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Mutualistic Symbiosis between Arbuscular Visicular Mycorrhizal Fungi with Cassava Plant *(Manihot esculenta Crantz)*

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Abstract: *Fertilization in cassava is necessary to improve soil nutrient availability, growth and tuber yield. However, cassava farmers, especially in sub-optimal (potential) land generally fertilize their plants with NPK fertilizer less than the recommended dosage. Therefore, nutrients from other sources are needed. This paper explains that some nutrients, especially P, could be substituted through mycorrhizal symbiosis. In return, mycorrhizal fungus obtains the energy from cassava as a host. Thus, Such an association is beneficial for both parties, the plant and its symbiont. This fungus forms vesicular and arbuscular structures in cortical tissues of the plant root and then is known as vascular-arbuscular mycorrhizae (VAM). The degree of growth and tuber yield Improvement due to mycorrhizae is determined by the genera and species of the VAM. The genetics of cassava also influence significantly the effectiveness of VAM. In addition to the improvement of nutrient absorption, VAM also enhances tolerance of cassava to drought, saline and some root diseases. Root disease tolerance of cassava due its symbiosis is assumed to be VAM's ability to curtail disease infection. The genera, species and the density of VAM affect the degree of retardation of disease infection, enhancement of nutrient absoption, tolerance to soil drought and tolerance to soil salinity.*

Keywords: *Cassava, drought, mycorrhizae, nutrient, salinity*

1. Introduction

Cassava (*Manihot esculenta* Crantz) is commonly planted by Indonesian farmers because it is an important food as a carbohydrate source after rice and maize. As a food source, cassava is consumed in non-processed form (boiled, fermented preparation called tapai, and fried in chips) or in processed form, flour and starch are processed into a variety of food products (Eleazu *et al.* 2014; Herlina and Nuraeni 2014; National Geographic 2017; Herawati 2015). In general, these plants are planted on infertile soils, such as in upland fields that are poor in P elements (calcareous soils) or in acid-reacting soils. Therefore, fertilizer application is needed to increase the growth and yield of cassava plants (Wargiono 2003).

Microbes as form of fungi are not only known as parasites which cause plants to become sick and even die.such as the fungus *Sclerotium rolfsii* in soybean plants or other plants (Yaqub and Shahzad 2005; Malinda *et al.* 2017). Remy *et al.* (1994) reported the presence of fungi in fossil plant roots that were around 400 million years old. Several studies from several decades ago revealed that sucha fungus was known as Glomales which was associated with plant roots and in fact the fungus could play a role to increase plant growth and production (Soedarjo and Habte 1993 and 1995; Habte and Soedarjo 1995 and 1996; Tong-jian *et al.* 2010). This association between fungi and plant roots is known as 'mycorrhizae', so they are also known as mycorrhizal fungi (Ruzicka *et al.* 2013).

Mycorrhizal fungi obtain energy from host plants (Solaiman and Saito 1997; Pfeffer *et al.* 1999) and host plants obtain additional nutrient uptake such as P from fungi which causes plants to grow and produce better, especially on soils that are P nutrient deficient (Soedarjo and Habte 1993 and 1995; Allen and Shachar-Hill 2009; Govindarajulu *et al*. 2005; Javot et al. 2007; Sankaralingam *et al.* 2016). Besides increasing P uptake, mycorrhizae also increase other nutrient uptake such as N, K, Mg, Cu, Zn (Guissou 2009; Yaseen*et al.* 2011). Mycorrhizal fungi also increase plant tolerance to water shortages (Aroca *et al.* 2008; Wu *et al.* 2011; Chitarra *et al.* 2016) and also increase disease resistance in various plants (Hwang *et al*. 1992; Liu *et al.* 2007; Poovarasan *et al.* 2013). Thus, this association is mutually beneficial and is known as mutualistic symbiosis (Ruzicka, *et al.*, 2013). This review discusses the important role of arbuscular vesicular mycorrhizae in order to enhance cassava growth and productivity.

2. VESICULAR ARBUSKULARMIKORIZAE

Vesicular-arbuscular mycorrhizae (VAM) is classified as endomycorrhizal, because vesicular and arbuscular structures are formed in the root cortex tissue of plants. This type of mycorrhizae infects plant roots and hyphae enter the cortical tissue (Figure 1). In cortical tissue, these mycorrhizae form arbuscular and vesicular structures. The tips of mycorrhizal hyphae that exist in the cortical tissue form like clumps of thread and are referred to as 'arbuscular'. While the tip of the hypha that forms a ball-like structure in cortical tissue is called the 'vesicular'. So, this type of mycorrhizae is known as Vesicular-Arbuscular Mycorrhizae (VAM). VAMfungi cannot be propagated with pure culture in the laboratory, but must always be associated with plant roots, because this type of fungus is an 'obligate'. The formation of vesicular and arbuscular indicates that symbiosis has occurred. Arbuscular and vesicular structures are common and are found in the roots of all plants, including cassava (Begoude *et al.* 2016).

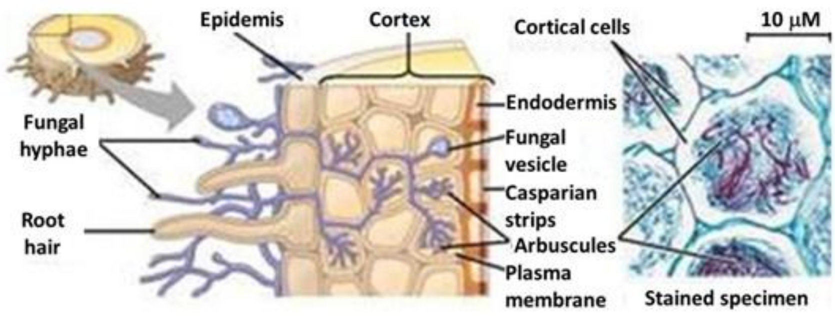

Figure1. *Mycorrhizal structures in plant roots in symbiosis with plants.*

(http://int.search.myway.com/search/AJimage.jhtml?&n=781bb8d6&p2=%5EY6%5Echryyy%5ETTAB02%5Eid&pg=AJimage&pn=1&ptb=0E8AF6B1-DB8D-4687-A10E B08C52D315F&qs=&searchfor=endomycorrhizae+image&ss=sub&st=tab&tpr=sbb&imgs=1p&filter=on&imgDetail=true (accessed 26 May 2017)

2.1. Effect of Mycorrhizae on Growth And Yields of Cassava

Plant growth and yield, including cassava, are determined by environmental and genetic factors. Environmental factors include the abiotic and biotic environments. Abiotic factors such as climate (air temperature, radiation, humidity, air pressure and rainfall) are not easily changed or modified to support optimal plant growth. Non-climatic abiotic factors such as soil fertility, soil acidity, salinity and relative soil moisture can be modified to improve plant growth. Plant experts define biotic factors in general are pests, diseases and weeds that can inhibit growth and reduce yields and even cause crop failure. The VAM fungus is also a biotic factor that can affect the growth and yield of cassava (Table 2) (Sridevi and Ramakrishnan, 2013).

Plant growth increase was due to improved nutrient uptake by host plants (Sridevi and Ramakrishnan 2013; Sankaralingam, *et al*., 2016). The Increaseplant growth is also caused by an increased of plant resistance to diseases (Poovarasan et al., 2013), resistance to water shortages (Chitarra et al. 2016) and resistance to saline conditions (Al-Khaliel, 2010). The benefits of mycorrhizae in enhancing plant growth and yield are also determined by mycorrhizal types and host species and even varieties of certain plant types (Azcon and Ocampo, 1981; Tawaraya, 2003; Ristiyanti, *et al.* 2014; Rias *et al.* 2014).

Table2. *Effect of Glomus fasciculatum inoculation on plant height (cm) at 150 days after planting and yield of cassava tubers (t / ha)*

Treatment	Plant height (cm)	Yield of cassava tubers (t/ha)
Control	131.93	16.790
Mycorrhizae (M)	140.67	20.571
25% NPK (25:25:40)/ha	149.53	26.124

100% NPK (100:100:160)/ha	192.53	33.120
M+25% NPK/ha	174.59	29.154
M+100% NPK/ha	198.19	34.152

Source: *Sridevi and Ramakrishnan (2013).*

2.2. Dependence of Cassava on Vesicular Arbuscular Mycorrhizal Fungi

The degree of plant dependence on vesicular arbuscular mycorrhizal (VAM) fungi is usually measured by the degree of infection, the degree of nutrient uptake and the rate of growth and yield of plants. The degree of plant dependence on VAM fungi is determined by the genotype of the host plant and also the VA mycorrhizal fungal genotype at the level of genus, species and even varieties (Table 1) (Azcon and Ocampo, 1981, Howeler 1982, Sharma *et al.* 1996, Bâi *et al.* 2000 , Kaeppler *et al.* 2000, Tawaraya K 2003, Zachée *et al.* 2008, Al-Qarawi and Al-Syahrani, 2010, Tong-jian *et al.* 2010, Jiang *et al.* 2013, He *et al.* 2014). The results of research by Graham and Syvertsen (1985) showed that plants abundant in fine roots were found to be have less dependant on VAM fungi than plants with few fine roots. Cassava has a few fine roots, so the cassava plants are highly dependant on VA mycorrhizal fungi (Howeler, 1982; Okon, 2011,). Cassava inoculation with VA mycorrhizae can significantly increase growth and yield (see Table 2) (Sridevi and Ramakrishnan 2013). However, the dependence of several cassava varieties on different VA mycorrhizae causes the growth to increase as well (Begoude *et al*. 2016).

Table1. *The degree of plant dependence on VAMfungi*

Type of plant	Type of VAMfungi	Depend-ence (%)	Sources
Cassava(*Manihot esculenta* Crantz) cv TMS 4(2)1425	*Glomus deserticola*	54	Okon et al. 2010
Cassava(*Manihot esculenta* Crantz) cv. TMS 30572	*Glomus deserticola*	77	Okon, 2011
Cassava (*Manihot esculenta* Crantz) cv. Nina	Mycorrhizal inoculum from cassava roots	51	Howeler , 1982
Cassava (*Manihot esculenta* Crantz) cv. Pata de Paloma	Mycorrhizal inoculum from cassava roots	29	Howeler , 1982
Cassava (*Manihot esculenta* Crantz) cv M Aus7	Mycorrhizal inoculum from cassava roots	37	Howeler , 1982
Cassava (*Manihot esculenta* Crantz) cv M Aus 8	Mycorrhizal inoculum from cassava roots	14	Howeler , 1982
Cassava (*Manihot esculenta* Crantz) cv M Aus 10	Mycorrhizal inoculum from cassava roots	16	Howeler , 1982
Cassava (*Manihot esculenta* Crantz) cv M Aus 20	Mycorrhizal inoculum from cassava roots	48	Howeler , 1982
Cassava (*Manihot esculenta* Crantz) cv M Aus 21	Mycorrhizal inoculum from cassava roots	37	Howeler , 1982
Cassava (*Manihot esculenta* Crantz) cv CMC 72	Mycorrhizal inoculum from cassava roots	27	Howeler , 1982
Rice (*Oryza sativa*)	Mycorrhizal inoculum from cassava roots	9	Howeler , 1982
Maize (*Zea mays* L.)	Mycorrhizal inoculum from cassava roots	15	Howeler , 1982
Acacia nilotica var. cupriciformis	*Glomus spp*	18	Sharma et al. 1996
Groundnut(*Arachis hypogaea* L.)	*Glomus sp.* and *Gigaspora sp.*	86	Zachée et al. 2008
Mungbean (*Vigna radiata*)	*Glomus caledonium* 90036	58	Tong-jian et al. 2010
Maize (*Zea mays* L) var. Mo17	Mixed of *G. etunicatum, G. clarum, G. intraradices, A. mellea*	40	Kaeppler et al. 2000
Maize (*Zea mays* L) var. B37	Mixed of *G. etunicatum, G. claroideum,, A. mellea*	62	Kaeppler et al. 2000
Maize (*Zea mays* L) var. W59M	Mixed of *G. etunicatum, G. clarum, G. intraradices, A. mellea*	76	Kaeppler et al. 2000

Bamboo hybrid, crosses between *Bambusa pervariabilis* and*B. grandis*	*Glomus intraradices* BEG 193	46	Jiang et al. 2013
Bamboo hybrid, crosses between *Bambusa pervariabilis* and*B. grandis*	*G. intraradices* BEG 141	35	Jiang et al. 2013
Bamboo hybrid, crosses between *Bambusa pervariabilis* andB. *grandis*	*G. mosseae* BEG 167	37	Jiang et al. 2013
Bamboo hybrid, crosses between *Bambusa pervariabilis* andB. *grandis*	*G.etunicatum* BEG 168	43	Jiang et al. 2013

Note: Dependency is measured by formula = (plant weight or yield of mycorrhizal seeds - weight of plants or yield of seeds without mycorrhizae) / (weight of plants or yields of bermororiza seeds) x 100%.

3. VAMFUNGI AND NUTRIENT UPTAKE ON CASSAVA

Cassava is generally planted on infertile (sub-optimal) soils in Indonesia, such as acid soils and upland soils that does not get technical irrigation (Saleh and Widodo, 2007), so the average production is lower than the yield potential of varieties which has been released by the Agency for Agricultural Research and Development (Balitkabi 2016).Fertilization is reported to increase cassava yields (Ispandi, 2003; Wargiono, 2003; Prasetyo et al, 2014). Table 2 shows that increased plant height and cassava yields due to NPK fertilization can largely be substituted by the inoculation of the VAM fungus, *Glomus fasciculatum* (Table 2) (Sridevi and Ramakrishnan, 2013). These results indicate that VA fungi help host plants increase nutrient uptake.

VAM fungus inoculation was reported to increase absorption of macro and micro nutrients by cassava plants and was followed by a significant increase in tuber yield (Table 3) (Carretero *et al*. 2009, Okon *et al*. 2010, Oyetunji *et al*., 2017).VAMfungi are also reported to increase growth and yields followed by increased nutrient uptake by other plants (Soedarjo and Habte. 1993 and 1995; Govindarajulu *et al*., 2005; Javot *et al*., 2007; Allen and Shachar-Hill, 2009; Guissou , 2009; Yaseen *et al*., 2011; Sankaralingam, *et al*., 2016). Thus, VA mycorrhizal fungi increase plant growth and yield through a mechanism of increasing nutrient uptake.

Table2. *Effect of Glomus fasciculatum inoculation on plant height (cm) at 150 days after planting and yield of cassava tubers (t / ha)*

Treatments	Plant height (cm)	Yield of cassava tubers (t/ha)
Control	131.93	16.79
Mycorrhiza (M)	140.67	20.57
25% NPK (25:25:40)/ha	149.53	26.12
100% NPK (100:100:160)/ha	192.53	33.12
M+25% NPK/ha	174.59	29.15
M+100% NPK/ha	198.19	34.15

Source: *Sridevi and Ramakrishnan (2013).*

3.1. The VAM Fungi Prevent Cassava Plants from Infectious Diseases

Plant diseases caused by viruses, fungi or bacteria are important obstacles to plant growth and yield and can cause crop failure. Important diseases of cassava are caused by white root fungus (*Rigidoporus microporus*), bacterial blight and bacterial wilt (Indonesia Bertanam, 2014). White root fungus disease also infected the roots of rubber plants and inoculation with mycorrhizal fungi was reported to reduce the degree of infection by white root fungi up to 66% (Putri *et al*., 2016). Likewise, as a root crop, cassava could be protected from damage of white root fungus by the presence of adequate amount of VA fungi in the soils.

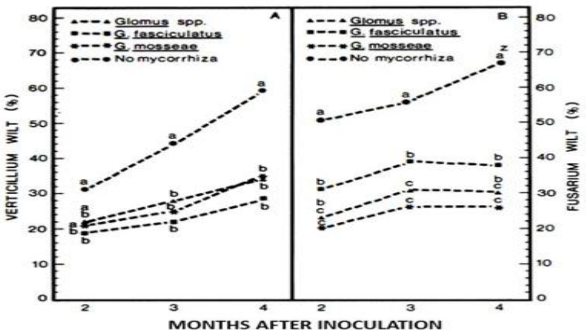

Figure2. *Effect of mycorrhizal inoculation on the degree of withering of alfalfa plants by Verticillium albo-atrum or Fusarium oxysporum f. sp. medicaginis.*

Source: *Hwang et al. 1992*

Fungal diseases caused by *Verticillium albo-atrum* or *Fusarium oxysporum f. sp*. medicaginis can cause the wilting of alfalfa plants and failureof harvest. Figure 2 shows that inoculation with mycorrhizal fungi reduces the degree of wilt in alfalfa caused by *Verticillium albo-atrum* (A) and *Fusarium oxysporum* f. sp. medicaginis (B) and the effectiveness of each type of mycorrhiza in suppressing the disease is different (Hwang *et al.* 1992). The VAM*G. margarita*fungi is effective in reducing the intensity of wilt disease in soybean plants caused by the fungus *Rhizoctonia solani* (Figure 3) and the degree of reduction depends on mycorrhizal dose (Rozy *et al.* 2004).

The damage intensity due to the Blood Disease Bacterium (BDB) in banana plants can also be significantly reduced by the use of the VAMfungi and the effectiveness of this fungus is determined by the mycorrhizalgenera (Suswati *et al*. 2013). The damage intensity due to wilt disease *Fusarium oxysporum* f. sp. Lycopersici in tomato plants decreased significantly with the inoculation of the VAMfungi (Figure 4 A and B) (Pedai *et al*. 2015). Salami and Olawole (2011) also reported the role of VA mycorrhizae in protecting jute plants from *Phytophthora infestans*. Until this review was made, there has been no study of the effect of mycorrhizae on decreasing damages by such a disease on cassava plants. However, from the results of studies on plants other than cassava, mycorrhizal inoculation was expected to suppress the disease infection, especially diseases caused by fungi.

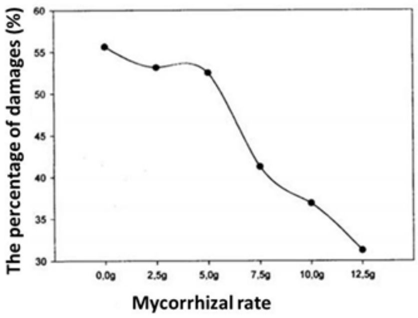

Figure3. *Effect of G. margarita mycorrhizal inoculation dose on the intensity of wilt disease (Rhizoctonia solani) on soybean plants.*

Source: *Rozy et al. 2004*

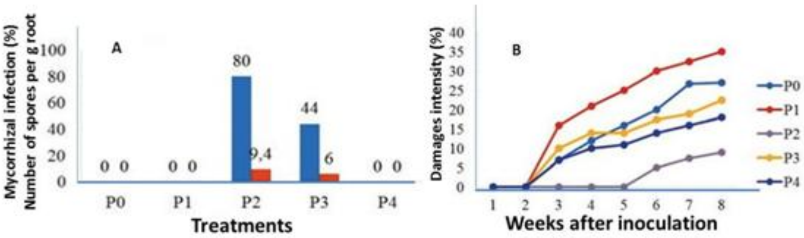

Figure4. *Degree of VA mycorrhizal fungal infection (%) in tomato root and spore density (spore / g) (A) and intensity of Fusarium oxysporum wilt attack f. sp. lycopersici in tomato plants (B). The blue and red histograms (A) are infections (%) and the number of VA mycorrhizal fungal spores per gram of root. PO: tomato plants without inoculation with mycorrhizal fungi and without inoculation with Fusarium oxysporum f. sp. lycopersici, P1: inoculation with Fusarium oxysporum f. sp. lycopersici, P2: inoculation with VA mycorrhizal fungi, P3: inoculation with Fusarium oxysporum f. sp. lycopersici and VA and P4 mycorrhizal fungi: inoculation with Fusarium oxysporum f. sp. lycopersici and sprayed with benomyl 0.2 g / l.*

Source: *Pedai et al. 2015*

3.2. VAM Fungus and Lack of Water in Cassava Plants

Water is the largest component of plants and as a basic material in the process of photosynthesis and evapotranspiration. Thus, lack of water will reduce the rate of photosynthesis and as consequently, the growth will be hampered and yields will decresease remarkably (Table 3) (Ruiz-Lozano *et al*. 1995, Mwanamwenge 1999, Cakir 2004). In drought stress conditions, plants usually produce more proline to prevent cell damage because proline is one of the important osmopolitans of plants (Yoshiba *et al* 1997). Thus, plants that are relatively tolerant of drought stress will produce relatively more proline than plants that are sensitive to drought stress. Table 3 shows that lettuce plants that established micororrhizal association contain higher levels of proline than those without micororrhizae. As a result, the mycorrhizal lettuce plants grow better and produce significantly higher biomass. Thus, mycorrhizal fungi increase plant tolerance to drought stress through increased levels of leaf proline. Proline is a general phenomenon produced higher in drought stress tolerant.

Table3. *Dry weight of lettuce plants in conditions of water adequacy and conditions of water shortage*

Treatments	Water adequacy			Water shortage		
	PDW (g/plant)	Root Infection (%)	Leaf Prolin (nmol/g)	PDW (g/plant)	Root Infection (%)	Leaf Prolin (nmol/g)
Control	0.56k	0	123g	0.15l	0	16.2g
$PO4^{-3}$	1.15j	0	148g	0.26l	0	16.4g
G. deserticola	6.00a	92.3a	79.3bcd	5.50bc	941a	119.6a
G. etunicatum	5.94ab	59.4d	62.8cde	4.98d	655cd	93.4ab
G. intraradices	5.43c	86.5ab	57.8def	4.52e	865ab	40.8ef
G. fasciculatum	5.08d	68.5cd	50.0def	4.39e	632cd	94.7ab
G. mosseae	4.54e	69.7c	38.3ef	3.87f	794b	79.4bcd
G. caledonium	3.53g	32.6f	30.5f	2.70h	272f	42.6ef
G. occultum	2.20i	42.0e	50.8def	0.67k	320f	87.9bc

Note: PDW = plant dry weight.

Figures in the same column followed by the same letter are not significantly different at the LSD 5% test level.

Source: *Ruiz-Lozano et al., 1995*

In addition to proline, mycorrhizae cause higher leaf chlorophyll content and an increase in leaf chlorophyll content is followed by an increase in dry weight of cassava plants as an indicator of plant growth rate (Table 4, Ekanayake *et al*. 2004). Leaf chlorophyll is a vital part of plants to carry out photosynthesis. The higher the chlorophyll content of leaves up to a certain level, the higher the photosynthetic activity and the more photosynthates produced. So that the VAM fungus can increase plant growth (see Table 4, Ekanayake *et al*. 2004).

Table4. *Effect of AV mycorrhizal inoculation on total chlorophyll content of cassava leaves*

Treatments	Chlorophyll content of cassava leaves (mg/l)	
	TMS 4(2)1425	TME1
	1 month after planting	
G. clarum	6.27a	6.03a
G. mosseae	5.49b	6.06a
Tanpa inokulasi	5.11b	5.31b
	2 months after planting	
G. clarum	6.95a	5.78a
G. mosseae	6.29a	5.78a
Tanpa inokulasi	5.05b	5.03b

Source: *Ekanayake et al. 2004.*

4. VAM Fungi and Salinity in Cassava Plants

Salinity is a term to describe the degree of salt (NaCl). Saline condition (high salt content) can inhibit plant growth and yield, including cassava (Al-khalil 2010). Decrease in plant growth and yield under saline conditions is associated with the decreased of photosynthetic rate, the decrease of leaf chlorophyll content, low nutrient uptake and Na accumulation in plant roots (Luiz-Rosano *et al.* 1996, Feng *et al.* 2002, Dudhane *et al.*2011). Interestingly, the presence of mycorrhizae in the roots of host plants was reported to increase plant growth and tolerance in saline conditions. Increased plant growth under saline condition is associated with an increase in the rate of photosynthesis, an increase in leaf chlorophyll content, an increase in plant nutrient content, an increase in leaf and plant moisture content, a decrease in the Na content of peanut plants (Tables 5 and 6, Luiz-Rosano *et al.*1996; Feng *et al.*2002; Cantos *et al.*2008, Sheng *et al.*2008, Al-Khaliel 2010, Dudhane *et al.*2011). However, mycorrhizal effectiveness in reducing the negative effects of saline conditions depends on the type of mycorrhizal fungi and plant genotypes (Ruiz-Rosano *et al.*1996; Carretero *et al.*2008, Al-khalil 2010).

Water and chlorophyll are important components in plant photosynthesis. Thus, the rate of photosynthesis of mycorrhizal plants is better than that of non-mycorrhizal plants and this condition will cause plants to grow and produce better.

Table5. *Effect of salinity on nutrient uptake and chlorophyll content of peanut leaves in inoculated or not inoculated conditions with VA mycorrhizal fungi (Glomus mosseae)*

NaCl (M)	InoculationmycorrhizalVA	Leaves nutrient concentration(%)				Chlorophyll(mg/g PFW))
		N	P	K	Na	
0.0	–	2.54	0.12	1.25	0.02	1.98
0.0	+	2.98	0.22	1.75	0.03	3.88
0.1	–	2.46	0.10	1.18	0.02	1.90
0.1	+	3.26	0.26	1.90	0.03	4.02
0.2	–	2.28	0.10	1.02	0.02	1.81
0.2	+	3.02	0.20	1.40	0.03	3.70
0.3	_	2.12	0.09	1.00	0.02	1.52
0.3	+	2.51	0.20	1.26	0.04	3.16
0.5	–	2.02	0.09	0.98	0.02	1.30
0.5	+	2.38	0.18	1.20	0.04	2.84

VA = vesicular arbuscular,

PFW = plant fresh weight

+ = inoculated with mycorrhiza; - = not inoculated with mycorrhizae;

Source: *Al-khalil 2010*

Table6. *Effect of VAM fungi on the fresh and dry weight of roots, stems and plants (roots + stems + leaves) cassava at 60 days after planting*

Cassava clones	Root(mg)		Stem(mg)		Leave(mg)		Plant(mg)	
	FW	DW	FW	DW	FW	DW	FW	DW
Clone SOM-1								
Mycorrhiza–	99	55	948	231	1,150	202	2,197	489
Mycorrhiza+	1 659	474	14 000	2 777	9 727	1 937	25 386	5 188
	**	**	**	**	**	**	**	**
Clone 05								
Mycorrhiza–	37	17	382	77	332	72	751	166
Mycorrhiza+	529	172	4 930	880	4 383	844	9 842	1 896
	**	**	**	**	**	**	**	**
Clone 50								
Mycorrhiza–	32	24	385	102	343	73	760	199
Mycorrhiza+	360	127	4 405	811	5 542	962	10 306	1 899
	**	**	**	**	**	**	**	**

FW = fresh weight; DW = dry weight

*** Significantly different at P = 0.01 from the Studied Test*

Source: *Carretero et al. 2009*

5. Conclusion

Vesicular-arbuscular mycorrhizal fungus (VAM) establishes symbiosis with plants, including cassava. This symbiosis is mutualistic and obligate, VAM fungi obtain energy sources from host plants and increase nutrient uptake by host plants. As a results, the mycorrhizal plants grow and yield better. Additionally, the mycorrhizal plants are more tolerant ~~of~~to diseases, water stress and salt stress (high salinity). However, the genetic background of VAM fungi and host plants significantly affected the symbiosis

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