

Responses of Cowpea Genotypes to Arbuscular Mycorrhiza

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ABSTRACT

A pot experiment was conducted under glasshouse conditions to evaluate response of some cowpea genotypes to Arbuscular Mycorrhiza. It was a 2 x 6 factorial experiment comprising factors of mycorrhizal inoculation (M) with AM fungal inoculum (M_1) and without AM fungal inoculum (M_0), and of plant genotypes (G) with G_f (a cowpea inbred line), G_m (a mung bean inbred line), G_{h1} , G_{h2} , G_{h3} and G_{h4} (the 1st, 2nd, 3rd and 4th generation of cowpea hybrids, derived from cross-breeding G_f x G_m as female and male parents, respectively), and arranged in a completely randomized design with 9 replicated pots, where plants were grown up for 14, 28 and 42 days before harvested serially. Responses of cowpea genotypes to colonization and contribution of mycorrhizal symbiosis varied greatly. The intensity of plant roots colonized by mycorrhizal fungi was highest on G_f , and it was descent on the cowpea hybrids following their generation order. On the other hand, the highest in plant growth response to mycorrhizal function was on G_m , followed by G_{h1} and G_{h2} . These results indicated that the cross-breed of cowpea and mung bean lines has generated cowpea hybrids that tend to benefit less from mycorrhizal symbiosis for their growth.

Keywords: arbuscular mycorrhiza; cowpea genotypes; mycorrhizal colonization; plant growth response; plant hybrids

INTRODUCTION

Under field conditions, roots of more than 80 % of agricultural plant species mainly legumes naturally form a mutuality symbiotic of Arbuscular Mycorrhiza (AM) (Tawaraya, 2003; Chawla et al., 2011), with a group of indigenous soil fungi of the phylum Glomeromycota (Schüßler, Schwarzott, & Walker, 2001). The symbiosis is found in almost all agricultural land, both in the tropics and sub-tropics.

AM is significant for improving growth of various crop plants particularly in low fertility soil (Clark & Zeto, 2000; Smith & Read, 2008). Through

their fungal external hyphae help plant roots to explore and absorb water and nutrients from bulk soil beyond depleted root zone resulting in increased P uptake, and other nutrients such as Ca, Cu, Mn and Zn. Furthermore, root colonization by AM fungi improves plant resistance to drought (Rohyadi, Nasrul, & Rachim, 2006), root pathogen attacks (Sastrahidayat, Djauhari, Saleh, & Muhibuddin, 2011), and other depressing environmental conditions (Mosse, 1981, 1986; Harrier & Watson, 2004).

Plant responses to AM considerably vary among species, varieties or cultivars (Tüfenkçi et al., 2012), even among genotypes within a plant species (Hacisalihoglu, Duke, & Longo, 2005). The variation also exists in genotypes of other plants from crosses, such as reported for the hybrids of chickpea (Hacisalihoglu, Duke, & Longo, 2005; Bazghaleh, Hamel, Gan, Tar'an, & Knight, 2015), cucumber (Tüfenkçi et al., 2012), and onions (Taylor et al., 2015).

Cowpea (*Vigna unguiculata* L. Walp.) is an important legume crop for food and fodder, and widely planted in tropical arid land around the world. Productivity of cowpea cultivated in Indonesia, particularly in West Nusa Tenggara province is low since neither improved cultivar nor modern agricultural technique was employed (Ujianto & Yakop, 2006).

Efforts to improve varieties of legumes including cowpea are being carried nationally in Indonesia. Currently a research group of Faculty of Agriculture, Mataram University has succeeded to get a number of new genotypes (hybrids) of cowpea as result of cross-breeding cowpea and mung bean inbred lines (Ujianto, Idris, & Yakop, 2012). Meanwhile, the research is ongoing to study the genetic characters of the new hybrids and it is very interesting to know more their mycotrophic responses to AM fungi. This is very important because AM may contribute to improve cowpea plant growth, especially under arid land conditions given that having low soil fertility.

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Cowpea is responsive and very dependent on AM to meet its needs of nutrient (Yaseen, Burni, & Hussain, 2011). Some previous studies indicated that cowpea cultivars, even their genotypes had different responses to AM (Mercy, Shivashankar, & Bagyaraj, 1990; Saidou, Singh, Abaidoo, Iwuafor, & Sanginga, 2012). Therefore, these new hybrids of cowpea may also show the differences.

The aim of this experiment was to determine to what extent the response of some new cowpea genotypes (hybrids) produced by the cross-breeding of cowpea and mung bean inbred lines to AM fungal colonization and function.

MATERIALS AND METHODS

The experiment was conducted at the Faculty of Agriculture, Mataram University from October to November 2015. It was a pot experiment performed under greenhouse conditions with daytime temperature between 23 and 32 °C.

Experimental Design

The layout of the experiment was a 2 x 6 factorial experiment. The first factor was mycorrhizal inoculation (M), consisting of M_0 (control, without inoculation) and M_1 (inoculation with AM fungi), while the second factor was genotypes of cowpea (G), consisting of G_f , G_m , G_{h1} , G_{h2} , G_{h3} and G_{h4} . The treatment combinations of the two factors were arranged in a completely randomized design, by placing each of them at 9 (nine) replicate pots, which were then grouped into three series of harvest with 3 pots for each harvest.

Biological Materials

Cowpea genotypes for this experiment were a cowpea inbred line (G_f), a common bean inbred line (G_m), and four cowpea hybrids as G_{h1} , G_{h2} , G_{h3} and G_{h4} . They are the 1st, 2nd, 3rd and 4th generation of derived hybrids from cross-breeding $G_f \times G_m$ (as female and male parents respectively). Seeds of these genotypes were obtained from Dr. Lestari Ujianto (Plant Breeding Laboratory, Faculty of Agriculture Mataram University, Lombok), while the commercial *Technofert*, produced by BPPT Jakarta was used as source of mycorrhizal inoculum. It contained about 11 spores g^{-1} inoculum.

Medium for Growing Plants

It was a mixture of soil and sand in 2:1 (w/w) ratio. The soil was Entisol, taken from a depth of 20 cm, and air-dried before mixed, while the sand was

black river sand. The mix was then sieved with 2 mm pores.

Experimental Procedure and Variable Measurement

Before using, planting medium was sterilized by heat steaming in an autoclave at 121 °C and 15 atm for 45 minutes twice with an interval of 24 hours. For M_1 treatments, the medium was mixed evenly with mycorrhizal inoculum at 9:1 ratio (w/w), whereas for M_0 treatment, the medium was not mixed at all. A 1000 g of each was then inserted into pots, made of PVC pipe (\varnothing 9 cm and height 18 cm). In the beginning of the experiment the planting medium was fertilized with Ruakura Solution (Smith, Johnston, & Cornforth, 1983) to have elemental composition: 59.4 NH_4 -N; 178.2 NO_3 -N; 36 P; 54 S; 214.2 K; 18.9 Mg; 114.3 Ca; 13.5 Na; 8.1 Cl; 2.7 Fe; 0.45 B; 0.45 Mn; 0.45 Zn; 0.036 Cu; and 0.009 Mo $mg\ kg^{-1}$ medium respectively.

Plant seeds were surface disinfection using NaOCl-10% solution, rinsed several times with sterilized distilled water, and germinated on wet tissue paper lined in container for three days. Germinated seed was sown singly into the planting pots. Subsequently, plants were grown for a period of 42 days. For maintaining moisture contents of the planting medium in pots at about field capacity some RO (reverse osmosis) water was added every two days by weighing.

Plants were harvested serially at 14, 28 and 42 days after sowing (das) by separating plant shoot from their roots. Plant shoots afterward were oven dried at 70 °C for 48 hours (Rohyadi, Smith, Murray, & Smith, 2004) and weighed for shoot dry weight.

Once harvested, plant roots from every pot were washed gently under tap water, drained using tissue paper and weighed. The roots were then cut into pieces along \pm 1cm, decomposed and mixed evenly. By weighing, 10 % of sample was taken from the mix. The samples were next prepared in small tubes for staining using trypan blue in lactoglycerol (Rohyadi, Smith, Murray, & Smith, 2004). Furthermore, the stained root samples were examined under a dissecting stereo microscope to measure the length of roots with and without mycorrhizal colonization based on the *gridline intersection* method of Brundrett, Bougher, Dell, Grove, & Malajczuk (1996). For total root length and total root length colonized by mycorrhiza were calculated by taking into account the samples and total of root fresh weights.

Intensity of root colonization by the AM fungi was calculated by the formula:

$$\text{Root colonization} = \frac{\text{Mycorrhiza l root length}}{\text{Total root length}} \times 100\%$$

Growth response to AM for plants at every genotype was calculated by the formulae:

$$GR_i = \frac{M_i - \overline{M_{oi}}}{\overline{M_{oi}}} \times 100 \%$$

Where: GR_i = Growth response of a genotype to AM; M_i = the shoot dry weight of a genotype plant with AM; $\overline{M_{oi}}$ = the average of shoot dry weight of a genotype control plant

Data Analysis

In general, the experimental data was analyzed by analysis of variance at 5 % significant level. For treatments showing a significant effect was then further tested using the method of Least Significant Difference (LSD) at $p = 5 \%$. Pearson product-moment correlation analysis was conducted between total root length and mycorrhizal colonized root length to shoot dry biomass of M_1 and M_0 plants respectively.

RESULTS AND DISCUSSION

Overall, the results of this study find out that there was much variability among cowpea genotypes tested in plant growth that was measured as shoots dry biomass and root length development. Factor of plant genotypes was more influential than that of mycorrhizal inoculation to alter the plant growth (Table 1 and Table 2). Besides that, the cowpea genotypes tested had positive responses to AM fungal inoculation, by forming colonization structures of mycorrhizae in their root tissues, and showing increased growth compared

to control plants without mycorrhizal inoculation. The responses, just then, greatly vary both between parental genotypes (G_f and G_m), the two parents to their hybrids, and among these hybrids (G_{h1} , G_{h2} , G_{h3} and G_{h4}) (Fig. 1 and Fig. 2). These results were for the most part in line with the results of preceding studies, which observed a high response of cowpea to AM fungal colonization and their positive contribution to growth improvement (Yaseen, Burni, & Hussain, 2011; Saidou, Singh, Abaidoo, Iwuafor, & Sanginga, 2012). Previously, Mosse (1986) classified cowpea into 'mycotrophic plants', which was greatly responsive to mycorrhizal colonization. Tawaraya (2003) noted cowpea was a type of legumes having a high degree of dependency on AM fungi to grow well.

There was no interaction effect between plant genotypes and mycorrhizal inoculation on shoot growth of plants. Both treatment factors affected the shoot growth independently. For the period of growing times, the plants grew in different extent among plant genotypes, while most the mycorrhizal inoculated plants (M_1) comparatively grew better than uninoculated control plants (M_0) mainly at 42 das (Table 1).

Similar to shoot growth, developed root system was observed for all plants along with period of growing times. Interaction effect between factors of plant genotypes and mycorrhizal inoculation on root development was not considerable up to 28 das, whilst the two treatment factors independently affected the root growth. It seems that much variability in the root length development existed among the plants, in which the variation basically was more affected by factors of plant genotypes than by of mycorrhizal inoculation. However, in average, mycorrhizal inoculation increased a number of root length density (Table 2).

Table 1. Shoot dry weight (g) of plant genotypes treated with and without mycorrhizal inoculation harvested at different plant ages

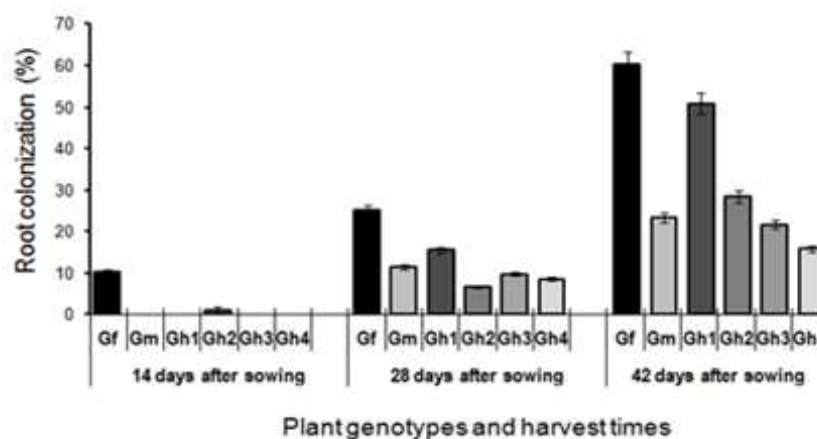
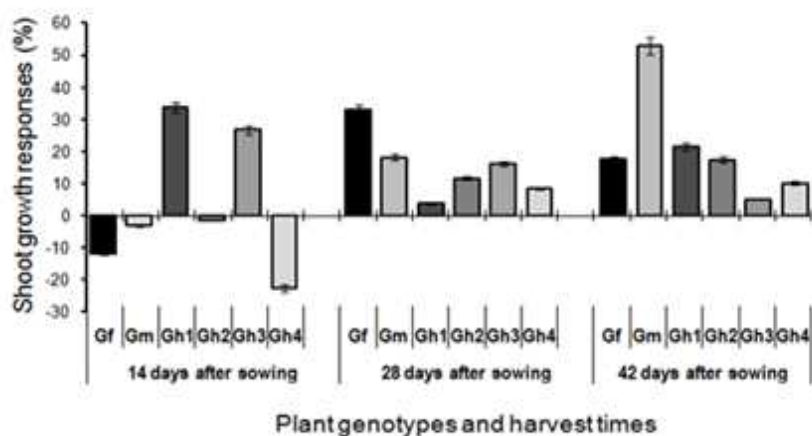
Plant genotypes		Harvest times (das)					
		14		28		42	
		M_0	M_1	M_0	M_1	M_0	M_1
Parents	G_f	1.0 ^{a*)}	0.9 ^a	3.4 ^{abc}	4.5 ^a	9.2 ^{bc}	10.8 ^b
	G_m	0.7 ^a	0.7 ^a	3.3 ^{abc}	3.9 ^{ab}	5.8 ^e	8.9 ^{bcd}
Hybrids	G_{h1}	0.5 ^a	0.7 ^a	2.2 ^c	2.3 ^c	7.5 ^{cde}	9.2 ^{bc}
	G_{h2}	0.7 ^a	0.7 ^a	2.1 ^c	2.4 ^c	7.9 ^{cde}	9.3 ^{bc}
	G_{h3}	0.5 ^a	0.6 ^a	3.4 ^{abc}	3.9 ^{ab}	14.0 ^a	14.7 ^a
	G_{h4}	0.8 ^a	0.6 ^a	2.6 ^{bc}	2.8 ^{bc}	8.1 ^{cde}	8.9 ^{bcd}
Average		0.7 ^a	0.7 ^a	2.8 ^a	3.3 ^a	8.8 ^b	10.3 ^a

Remarks: *) Data in the same appropriate column followed by the same superscript letter are non-significantly different based on LSD test at $p = 5 \%$; M_1 and M_0 : plants with and without mycorrhizal inoculation; das: days after sowing

Table 2. Root length (cm) of plant genotypes treated with and without mycorrhizal inoculation harvested at different plant ages

Plant genotypes	Harvest times (das)						
	14		28		42		
	M ₀	M ₁	M ₀	M ₁	M ₀	M ₁	
Parents	G _f	142 ^{bc} *)	205 ^a	232 ^{abc}	252 ^{ab}	405 ^b	473 ^a
	G _m	99 ^e	90 ^e	124 ^g	155 ^{efg}	204 ^h	279 ^{fg}
Hybrids	G _{h1}	106 ^{bcd}	116 ^{bcd}	171 ^{defg}	188 ^{cde}	369 ^{bc}	396 ^b
	G _{h2}	84 ^e	154 ^b	174 ^{def}	265 ^a	300 ^{efg}	355 ^{bcd}
	G _{h3}	76 ^e	85 ^e	168 ^{defg}	207 ^{bcd}	334 ^{cde}	332 ^{cde}
	G _{h4}	75 ^e	71 ^e	183 ^{cdef}	210 ^{bcd}	314 ^{def}	366 ^{bc}
Average	97 ^b	120 ^a	176 ^b	213 ^a	321 ^b	367 ^a	

Remarks: *) Data in the same appropriate column followed by the same superscript letter are non-significantly different based on LSD test at p= 5%; M₁ and M₀: plants with and without mycorrhizal inoculation; das: days after sowing

**Fig. 1.** Intensity of mycorrhizal colonization on the roots of cowpea genotypic plants at different harvest times**Fig. 2.** Shoot growth responses of cowpea genotypic plants to mycorrhizal colonization at different harvest times

Furthermore, from microscopic examination, there was no any colonization structure of mycorrhizae measurable on roots of uninoculated control plants as was evident on that of plants with mycorrhizal inoculation. The percentage of root length colonized by mycorrhizal fungi considerably varied among plant genotypes, which increased along with plant growth (Fig. 1). At 14 das (days after sowing), most the roots have not been colonized excepting on G_f and G_{h2} , with intensity of about 10 and 1 % respectively. Root colonization was observable for all genotypes at 28 das, and its rates significantly increased at 42 das.

The extent of mycorrhizal root colonization significantly differed between parental genotypes (G_f and G_m), the two parental inbred lines to their hybrids, and among these hybrids (G_{h1} , G_{h2} , G_{h3} and G_{h4}) tested that increased by plant age. At 42 das, the female parent G_f (a cowpea inbred line) revealed the highest response to mycorrhizal inoculation by rapidly forming mycorrhizal colonization compared to other genotypes; otherwise the male parent G_m (a mung bean inbred line) was less in responding mycorrhizal infection and colonization. Moreover, it is clearly found that cowpea hybrids, generated from such parental genotypes ($G_f \times G_m$), had decreased level of response to mycorrhizal infection and colonization compared to that on their responsive parental genotype G_f . The rate of mycorrhizal root colonization among these hybrids in descending order was $G_{h1} > G_{h2} > G_{h3} > G_{h4}$.

The different response to mycorrhizal infection and colonization between the two parental genotypes and among their hybrids as well found in this study is lined up with Khalil, Loynachan, & Tabatabai (1994), stating that modern plant cultivars respond less to mycorrhizal colonization than their parental cultivar origins. Mercy, Shivashankar, & Bagyaraj (1990) from their experiment with a number of cowpea genotypes explained that the presence of genes regulating plant responses to mycorrhizal colonization was free and could be derived. Therefore, the descending response to mycorrhizal infection by cowpea hybrids also indicated that genes derived from G_m was more expressed on controlling mycorrhizal colonization than that ones from G_f .

Moreover, the difference in growth response of cowpea genotypes in relation to the effect of mycorrhizal inoculation on their shoot growth was shown by Fig. 2. The growth responses of G_m , G_{h2} ,

and G_{h4} increased from time to time. It is obvious that G_m had the highest growth response, while G_f , G_{h1} and G_{h3} showed an altering response pattern. Response of G_f was initially negative at 14 das, increased at 28 das, and then decreased at 42 das. Response of G_{h1} was quite high at 14 das, then decreased significantly at 28 das, and increased again at 42 das. While for G_{h3} , its response initially was high at 14 and 28 das, but then decreased at 42 das.

The existence of a large variation in the growth response among the cowpea genotypes clearly clarifies possibility about the nature of genotypic expression on plant responses to mycorrhizal symbiosis (Mercy, Shivashankar, & Bagyaraj, 1990). Changes in response of modern cultivar to AM fungi have previously been reported for some crops, such as wheat cultivars (Hetrick, Wilson, & Cox, 1992; Zhu, Smith, Barritt, & Smith, 2001), chickpea genotypes (Hacisalihoglu, Duke, & Longo, 2005; Bazghaleh, Hamel, Gan, Tar'an, & Knight, 2015), onions genotypes (Taylor et al., 2015), and cucumber hybrids (Tüfenkçi et al., 2012). Hetrick, Wilson, & Cox (1992) demonstrated a strong genetic basis for differences among wheat cultivars in responding mycorrhizal colonization and function. Some modern varieties showed either no response to or reduced growth due to mycorrhizal colonization. Correspondingly, Zhu, Smith, Barritt, & Smith (2001), when testing a number of wheat varieties released at different years found that the newer varieties had less response to colonization and contribution of AM fungi compared to the older varieties.

The difference in response among the genotypes of cowpea plant found in this study, and other plant species in prior studies (Hacisalihoglu, Duke, & Longo, 2005; Tüfenkçi et al., 2012), may be resulting from the effect of some other plant traits. Mosse (1981) mentioned morphological characteristic of plant roots as well as root development might influence plant responses to AM fungi. This present study found that plant growth as indicated by shoot dry weight closely correlated to total root length, as found on control plants (M_0) as well as on mycorrhizal plants (M_1). In this case, *Pearson's correlation coefficient* between the two variables was $r = 0.86$ for M_0 ($p < 0.01$) and $r = 0.82$ for M_1 ($p < 0.01$) respectively. A positive correlation was also shown for shoot dry weight and colonized root length on mycorrhizal plants (M_1) ($r = 0.71$; $p < 0.01$).

These really indicates that plant root development has a close relationship with their biomass shoot weight, in which the correlation coefficient in the uninoculated mycorrhizal control plants is higher than in mycorrhizal plants. The data suggest that in order to grow optimally, plants without mycorrhiza require root length longer (resulting from extensive growth) compared to plants with mycorrhiza.

Physiological status of the plant is also very influential for regulating plant responses to AM fungal colonization and contribution. Some recently reports indicate that requirements for nutrients especially phosphorus might affect plant response to mycorrhizal infection (Mosse, 1986; Tawaraya, 2003; Wang, Zhao, & Bücking, 2016). Yaseen, Burni, & Hussain (2011) found that differences in growth response of several genotypes of cowpea to AM related to P supply.

In addition, plant responses to mycorrhizal function may be influenced by many other factors that also involves species of AM fungi and environmental conditions, but the characteristics of plant (root morphology and physiology) may have an important role in controlling the activity of AM fungi on internal root tissues (Linderman & Davis, 2004). Parke & Kaeppeler (2000) stated that changes in mycorrhizal response in the new plant genotypes basically related to how plant genes inherited during breeding process expressed, or else the changes could be favored by a range of environmental factors that influence genetic expression. Tawaraya (2003) concluded that despite the genetic expression implementation of plant growth was also greatly affected by environmental conditions.

Data in this study on decreasing responses of cowpea hybrids to mycorrhizal symbiosis refresh the reflection of Eason et al. (2001) that "plant-breeding programmes have not selected for plants based on their ability to form effective AM association", by ignoring genetic traits to form mycorrhizal symbioses. Accordingly, plant hybrids or cultivars generated from the cross-breeding may loss their mycotrophic traits, so unable to employ effectively nutrient sources available in soil. Indeed, they become either more susceptible to nutrient deficiencies or being highly dependent on external nutrient's supply for maximum growth.

Since the function of mycorrhizae are very beneficial for plant growth as described above, it is really suggested for plant breeders to consider

not only plant traits for production, but also for their ability to form a mutuality symbiosis with beneficial soil microbes, including AM-forming fungi (Rengel, 2002). Hopefully, the new plant cultivars obtained from the plant-breeding programmes may be higher in production, and else prominently able to use efficiently various sources of nutrients and water in bulk soil. The problem is such varieties have not been developed so far. Therefore, collaboration between mycorrhizal researchers, plant breeders and plant physiologist is highly required to work together to breed the expected cultivars.

CONCLUSION AND SUGGESTION

This study has shown that responses of cowpea genotypes to arbuscular mycorrhizal fungi are substantially different between the two inbred line parents, among the two parents and their hybrids, and among the hybrids. The highest response to mycorrhizal colonization was on the female parent's genotype G_f (cowpea inbred line), and the response in effect decreased on their hybrid genotypes by degrees in the following order of $G_{h1} > G_{h2} > G_{h3} > G_{h4}$. Instead, the highest growth response to mycorrhizal contribution was shown by male parent's genotype G_m (mung bean inbred line), followed by the first and the second generation of the hybrids (G_{h1} and G_{h2}).

Further experiments are needed to study in detail the function of AM symbiosis on growth and production of these cowpea genotypes in the field.

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