3 and Selayar, while the genotypes M6, SO8 and Nias have insignificant effect. Weights of the harvested seeds per plot on those five genotypes are higher on inoculation treatment of 150 spores than inoculation of 75 mycorrhizal spores. However, based on mean values of the combination 32 treatments of mycorrhizal inoculation on mean of control showed that wheat, which has the mycorrhizal inoculation treatment, has resulted insignificant difference on weight of harvested seeds per plot with the control (Table 4 and 5).

Table 1. Recapitulation of significancy for each observation character on mycorrhizal inoculation, the amount of mycorrhizal spores, and wheat genotype

Treatment	DF	DH	PH	NL	DS	NP/T	NPP/T	PL	NSp/P	NS/P	WS	WHS/P	SPRV	SPRH	PCM
Interaction of 3 Factors															
C*D*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
Interaction of 2 Factors															
C*D	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
C*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	ns	ns
D*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
Single treatment															
C (mycorrhizal inoculation)	ns	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns
D (amount of mycorrhizal spores)	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*	*
G (wheat genotype)	*	*	*	*	*	*	*	*	*	*	*	*	*	ns	*

Note : DF = Days of flowering, **DH =** Days of harvest, **PH =** Plant height, **NL** = numbers of leaf, **DS** = Diameter of stem, **NP/T** = number of plantlets per tribus, **NPP/T =** Numbers of productive plantlet per tribus, **PL** = Panicle length, **NSp/P** = Numbers of Spikelet per Panicle, **NS/P =** Numbers of seed per panicle, **WS =** Weight of 1000 seeds, **WHS/P =** Weight of harvested seeds per plot, **SPRV =** Amount of spores when the plant in vegetative phase, **SPRH =** Amount of spores when the plant in harvest phase, **PCM** = Percentage of mycorrhizal colonization, ns = not significance difference

Table 2. Mean of agronomic character on wheat.

Treatment	DF		DH		PH		NL		DS		NP/T		NPP/T		PL		NSp/P		NS/P		WS	
Mycorrhizal inoculation method																						
C1 (seedling inoculation)	61.65		108.31		87.46	b	88.51		3.31		10.96		10.56	a	9.89		19.45		46.24		40.53	
C2 (field inoculation)	61.71		108.77		86.02	a	86.93		3.21		12.25		11.86	b	9.94		19.45		45.67		39.43	
LSD 5%	ns		ns		0.82		ns		ns		ns		1.28		ns		ns		ns		ns	
Amount of mycorrhizal spores																						
D1 (75 spores)	62.54		109.31		85.71	a	86.83		3.27		11.70		11.24		9.84		19.28		46.86		40.32	
D2 (150 spores)	60.81		107.77		87.77	b	88.61		3.25		11.51		11.18		9.99		19.62		45.05		39.65	
LSD 5%	ns		ns		2.03		ns		ns		ns		ns		ns		ns		ns		ns	
Genotype																						
G1 (genotype M4)	65.00	cd	113.58	d	95.12	d	91.96	bc	3.38	d	10.90	b	10.44	b	9.80	c	21.83	e	47.03	c	44.01	d
G2 (genotype M6)	66.33	d	113.25	d	93.86	d	100.08	c	3.69	e	9.17	a	8.75	a	10.78	e	19.87	cd	61.06	e	43.54	cd
G3 (genotype M7)	56.42	a	97.83	a	78.33	ab	70.24	a	3.09	bc	12.16	bc	11.93	c	9.46	b	17.21	a	41.53	b	36.79	b
G4 (genotype M9)	58.92	b	108.83	c	80.79	bc	88.12	b	3.04	ab	12.85	c	12.62	c	9.05	a	19.45	c	41.57	b	34.81	a
G5 (genotype SO3)	65.58	cd	108.83	c	92.73	d	94.40	bc	3.61	e	9.15	a	8.34	a	10.73	e	19.31	c	55.79	d	43.33	cd
G6 (genotype SO8)	59.92	b	111.75	d	94.50	d	93.13	bc	2.93	a	14.81	d	14.37	d	9.53	bc	19.31	c	36.17	a	37.31	b
G7 (genotype Nias)	56.58	a	100.67	b	81.72	c	62.88	a	3.19	c	11.84	bc	11.60	bc	10.17	d	18.18	b	46.40	c	37.57	b
G8 (genotype Selayar)	64.67	c	113.58	d	76.87	a	100.97	c	3.14	bc	11.97	bc	11.65	bc	9.82	c	20.46	d	38.10	a	42.51	c
LSD 5%	1.60		2.66		2.84		10.06		0.14		1.30		1.33		0.32		0.59		3.27		1.21	
Control vs Treatment																						
Control	61.21		108.75		84.51		84.79		3.26		11.76		11.56		9.82		19.28		44.43		39.57	
Treatment	61.68		108.54		86.74		87.72		3.26		11.61		11.21		9.92		19.45		45.96		39.98	
LSD 5%	ns		ns		ns		ns		ns		ns		ns		ns		ns		ns		ns	

Note: **DF** = Days of flowering, **DH =** Days of harvest, **PH =** Plant height, **NL** = Number of leaf, **DS** = Diameter of stem, **NP/T** = Number of plantlet per tribus, **NPP/T =** Number of productive plantlets per tribus, **PL** = Panicle length, **NSp/P** = Number of Spikelet per panicle, **NS/P =** Number of seeds per panicle, **WS =** Weight of 1000 seeds, ns = not significance difference.

Table 3. Mean for weight of the harvested seeds per plot (g) of wheat as a result of interaction in treatment between the mycorrhizal inoculation and wheat genotypes

Genotype	Mycorrhizal inoculation			
	C1 (seedlings)		C2 (field)	
G1 (genotype M4)	515.65	de	466.21	abcd
G2 (genotype M6)	560.43	e	455.55	abcd
G3 (genotype M7)	465.97	abcd	428.33	ab
G4 (genotype M9)	432.29	ab	433.32	ab
G5 (genotype SO3)	432.94	ab	508.01	cde
G6 (genotype SO8)	548.73	e	477.74	bcd
G7 (genotype Nias)	403.38	a	418.87	ab
G8 (genotype Selayar)	465.21	abcd	445.09	abc
LSD 5%	68.96			

Note : Numbers followed by the same letter show insignificant difference based on LSD Test of 5%

Table 4. Mean for weight of the harvested seeds per plot (g) of wheat as a result of interaction in treatment between the amount of mycorrhizal spores and wheat genotypes

Genotype	Amount of mycorrhizal spores			
	D1 (75 spores)		D2 (150 spores)	
G1 (genotype M4)	447.61	abcde	534.25	fg
G2 (genotype M6)	514.71	efg	501.28	defg
G3 (genotype M7)	407.92	ab	486.38	cdef
G4 (genotype M9)	397.77	a	467.84	bcdef
G5 (genotype SO3)	381.73	a	559.22	g
G6 (genotype SO8)	512.56	efg	513.91	efg
G7 (genotype Nias)	386.84	a	435.41	abcd
G8 (genotype Selayar)	418.79	abc	491.52	defg
LSD 5%	68.96			

Note : Numbers followed by the same letter show insignificant difference based on LSD test of 5%

Table 5. Comparison of mean for weight of the harvested seeds per plot (g) of wheat between treatment and control

Treatment	Weight of harvested seeds per plot (g)
Control vs Treatment	
Control	417.42
Treatment	466.11
LSD 5 %	ns

Note : Numbers in the same column and followed by the same letter show insignificant difference based on LSD test of 5%, ns = not significance difference

3.3 Amount of Mycorrhizal Spores

Observation on the amount of spores was done to study the extent of mycorrhizal breed in any condition of medium along with any existed host. Result for analysis of variance shows significant interaction among treatment of inoculation method, amount of mycorrhizal spores and wheat genotypes on the amount of spores during the plant in vegetative phase (30 DAP).

For character on the amount of spores during the plant in vegetative phase, interactions occur among treatments of mycorrhizal inoculation, amount of mycorrhizal spores, and wheat genotypes. Based on interaction of those three treatments, they showed that response of the increasing amount of spores during the plant in vegetative phase was more positive by the increasing amount of the inoculated spores and the field inoculation method in comparison with seedling inoculation method (Table 6).

Amount of the inoculated mycorrhizal spores has significant effect on the amount of spores, while the inoculated method and wheat genotype have insignificant effect on the amount of spores during harvest time. Inoculation of 150 spores resulted more spores during the harvest phase, and it showed significant difference in comparison with inoculation of 75 mycorrhizal spores (Table 7).

Table 6. Mean for the amount of spores during the plant in vegetative phase as a result of interaction of treatments among inoculations, amount of mycorrhizal spores, and wheat genotypes

Genotype	C1 (Seedling inoculation)				C2 (Field inoculation)			
	D1 (75 spores)		D2 (150 spores)		D1 (75 spores)		D2 (150 spores)	
G1 (genotype M4)	440.00	ab	534.67	abcdef	665.33	efghi	770.67	hij
G2 (genotype M6)	460.00	ab	651.33	defgh	659.33	defghi	868.67	jk
G3 (genotype M7)	589.67	bcdefg	687.33	fghi	588.67	bcdefg	1088.00	lmn
G4 (genotype M9)	429.00	ab	660.67	defghi	833.33	ij	1025.33	klm
G5 (genotype SO3)	373.33	a	564.33	bcdef	462.67	abc	1052.00	lm
G6 (genotype SO8)	466.00	abc	527.00	abcdef	641.00	cdefgh	925.00	jkl
G7 (genotype Nias)	430.33	ab	501.33	abcde	781.00	hij	1233.67	n
G8 (genotype Selayar)	573.00	bcdef	482.67	abcd	756.67	ghij	1141.67	mn
LSD 5%	179.97							

Note: Numbers followed by the same letter show insignificant difference based on LSD test of 5%

Table7. Mean for the amount of spores during the plant in harvest phase and percentage of mycorrhizal colonization on the treatments of inoculation methods, amount mycorrhizal spores, and wheat genotypes

Treatment	SPRH		PCM	
Mycorrhizal inoculation method				
M1 (seedling inoculation)	420.67		65.00	
M2 (field inoculation)	407.90		67.29	
LSD 5%	ns		ns	
Amount of mycorrhizal spores				
D1 (75 spores)	348.00	a	63.54	a
D2 (150 spores)	480.56	b	68.75	b
LSD 5%	101.84		2.38	
Genotype				
G1 (genotype M4)	413.92		60.00	ab
G2 (genotype M6)	422.42		62.50	ab
G3 (genotype M7)	418.92		56.67	ab
G4 (genotype M9)	391.58		55.00	a
G5 (genotype SO3)	456.50		73.33	cd
G6 (genotype SO8)	420.25		66.67	bc
G7 (genotype Nias)	394.25		73.33	cd
G8 (genotype Selayar)	396.42		81.67	d
LSD 5%	ns		10.58	
Control vs Treatment				
Control	261.25	a	51.25	
Treatment	414.28	b	66.15	
LSD 5%	104.15		ns	

Note : SPRH = Amount of spores when the plant in harvest phase, **PCM** = Percentage of mycorrhizal colonization. Numbers in the same column and followed by the same letter show insignificant difference based on LSD test of 5%, ns = not significance difference

3.4 Percentage of Colonization

Single treatment for the amount of inoculated spores and wheat genotypes has affected on percentage of colonization. Based on mean for combinations of 32 mycorrhizal inoculation treatments toward mean of control showed that the wheat, which was given the mycorrhizal inoculation treatment, resulted percentage of colonization that has insignificant difference with the control. Table 7 shows that inoculations of 150 spores resulted significant difference and higher percentage of root colonization than inoculation of 75 spores. Selayar genotype has insignificant difference with SO3 and Nias has higher percentage of mycorrhizal colonization than other genotypes.

IV. Discussion

Based on result for the analysis of variance, it showed that single treatment through mycorrhizal inoculation has significant effect on some characters, such as plant height, number of productive plantlets per tribus, and the amount of spores during the plant in vegetative phase. The inoculation method relates to the spore placement, the spacing between inoculums and roots of the host. Sastrahidayat (2011), stated that the spores placement affected the emergence of root's infection of the host. The closer to the inoculated roots, the better possibility of the infection is to be succeeded. Formation of such infection or colonization is highly important in relation to improvement of growth and yield of the plant by VMA. VMA that infected the rooting system of the host will produce external hypha tissues, which grow extensively, and penetrate the sub soil layer, so that it will increase capacity of the roots in absorbing nutrients and water (Cruz et al., 2004).

Single treatment for the amont of inoculated mycorrhiza spores has significant effect on some characters, such as plant height, weight of harvested seeds per plot, amount of spores during the plant in vegetative phase and harvest time, as well as percentage of mycorrhizal colonization. Tendency for the increasing some characters of the observation occurred when the inoculation was increased from 75 to 150 mycorrhizal spores. Hetrick (1984) reported that inoculums density could affect roots colonization and spores production. Daft dan Nicolson (1972) also stated that the increasing inoculums level could increase percentage of roots colonization to a given optimal point. Inoculum, 150 spores, gives some opportunity to the spores to infect the roots to be bigger than 75 spores on percentage of the mycorrhizal colonization. Moreover, Sanders dan Sheikh (1983) stated that propagule density is one of factors that affect primary infection besides the spores germination, velocity of the hypha growth in the medium and the growth rate of the roots.

The research showed that the amount of mycorrhizal spores is higher during the plant in vegetative phase than during the harvest phase.According to Johnson-Green et al., (1995) if symbiosis between VMA and the host is important, therefore the best growth of the host is the peak activity of the VMA. During the vegetative phase, the wheat's growth forms intensive rooting and leaf as photosynthetic organ. Yield of the photosynthetic is carbohydrate, which is immediately translocated to the rooting system and then can be utilized by VMA for spores' germination. Furthermore, Tarmedi (2006) stated that the mycorrhizal formation may occur due to the root's exudates will stimulate the growth of VMA. Such VMA will colonize with the rooting system and increase translocation of the nutrients to the top parts of the plant, which will increase the photosynthetic rate. During the vegetative phase or active growth of wheat, it is presumed that the roots' exudates are higher than during the harvest time, so that development for the amount of spores is higher as well.

Rumondang (2011) has been reported that the hole system inoculation technique has higher capability or percentage of infected roots, the infected roots are more concentrated, and the infected roots process is faster than the layering inoculation technique on teak (Tectona grandis L.). Furthermore, Umam (2008) stated that the application of line inoculation on teak's seedling is less effective due to the existence of VMA's propagule that has farther distance toward the seedling roots. In this research, mycorrhizal inoculation through seedling or directly in the field, have tried to place the inoculums as close as possible to the host's roots. It can be seen on character of percentage of mycorrhizal colonization, the colonization belongs to high category (>30%). There was no significant difference between both inoculation methods and both methods are effective in mycorrhizal colonization process on wheat's roots. However, high colonization could not always increase the yield due to the interaction with genotypes as well as the amount of the inoculated spores and the environmental effects.

In this research, comparison between mean values for combination of 32 mycorrhizal inoculation treatments toward mean values of the control showed that all characters of growth and production of wheat did not statistically bring about significant difference, except the amount of spores, both during the vegetative phase and the harvest phase. There are some items that cause the mycorrhizal treatment does not have any effect on the wheat's growth, such as no soil sterilization. Cahyani (2009) revealed that soil sterilization is absolutely required in conducting any research in order to find out the effect of a given microorganism inoculation without any influence of endogenous microorganism. The research used soil that contains endogenous mycorrhizae based on early observation on the related soil before planting. Mean for the amount of spores was 285 mycorrhizal /100 g soil. Fakuara (1988) stated that the experiment in unsterile pots, which are made of earth, enable the emergence of pure vesicular-arbuscular mycorrhizal fungi mycorrhizal fungi in it. Result of the research by Muis et al., (2013) showed that due to the used soil was not sterilized first, it caused percentage of the infected roots between the plants without inoculation and the inoculated mycorrhizae did not cause significant difference with category for the number of infected roots was medium.

Mycorrhizal inoculation on wheat is not effective, and it is also caused by the genetic effect of the wheat itself. Thompson dan Troeh (1994) stated that there are three main factors, which determine the success of VMA inoculation at the field, the dependency of the plant to mycorrhizae, nutritive status of the soil, and

potency of the VMA inoculums. Also, it depends on the population density of the endogenous VMA. Baylis (1975) stated that plant, which has big roots, more depends on the mycorrhizae than the plant with the rooting system that has long and more root hairs. Moreover, as stated by Mosse (1986) that plant has diverse requirements and response to phosphate and dependency on mycorrhizae. Different dependency between various kinds of plants can be categorized into high, medium, and less (low) dependency. Wheat belongs to group that less dependent to mycorrhizae. However, wheat is able to reach optimal growth and production at the definite level of soil fertility, along with mycorrhizae or not. This is different from the cassava and orange, which have high dependency, as well as soybean and corn that have medium dependency.

Research on mycorrhizal inoculation in wheat species have been conducted frequently. Kapulnik dan Kushnir (1991) stated that mycorrhizal dependency on wheat is different among wild, primitive, and modern cultivars. Cultivars, which were released before 1950, showed higher value of mycorrhizal dependency than cultivars that were released after 1950 (Hetrick et al., 1993).

Comparison between modern and aged cultivars of wheat showed that modern cultivar has lower response to mycorrhizae than the aged cultivar. It indicates that plant breeding program may reduce responsiveness of the wheat to arbuscular mycorrhizal fungi (Zhu et al., 2001). This research used wheat genotypes that derived from plant breeding at the Research Center on Cereal and introduction from Slovakia, which is categorized as modern cultivar. The effect of such mycorrhizal treatment on some wheat’s genotypes, which were used in this research, has shown low responsiveness of the plant toward mycorrhizal inoculation on the growth character.

Relating to the nutrition status of the soil, the research used fertile soil based on the analysis result on soil that has been done at the Chemistry Laboratory, Department of Soil Science, Faculty of Agriculture, University of Brawijaya (data unpresented). Sample of the soil without mycorrhizal inoculation as well as with mycorrhizal inoculation have the same nutrient status, pH is rather acid, medium availability of N, P is very high and K is high. Husin (2003) stated that the plant’s response to VMA would be more clearly if it is planted in poor nutrient soil, such as Ultisol, but the plant’s response is no longer obvious if it is planted in fertile soil.

Growth and development of wheat are optimal during both vegetative and generative phases. They are supported by the appropriate growing requirements, particularly temperature, which is highly influential on growth and yield of the plant because wheat is originated from sub tropical area. Temperature during the research ranged 21.9 – 24.0 ºC, as optimal temperature for the wheat’s growth Wiyono (1980) has been stated that optimal temperature for the wheat’s growth ranges 15 - 25°C.

V. Conclusion

1. Seedling inoculation and field inoculation are effective in increasing mycorrhizal colonization and high colonization will not be able to increase the growth and production of the wheat.
2. Single treatment on the amount of the inoculated mycorrhizal spores has significant effect on characters of plant height, weight of harvested seeds per plot, amount of spores during the plant in vegetative phase and harvest time, as well as percentage of mycorrhizal colonization.
3. Single treatment of wheat genotype always has significant effect on characters of growth and production.

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