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Growth Responses of Micropropagated Cassava Clones as Affected by *Glomus Intraradices* Colonization

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ABSTRACT

This study reports the effectiveness of an arbuscular mycorrhizal (AM) fungus *Glomus intraradices* on three clones (SOM-1, 05 and 50) of cassava (*Manihot esculenta*). Arbuscular mycorrhizal inoculation increased plant resistance to transplant stress from "in vitro" to "ex vitro" conditions and plant biomass (shoot and root) production was greatly enhanced by AM-colonization. The magnitude of AM growth stimulation over control clones was: 861% (SOM-1), 1042% (05) and 854% (50). Arbuscular mycorrhizal colonized cassava plants increased cassava water uptake in terms of percentage, 62% in clone SOM-1, 24% in clone 05, and 157% in clone 50. The highest effect of AM-colonization on water content in root of clone 50 was correlated with the greatest increment in leaf tissue production (1218% over control) and with the maximum shoot/root ratio determined. The biomass distribution between shoot and root was changed by AM symbiosis and such effect varied for each clone that may be caused by mycorrhizal changes in macro/micro-nutrients translocation/compartimentation. Cassava dependence on AM symbiosis was greatest in clone SOM-1 since AM-colonization provided the highest stem (weight, length, and diameters), leaf (weight and number), bud number, and root weight. These results lead to practical applications because AM inoculation is crucial for improving cassava yield (shoot and root) and nutrition irrespective of the clone involved. Thus, importance of AM symbiosis in micropropagated cassava clones is of great practical interest in agriculture and allows the selection of the most suitable clone for dry environments due to the particular effect on root water content that improves drought adaptation.

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INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a food crop of great importance in underdeveloped countries. Cassava is acknowledged as an important food in many subtropical and tropical areas. Previously, arbuscular-mycorrhizal (AM) symbiosis has been shown to affect the growth and nutrition of cassava plants (Azcón-Aguilar et al., 1997; Carretero et al., 1997). The mycorrhizal effectiveness on cassava agree well with the mycotrophy and plant dependence of AM–colonization for reaching the optimum development as reported by Smith et al. (1992) for these plants.

Thus, cassava species are considered to be very reliant upon their fungal symbionts in less fertile environmental conditions. Nevertheless, little information is available on the behavior of this symbiosis to the tolerance of "in vitro" obtained cassava clones when transplanted to outside soil conditions and on the further plant development. Therefore, although cassava plants are responsive to AM fungi they may exhibit, as other plants do, a range of clone-dependent responses to AM association.

Previous studies reported the relevance of AM associations on host plant species, other than cassava and showed plant genotype effect in relation to the AM fungal compatibility and effectiveness (Estaun et al., 1987). Regarding cassava production and the problems associated with poor and stressed soils in many areas, the knowledge about susceptibility and effectiveness of cassava clones to AM-association is not well-documented although considered very important. Previous information showed that it is not possible to generalize on interactions between symbionts because each partner needs a particular study (Azcón and Ocampo, 1981; Monzón and Azcón, 1996).

In this paper, we have studied the influence of AM colonization by *Glomus intraradices* on three clones of *Manihot esculenta* (SOM-1, 05, and 50) grown in vitro, after ex vitro transplanting. We have examined the resistance to transplanting stress of inoculated and non inoculated plants as well as the further development of the plants in outside conditions.

MATERIALS AND METHODS

In vitro plants of the cassava clones SOM-1, from Somalia (sent by Prof. P. Fiorino of the University of Florence, Italy), 05 (with identification no. 7902) and 50 (identification "Bonova rouge"), both from the Ivory Coast (IRAT Mouaké) sent by L'ORSTOM of Montpellier, France, were used as plant material. These

plants were subcultured in our laboratory to reach the necessary number of plants for the different experiments carried out.

Ninety-six in vitro grown plantlets of each clone were individually transplanted to 300 mL pots (Cantos et al., 1993) filled with a steamy sterilized sandy soil. Half of the pots (48) were previously inoculated with arbuscular mycorrhiza *Glomus intraradices* fungus (isolate 11AG8903). Soil was sterilized by steaming for three sterilization cycles (100°C for 1h in 3 consecutive days).

Mycorrhizal inoculum was multiplied in an open pot culture of *Allium cepa* and after six months of culture the shoots were eliminated and the underground part (mycorrhizal roots plus soil possessing fungal spores and mycelium) was stored for 3–6 months in polyethylene bags at 5°C. Inocula consisted of thoroughly mixed rhizosphere samples containing spores, hyphae, and mycorrhizal root fragments, having about 80% of AM colonization. Twenty grams of inoculum were added to appropriate pots. Non-mycorrhizal treatments received the same amount of autoclaved inoculum together with a 2-mL aliquot of a filtrate (<20 μm mesh) of the AM inoculum to provide a general microbial population free of AM propagules.

After 8 d of initial adaptation, the surviving plants of each group (inoculated and non inoculated) were transferred to pots of 2000 mL of capacity containing 1200 g of the same sterilized soil, AM inoculated, or not, correspondingly. This initial hardening was performed in a growth chamber at 22 ± 2°C, 16 h photoperiod and 111 μE m^{-1} s^{-1} of light intensity.

After 15 d of hardening the plants were transferred to the greenhouse and homogeneously irrigated with nutritive 20% Hoagland's solution for 60 d. The temperature in the greenhouse was maintained over 20°C by electrical heating. The relative humidity was registered along the experiment ranging 60–90%. Photoperiod and light conditions were those of natural climate at late spring.

Physical and chemical characteristics of the soil (Table 1) were determined by standard soil analysis methods. The soil samples were air dried, ground, and sieved (through 2 mm mesh) and then the following parameters were determined: texture (Bouyoucos method), bulk density (cylinder method), particle density (pycnometer method), pH (in saturated paste extract), carbonate (Bernard calcimeter), organic matter (Walkley and Black method), nitrogen (N) (Kjeldahl method), extractable phosphorus (P) (Olsen method), available potassium (K) (flame photometry), available calcium (Ca) and magnesium (Mg), as well as manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) by inductively coupled plasma emission spectroscopy.

The influence of AM colonization on the percentage of surviving cassava clones after 15 days of transplanting was determined.

At the end of the experiment, the fresh and dry weight, and water content of root, stem, leaf, and total plant, as well as stem length and diameter, and leaf and bud number were measured in non-mycorrhizal and mycorrhizal plants. Also, the following nutrients were determined: N by Kjeldahl, chlorine (Cl),

Table 1
Physicals and Chemicals characteristics of the soil used as substratum

	Sand	75.8	%
Texture	Loam	8.0	%
	Clay	16.2	%
Bulk Density		1.37	g ml^{-1}
Particle Density		2.62	g ml^{-1}
Capillary Potential	1/3	13.8	atm
	15	10.1	atm
Total Porosity		47.9	%
pH		7.45	
Carbonates		<1	%
Organic Matter		0.8	%
N (total)		0.03	%
C/N		7.8	
$N\text{-}NO_3$		54	mg Kg^{-1} of soil
Extractable P		16	mg Kg^{-1} of soil
Available K		199	mg Kg^{-1} of soil
Available Ca		161	mg Kg^{-1} of soil
Available Mg		113	mg Kg^{-1} of soil
Manganese		23	mg Kg^{-1} of soil
Iron		20	mg Kg^{-1} of soil
Copper		9	mg Kg^{-1} of soil
Zinc		50	mg Kg^{-1} of soil

and P by colorimetry, and sodium (Na), Ca, Mg, K, Fe, Cu, Mn, and Zn by inductively coupled plasma emission spectroscopy.

The tissue water content (% hydration) was calculated from the formula

$$H = \frac{fw - dw}{fw} \qquad (fw = \text{freshweight}; dw = \text{dryweight}).$$

The mycorrhizal presence in AM-inoculated root was checked. Roots from each non-mycorrhized and mycorrhized plants were treated with hot 10% potassium hydroxide (KOH), stained with 0.05% Trypan blue in lactic acid, de-stained with 50% glycerol (Phillips and Hayman, 1970), mounted on slides, and observed directly under a microscope.

Student t and multifactor analysis of variance were used for statistical analysis of the results.

Table 2
Effect of mycorrhizal inoculation (Myc^+) on cassava plants survival through the ex vitro adaptation phases [after 8 days (first transplanting) or 15 days (second transplanting)]

	Clone					
	SOM-1		05		50	
	Myc^-	Myc^+	Myc^-	Myc^+	Myc^-	Myc^+
No. of transplanted plants	48	48	48	48	48	48
No. of surviving plants after first transplanting	40	45	44	48	44	48
% survival	83.3	93.7	91.6	100	91.6	100
No. of surviving plants after second transplanting	37	40	43	43	34	41
% survival	77	83.3	89.6	89.6	71	85.4

RESULTS

The physico-chemical characteristics of the soil used are presented in Table 1. Mycorrhizal colonization was important in increasing cassava survival after transplanting (Table 2) and this effect was observed at the two adaptation phases after transplanting. The clone SOM-1 was sensitive to the two transplanting phases and survived 77% of the non AM-inoculated plants and 83% of AM inoculated plants. For clone 05, AM inoculation did not affect plant survival (after 15 days); and in clone 50, the mycorrhizal effect (after 15 days) increased 20% plant survival (Table 2). This was the highest AM response on transplanting tolerance after such short period of time after the inoculation.

Cassava plant biomass yield (shoot and root) was greatly increased by AM colonization (Table 3). The increase in dry weight was observed for SOM-1 ranging from 762% (root) to 1102% (shoot), for clone 05 ranging from 912% (root) to 1043% (shoot), and for clone 50 ranging from 429% (root) to 695% (shoot). As shown, the growth stimulation was clone-dependent.

The influence of the clone on the response to mycorrhizal can be observed from a number of features. The AM colonization produced the highest root increase in clone 05 while the highest response for stem growth was found in SOM-1. Leaf dry weight was more affected in clone 50.

The relative proportion of root/stem/leaf was also changed by AM colonization (Table 3). The mycorrhizal effect on biomass distribution was higher for clone 50, in which root and stem proportion decreased by 1.8 and 1.18 fold, respectively, while leaf proportion increased 1.4 fold.

The AM colonization increased water content, particularly in roots, indicating the effectiveness of AM fungus on the uptake of water by cassava

Table 3
Effect of mycorrhizal colonization (Myc^+) on the fresh and dry weight (in mg), of root, stem, leaf, and total plant of the three cassava clones, growing in greenhouse during 60 days

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

Student's t test; **mean significant differences $P \leq 0.01$.

plants (Table 4). Colonized cassava plants increase of root water content was 62%, 24%, and 57% for SOM-1, 05, and 50, respectively. The effect of *G. intraradices* on stem length was different in each one of cassava clones as well. Those increases ranged from 223% in SOM-1 to 59% in clone 50 (Table 5).

Similar features can be observed regarding stem diameter (Table 5). On the contrary, the ability of *G. intraradices* in increasing leaf and bud numbers was the highest in clone 50 (150% for leaf and 36% for bud number) and the lowest in clone 05 (36% and 13%, respectively).

The growth parameters determined were differently improved by the AM colonization in each clone as well as the percentage of biomass distribution. No differences were observed in the percentage of AM-colonized roots (data not shown).

Nitrogen concentration increased in roots and decreased in leaves after mycorrhizal-colonization in the three cassava clones assayed. Such AM effect was stronger in SOM-1 than in the other two clones. The AM effect on P and K concentration was not relevant in any case (Table 6).

Table 7 shows that AM-colonization changed the translocation factor for N and K in the three cassava clones. For N, AM depressed such value particularly in the clone SOM-1 and for K the opposite effect was observed in clones SOM-1 and 50. Differences in Ca and Mg among clones were not relevant.

Table 4
Effect of mycorrhizal colonization (Myc^+) on the water content (%) of root, stem, leaf, and total plant of the three cassava clones, growing in greenhouse during 60 days

Clone	Root	Stem	Leaf	Plant
SOM-1				
Myc^-	44.2	74.9	82.7	77.6
Myc^+	71.6	80.3	80.1	77.6
	**			
05				
Myc^-	54.2	79.8	78.3	77.7
Myc^+	67.4	81.8	80.7	80.6
	**			**
50				
Myc^-	25.2	73.5	78.7	73.8
Myc^+	64.7	81.5	82.6	81.7
	*			**

Student's t test; **mean significant differences $P \leq 0.01$; *mean significant differences $P \leq 0.05$.

Table 5
Effect of mycorrhizal-colonization (Myc^+) on the stem length and diameter, and leaf and bud number of the three cassava clones growing in greenhouse during 60 days

Clone	Stem length (cm)	Diameter (mm)	No. of leaves	No. of buds
SOM-1				
Myc^-	22	3	5	15
Myc^+	71	8	10	20
	**	**	**	*
05				
Myc^-	16	2	5	16
Myc^+	44	5	8	18
	**	**	*	ns
50				
Myc^-	22	3	4	14
Myc^+	35	4	10	19
	*	*	**	ns

Student t test; **mean significant differences $P \leq 0.01$; *mean significant differences $P \leq 0.05$. ns: non significant.

Table 6
Effect of AM colonization (Myc$^+$) on N, P and K concentration (% dw) in root (R), stem (S) and leaves (L) in the three cassava clones

	N			P			K		
Clone	R	S	L	R	S	L	R	S	L
SOM-1									
Myc$^-$	0.90	1.96	4.43	0.16	0.07	0.13	0.49	1.67	2.06
Myc$^+$	2.69 **	1.12	3.49	0.18	0.08	0.23	0.33	0.71	2.48
05									
Myc$^-$	0.84	1.79	4.12	0.17	0.08	0.15	0.27	2.30	1.79
Myc$^+$	1.18	1.72	3.46	0.16	0.07	0.18	0.34	1.84	1.85
50									
Myc$^-$	1.03	1.84	4.10	0.20	0.09	0.15	0.46	2.41	1.82
Myc$^+$	1.87	2.42	3.46	0.15	0.10	0.18	0.29	2.16	1.85

Student's t test; **mean significant differences $P \leq 0.01$.

Nevertheless, AM-colonization particularly increased Ca in the leaves of the three clones (Table 8).

Concentration of micronutrients (Cu, Fe, Mn, and Fe) is particularly high in root tissue, especially Fe. In all clones, the concentration of Fe and Zn was highly decreased by AM-colonization in roots. Similar AM effect was observed in Mn (clones 05 and 50) but the AM decreasing effect was less strong than with Fe (Table 9). On the contrary, AM plants showed higher Cu (particularly in the roots).

Table 7
Effect of AM colonization (Myc$^+$) on translocation factor (from root to shoot) of nutrients (N, P, K) in the three cassava clones

Clone	N%	P%	K%
SOM-1			
Myc$^-$	7.10	1.25	7.60
Myc$^+$	1.71	1.72	12.70
05			
Myc$^-$	7.03	1.35	14.80
Myc$^+$	4.05	1.56	10.80
50			
Myc$^-$	5.77	1.20	9.19
Myc$^+$	3.14	1.87	13.82

Table 8
Effect of AM-colonization (Myc^+) on Ca and Mg concentration (% dw) in root (R), stem (S) and leaves (L) in the three cassava clones

	Ca			Mg		
Clone	R	S	L	R	S	L
SOM-1						
Myc^-	2.35	1.17	2.05	0.43	0.13	0.40
Myc^+	2.44	1.46	2.84	0.44	0.15	0.42
05						
Myc^-	2.05	1.71	2.35	0.34	0.18	0.40
Myc^+	2.03	1.32	2.82	0.38	0.19	0.39
50						
Myc^-	2.28	1.44	1.81	0.41	0.22	0.33
Myc^+	1.92	1.47	2.81	0.42	0.28	0.39

DISCUSSION

One goal of this work was to know the influence of AM-colonization on the survival and development of cassava clones after transplanting from in

Table 9
Effect of AM colonization (Myc^+) on Cu, Fe, Mn and Zn concentration in root, stem and leaves in the three cassava clones

	Cu		Fe		Mn		Zn	
	Myc^-	Myc^+	Myc^-	Myc^+	Myc^-	Myc^+	Myc^-	Myc^+
				Clone SOM-1				
Root	33	42**	4132	1645**	436	356	141	64**
Stem	18	19	86	107	73	43*	99	23
Leaves	18	35	303	290	143	111	77	61
				Clone 05				
Root	36	43	4315	1544**	526	241**	133	56**
Stem	13	10	185	96**	81	59**	48	35
Leaves	16	21	257	260	145	130	80	59
				Clone 50				
Root	67	73	6221	1797**	544	357**	252	100**
Stem	16	13	166	192	89	74	92	58
Leaves	13	19	234	237	174	164	55	70

Student's t test; **mean significant differences $P \leq 0.01$; *mean significant differences $P \leq 0.05$.

vitro to ex vitro conditions. The results show that the mycorrhizal effect was greater on increasing plant growth values than on cassava survival after ex vitro transplanting and after the second adaptation period. Nevertheless, the small differences found between the survival of inoculated and control plants might be due to the short time elapsed from the AM inoculation. At this initial stage, a low colonization is produced and its effectiveness would require more time to be noted.

Early AM inoculation, before the emission of roots, allows the beneficial influence of the fungus to be gained from the early growth stage. Jaizme-Vega et al. (1997) described in banana plants the AM benefit from the earliest period. Vidal et al. (1992) reported that only 4% of the inoculated plants of avocado were lost after transplanting compared to 7% of control lost. These results are in agreement with other results showing that AM inoculation increased resistance to transplanting stress. Results also suggest that the effectiveness of AM colonization was influenced by the cassava clone involved.

Compared to non-mycorrhizal plants, AM-colonization increased root, stem and leaf growth in the three cassava clones but each clone showed specific features after *G. intraradices* colonization. Comparatively, AM-colonization produced the highest root improvement (by 912%) in clone 05, but it did not provoke the highest stem or leaf development. Both stem and leaf were particularly increased by AM-colonization in clone SOM-1 (stem, by 1102%) and in clone 50 (leaf by 1218%). These results evidenced that *G. intraradices* effect is clone-dependent.

The variation of the effect in increasing root, stem, leaf, water content, and proportion of plant biomass distribution in each cassava clone indicates the different physiological compatibilities between cassava plant clones and the AM fungus (Monzón and Azcón, 1996). Particular mechanisms conferring differences in improving the growth of specific cassava tissues could be due to variations of the physiological characteristics of the association. However, no consistent relationship was found between the infectivity level and the cassava growth response.

The mechanisms underlying the growth improvement mediated by AM colonization could be based on nutrients uptake or/and other physiological processes as increased carbon dioxide exchange rate. The enhancement of water content in mycorrhizal root changed from 62% (clone SOM-1) up to 157% (clone 50). This higher water status in mycorrhizal roots (particularly in clone 50) could be more advantageous than other AM effects for long-term plant survival under drought or saline conditions. According to the results, differences in the characteristics of external hyphae such as length or distribution (not determined here) could be expected since AM extraradical mycelium makes important contribution to water uptake by the host plant (Ruiz-Lozano et al., 1995a; 1995b). Leaf area and net photosynthesis are closely related. The mycorrhizal effectiveness in connecting increased leaf expansion with increased water in roots found in clone 50 was not so clearly observed in the other two

clones. These differences in mycorrhizal response among clones may be related to differences in competition for the available water for transpiration and for cell expansion. In addition, a noticeable difference about AM-response in clone 50 is the increased shoot/root ratio. This value was greater in AM plants irrespectively the cassava clone involved but shoot/root ratio increases were lower in SOM-1 and 05 clones than in clone 50 that increased nearly to 100%. This AM activity is an indication of a greater nutrient transport to the upper tissues.

The usual situation for cassava plants is to grow in soils where nutrients and water are limiting factors. Under such stress conditions, it is necessary to maximize the efficiency of the uptake and use of water and nutrients. Thus, the effect of AM symbiosis in each clone can be attributed to complementary mechanisms such as an increased nutrients translocation, root growth and activity or compartmentation. Physiological root characteristics are very important in improving plant nutrition and water uptake that is critical for plant growth and resistance to the environmental stresses.

The relative uptake and allocation of macro and micronutrients were different in AM-colonized plants. Nutrient concentration also varied among plant parts. The Fe, Mn, and Zn concentrations decreased in AM plants. These micronutrients were more reduced in the root than in the other plant parts in response to AM infection which indicate the alteration of mineral allocation due to AM fungus. The depletion of these trace elements in root was correlated with a greater N concentration. Mechanisms mediating nutrients assimilation on exclusion by AM-colonization are not known and it would be interesting to further study these nutrients/plant/fungus interactions.

Nutrients requirements and their interactions in cassava clones may have been different for AM plants. It is unclear if Fe, Mn, and Zn decrease was the result of lower requirements of these elements by AM-colonized cassava plants, or if this AM effect was incidental to the lower uptake by the fungus. Nevertheless, micronutrient assimilation and interactions is an unexplored area of importance in mycorrhizal research.

Often, increased P has a detrimental effect on micronutrient uptake (Cu, Zn, etc.) as Murphy et al. (1981) reported. But here, in cassava clones, AM-colonization did not enhance the P status of the host and the uptake of these micronutrients decreased as well.

Comprehensive investigations on interactive symbiosis in each clone and the mechanisms involved are required. Such knowledge should provide insights into the functional significance of differences between plants and AM-symbiont combinations. The genetic variation within cassava clones points out the high potential of the AM symbiosis to adapt plants to detrimental conditions and the AM colonization provided a suitable system for advantageous cassava growth in infertile areas.

Despite the lack of specificity in the AM association, the AM fungus differed in its ability to enhance growth by the host plants even when host

differences were minimum (at the clone level) and the extent of AM-colonization was similar. To optimize cassava growth in low fertile soils, plants need to be AM-colonized but the effectiveness of this symbiosis is greatly influenced by the host plant.

Regardless of the cassava clone used, AM colonization is a critical factor for plant growth. The dependency of plants on AM-colonization has been often related to root development; plants with reduced root system display a high dependence whereas those with extensive root system, as grasses, have a low degree of AM dependence (Hayman, 1982). Thus, in cassava clones, having different root growth, changes in AM-dependence could be expected. Regarding these results, it is curious that the clone SOM-1 showed the greatest degree of mycorrhizal dependency and the highest root growth and the benefit that clone 50, with the lowest root growth, obtained from AM-association was the lowest which is in disagreement with that previously stated for most of the plants.

Results show that AM fungus has an important role in agriculture providing balanced nutrition for plants and promoting water uptake and growth. This microbial activity may reduce the need for chemical fertilizer and increase tolerance to nutrient and water deficiencies. The greatest root development is very important in the active growth periods.

Under the present experimental conditions, the dry weight of aerial and underground tissues of colonized cassava plants were 10.6 fold (SOM-1), 11.4 fold (05), and 9.5 (50) fold than in control plants. Thus, in nursery cassava plants raised by micropropagation should be systematically inoculated.

The AM colonization produced higher root biomass and this could mean a greater water uptake in these plants as results show. Nevertheless, the mycorrhizal effectiveness on root growth was more relevant on clone 05 but the effect on water improvement by roots was strongest in clone 50. These results might suggest that this cassava clone (50) when mycorrhizal could tolerate drought more efficiently than the other two clones resulting in better tolerance to water limiting conditions.

These results could lead to practical applications since AM inoculation seems to be essential for improving cassava yield and also selecting the most suitable clone for dry environments since AM colonization improved the adaptation to drought.

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