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Arbuscular-Mycorrhizal Contributes to Alleviation of Salt Damage in Cassava Clones

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ABSTRACT

This study determined how arbuscular-mycorrhizal (AM) colonization by *Glomus intraradices* affected plant biomass and salt tolerance (in terms of growth) of three cassava clones (SOM-1, 05, and 50). Survival, root, stem and leaf production, and nutrient accumulation were determined in AM -inoculated and non-inoculated cassava clones under a range of sodium chloride (NaCl) levels (0, 68.4, or 136.8 mM) in the medium. The AM colonization stimulated plant growth and increased survival at 136.8 mM of salt. Clone SOM-1 showed to be the most salt tolerant of the three clones tested. *G. intraradices*-inoculation was important not only for growth promotion, but also played a crucial role in protecting cassava clones against salt (particularly the most salt sensitive clones). Mycorrhizal clones growing under 136.8 mM of NaCl showed greater dry weight than non-mycorrhizal clones growing without salt. Results show that AM-colonization provides a biological mechanism by which cassava clones increased plant biomass and salt tolerance being required for the best cassava clone development under non-stress and stress conditions.

Keywords: cassava, *Glomus intraradices*, mycorrhiza, salt stress

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important food crop for over 500 million people in some 60 subtropical and tropical countries, generally underdeveloped. Its radical system forms tubers very rich in digestible starch

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and vitamin (C). It is a rustic crop, well adapted to poor soils, but osmotic stresses such as drought or salinity are limiting factors for its cultivation. The need for cassava as a food requires new cultivation soils but some of these are saline. Then, it is a major goal to have cassava plants more tolerant to salt. In sustainable agriculture, solutions to salinity-alkalinity problems should include both plant breeding for salt tolerance and the application of biological factors such as mycorrhizal fungi. In this respect, it is very important to study the effectiveness of arbuscular mycorrhizal fungi (AM) under saline-alkaline soils and their effects on host plants.

Arbuscular mycorrhizal (AM) symbiosis normally increases plant growth, especially under poor fertility conditions (Azcón and Barea, 1997). This plant growth improvement favors the adaptation of the plant affected by biotic and/or abiotic stress conditions (Davies et al., 1992; Barea et al., 2002). Mycorrhizal colonization may be a key factor for plant survival in stressed environments by enhancing stress tolerance and by improving essential nutrients uptake (Jeffries et al., 2003).

Salinity negatively affects the energetic, hydric, and nutritional equilibria of the plant (Pasternak, 1987). Munns (1993) indicated that plant growth in saline medium is affected first by an osmotic stress and then by toxic and nutritive stresses. Mycorrhizal colonization is able to compensate such disequilibria (Ruiz-Lozano, 2003; Marulanda et al., 2006). The plant water content improvement due to mycorrhization seems to be related in part, to a better nutritional status. Mycorrhizal colonization is involved in nutrients uptake such as nitrogen (N) (Tobar et al., 1994a; b; Azcón et al., 1996), potassium (K) (Vivas et al., 2003a; b), phosphorus (P) (Azcón and Barea, 1997), copper (Cu), and zinc (Zn) (Díaz et al., 1996; Smith and Read, 1997; González-Guerrero et al., 2005). However, AM colonization may also increase resistance of the host plant to abiotic stresses (as drought, salinity or heavy metals) by other mechanisms (Ruiz-Lozano et al., 1995a; b; Azcón and El-Atrash, 1997; Vivas et al., 2003a; b). Decreased chloride (Cl)–ions accumulation, (Pfeiffer and Bloss, 1988), control of proline synthesis (Ruiz-Lozano et al., 1996), and a decline in antioxidant compounds (glutathione, ascorbate) accumulation (Marulanda et al., 2006) have also been related to the effects of mycorrhiza on plant salt tolerance.

According to Clark and Zeto (2000), the reasons for this plant-fungus behavior are a greater colonization of soil volume, greater absorbing surface, and the excretion of some chemical compounds.

Although the symbiotic association between AM fungi and their host plant is considered as non specific, studies confirmed the differences within plant and/or AM fungus species and even within geography or ecotype isolates (Monzón and Azcón, 1996; Smith and Smith, 1997; Bago et al., 1998). Mycorrhizal plants could be affected by the salt more or less severely according to the intensity of the stress, plant tolerance and AM fungal adaptation (Ruiz-Lozano and Azcón, 2000). Differences in the characteristics and/or

effect of particular interactions need to be studied in order to select those having the highest effectiveness (Douds and Millner, 1999). But ecophysiological plasticities of plant/fungus interaction under saline stress conditions are not well understood yet and require further study. The potential contribution of AM fungus to protect colonized cassava clones against salinity levels is an interesting topic to be studied.

The objective of this work was to determine the effectiveness of mycorrhizal symbiotic (in terms of survival, and root, stem and leaf growth, and water content) of three cassava clones (SOM-1, 05, or 50) in greenhouse conditions. It was evaluated how the mycorrhizal colonization by *Glomus intraradices* affected the salt tolerance of these clones (in terms of plant growth) when they were cultivated within a range of NaCl levels (0, 68.4, or 136.8 mM) applied to the growing medium. Water content, sodium and chloride accumulation in the plant were used as possible mechanisms involved in the protection of AM colonization from moderate (68.4 mM of NaCl) to severe (136.8 mM of NaCl) salt application.

MATERIALS AND METHODS

In vitro plants of the cassava clones SOM-1, from Somalia (sent by Prof. P. Fiorino of the Univ. of Florence, Italy), 05 (with identification no. 7902), and 50 (identification "Bonova rouge"), both from Ivory Coast (IRAT Mouaké), and sent by L'ORSTOM of Montpellier (France), were used as plant material. These plants were subcultured in our laboratory to reach the necessary number for the different experiments carried out.

For this experiment, 96 in vitro plants of each clone were transplanted to 300 ml pots (Cantos et al., 1993) filled with a steamy sterilized sandy soil. Physical and chemical characteristics of the soil were determined by standard soil analysis methods. It was air dried, ground, and sieved trough 2 mm mesh and the following parameters 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), N (Kjeldahl method), extractable P (Olsen method), available K (flame photometry), available calcium (Ca) and magnesium (Mg), and manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) (plasma emission techniques) (Table 1).

Soil was sterilized by steaming for three sterilization cycles (100°C for 1h in 3 consecutive days) and after the first adaptation, the surviving plants of each clone were divided into two groups and transplanted (second adaptation) to 2000 mL pots filled with 1200 g of the same sterilized soil. One group was inoculated with arbuscular mycorrhiza *Glomus intraradices* fungus (isolate 11AG8903). Mycorrhizal inoculum was multiplied in an open pot culture of *Allium cepa* and after six months of plant growth the shoots were eliminated

Table 1
Physicals and Chemicals characteristics of experimental soil

Texture	Sand	75.8	%
	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 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.

During this second adaptation period, the plants were irrigated with 20% Hoagland solution and after 15 d each clone (mycorrhizal and non mycorrhizal plants) was divided into three groups: i) control (which continued to be irrigated with 20% Hoagland solution (0.46 dSm^{-1}); ii) irrigated with 20% Hoagland solution plus 68.4 mM of NaCl (7.51 dSm^{-1}); and iii) irrigated with 20% Hoagland solution plus 136.8 mM of NaCl (14.4 dSm^{-1}). The addition of NaCl was gradual, beginning with 34.2 mM, and then increasing in 34.2 mM every two days until reaching the desired 68.4 or 136.8 mM. These final concentrations were maintained until the end of the experiment (50 d). The temperature of the greenhouse was maintained over 20°C with an 18/6 h light/dark

Table 2
Influence of the NaCl treatments on the salinization of the nutritive solution and the substratum used as culture medium

	NaCl (mM)		
	0	68.4	136.8
Solution εC (dSm^{-1})	0.46	7.51	14.4
Solution SAR	0	62	124
Substratum εC (dSm^{-1})	2.3	10.5	17.5
Substratum SAR	1.3	12.1	16.4
Substratum OP (atm.)	−0.83	−3.8	−6.1

period, photosynthetic photon flux (PPF) ca. 500 μmol m^{-1} s^{-1} throughout the experiment. Humidity ranged 60–90%.

In this experiment, in each cassava clone shoot and root fresh and dry biomass was measured in AM inoculated and non-inoculated plants growing at the three saline levels. Additionally, nutrient concentration of root, stem, and leaf were analyzed. Determination of N was performed by Kjeldahl method, P and Cl by colorimetric methods, and K, Na, Ca, Mg, Fe, Cu, Mn, and Zn by plasma emission techniques (Hamilton et al., 1980).

In this experiment the mycorrhizal presence in AM-inoculated root was checked. Roots from each non-mycorrhized and mycorrhized plants were treated in 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, smashed, and observed directly under the microscope.

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

RESULTS

Some physical and chemical characteristics of the substratum used are shown in Table 1 and the influence of the NaCl treatments on the salinization of the nutritive solution and the substratum are indicated in Table 2.

No losses of cassava plants, mycorrhized or not, were recorded in the control or in the 68.4 mM salt treatments, so no differences could be established between clones or AM-colonization. On the contrary, plant losses occurred with the applications of 136.8 mM of NaCl (Table 3). When non mycorrhizal, the cassava plants could not resist the 50 d of treatment with 136.8 mM of NaCl. In these conditions, clone SOM-1 showed more tolerance to salt applications presenting a 30.7% of survival after 45 d of culture while no plants of the other two clones could survive beyond 35 d.

Table 3
Influence of mycorrhizal-colonization (Myc^+) on the survival percentage of cassava clones along the culture in medium supplied with 136.8 mM of NaCl

Clone		Days after 136.8 mM NaCl application					
		0	30	35	40	45	50
SOM-1	Myc^-	100 Aa	100 Aa	76.9 Aab	46.1 Bb	30.7 Bb	0
	Myc^+	100 Aa	100 Aa	100 Aa	36.7 Bb	35.7 Bb	35.7 Bb
05	Myc^-	100 Aa	85.7 Aa	21.4 Bb	0	0	0
	Myc^+	100 Aa	100 Aa	92.3 Aa	23 Bb	0	0
50	Myc^-	100 Aa	100 Aa	25 Bb	0	0	0
	Myc^+	100 Aa	100 Aa	91.6 Aa	25 Bb	0	0

Student's t-test. Different letters mean significant differences $P \leq 0.05$ (Capital letters between mycorrhizal or non-mycorrhizal treatments, small letters between clones).

The AM-colonization improved survival of all the clones. Clone SOM-1 was again the most tolerant with a 35.7% survival at the end of the experiment (50 d), while the other two clones presented more than 90% of survival after 35 d of transplanting and nearly 25% at day 40, although no plants of clones 05 and 50 could survive beyond day 40.

From these results it is possible to establish that SOM-1 was the most salt tolerant clone and that mycorrhizal improved salt tolerance in all the clones.

Biomass production of the surviving plants was affected negatively by the salt treatments which decreased fresh and dry weight of the aerial tissues (Tables 4 and 5). On the contrary, root biomass was not affected by the salt. As in plant survival, mycorrhizal compensated partly the negative effect of the salt applications on biomass production but its effectiveness decreased with the increasing salinity.

Salinity also modified water content of the plants. Therefore, increasing salt applications increased water content of the roots and provoked water losses in the leaves, while stem water content remained unaffected (Table 6). Only clone SOM-1 showed lower reduction in the leaf water content, particularly when mycorrhized.

The shoot-root ratio decreased with NaCl concentration but mycorrhizal contributed to increase this ratio, particularly at the moderate salt level (Table 7).

The presence of NaCl also influenced the nutritive status of the plants. Since no important differences among clones were found, Table 8 shows the nutrient contents at the different salt levels of root, stem and leaf of mycorrhizal and non-mycorrhizal plants as an average of the 3 clones. As expected, Na and Cl contents increased with salt applications but no clear effect of mycorrhizal was observed. Also it was observed that salt treatments increased N contents in roots and stems. In the roots, mycorrhizal plants showed higher N contents than

Table 4
Effect of NaCl level (0, 68.4 or 136.8 mM) and/or mycorrhizal inoculation (Myc^+) on the fresh weight (mg) of three cassava clones

		SOM-1 NaCl mM			05 NaCl mM			50 NaCl mM		
		0	68.4	136.8	0	68.4	136.8	0	68.4	136.8
Root	Myc^-	100	196	380	85	189	176	36	196	172
	Myc^+	155**	194	362	91	52	307	64	205	159
Stem	Myc^-	984	738	773	649	425	359	441	486	333
	Myc^+	1902**	1041*	842	881	843**	578	606	572	358
Leaf	Myc^-	1229	605	438	988	342	166	727	444	121
	Myc^+	3098**	1109*	707	1612*	876*	270	1109	578	252
Plant	Myc^-	2313	1539	1592	1721	956	701	1204	1126	626
	Myc^+	5155**	2344*	1911	2583	1971**	1151*	1775	1294	769

Analysis of variance, F-test. Asterisks indicate significant differences between Myc^- and Myc^+ for each organ and salt treatment. $^*P \leq 0.05$. $^{**}P \leq 0.01$.

non-mycorrhizal. In the leaves, the opposite effect was observed. P contents were not modified by the salt treatments or the AM-inoculation.

Micronutrients (Cu, Fe, Mn, and Zn) accumulated mainly in the roots. Fe showed the highest values in non-mycorrhizal plants irrespectively the salt level in the medium. A decreasing AM effect was also observed with Zn. Manganese concentration in AM plants followed similar decreasing trend but this AM effect was noted for Cu only in roots under salt levels (Table 8).

Table 5
Effect of NaCl levels (0, 68.4, or 136.8 mM) and/or mycorrhizal inoculation (Myc^+) on the dry weight (mg) of three cassava clones

		SOM-1 NaCl mM			05 NaCl mM			50 NaCl mM		
		0	68.4	136.8	0	68.4	136.8	0	68.4	136.8
Root	Myc^-	50	42	69	33	44	21	14	36	29
	Myc^+	85**	43	72	37	41	46**	29*	34	29
Stem	Myc^-	209	126	151	103	79	59	60	77	61
	Myc^+	361**	185	158	157	169**	108*	174	109	63
Leaf	Myc^-	205	132	230	165	85	108	108	95	86
	Myc^+	499**	205	246	248	170**	172	168	110	130
Plant	Myc^-	464	300	450	301	209	187	181	208	175
	Myc^+	946**	433	476	443	381**	326*	371	253	222

Analysis of variance, F-test. Asterisks indicate significant differences between Myc^- and Myc^+ for each organ and salt treatment. $^*P \leq 0.05$. $^{**}P \leq 0.01$.

Table 6
Effect of NaCl levels (0, 68.4, or 136.8 mM) and/or mycorrhizal inoculation (Myc^+) on the water content (%) of three cassava clones

		SOM-1 NaCl mM			05 NaCl mM			50 NaCl mM		
		0	68.4	136.8	0	68.4	136.8	0	68.4	136.8
Root	Myc^-	50	78	79	61	75	89	57	80	80
	Myc^+	45	79	79	56	82	84	50	79	82
Stem	Myc^-	80	83	80	84	82	82	87	82	78
	Myc^+	81	83	81	83	81	82	72	83	83
Leaf	Myc^-	81	68	45	83	64	30	85	69	26
	Myc^+	84	81	54	84	76	30	85	72	32
Plant	Myc^-	80	80	71	82	77	71	86	78	69
	Myc^+	82	82	74	83	80	72	81	80	71

DISCUSSION

No previous information exists about the mycorrhizal effects on cassava clones under saline conditions. AM fungi have been shown to decrease plant field losses in saline soils (Al-Karaki, 2000). Since excess of salinity negatively affect yield, plant salt tolerance is an important concern in arid and semiarid zones. The results presented here demonstrate that cassava clones have different growth potential as well as tolerance/resistance to the levels of NaCl used in this study.

The three cassava clones could tolerate salt levels up to 68.4 mM of NaCl without losses of plants after 50 d of culture in this saline condition. With salt applications of 136.8 mM, the clone SOM-1 showed to be more tolerant than the other two clones, both in mycorrhizal and non-mycorrhizal conditions (Table 3). The AM inoculation helped to overcome salt stress allowing a 35.7% of the SOM-1 plants to survive at the end of the experiment. The other two clones (05 and 50) also improved survival at 136.8 mM of NaCl compared to

Table 7
Effect of NaCl levels (0, 68.4, or 136.8 mM) and/or mycorrhizal inoculation (Myc^+) on the shoot/root dry weight of three cassava clones

	SOM-1 NaCl mM			05 NaCl mM			50 NaCl mM		
	0	68.4	136.8	0	68.4	136.8	0	68.4	136.8
Myc^-	8.3	6.1	5.5	8.1	3.75	7.9	11.9	4.8	5.0
Myc^+	10.1	9.1	5.6	11.0	8.3	6.1	11.8	6.44	6.6

Table 8
Effect of NaCl levels (0, 68.4, or 136.8 mM) and/or mycorrhizal inoculation (Myc^+) on the nutrient contents of three cassava clones

NaCl (mM)		% d.w.							ppm			
		N	P	K	Ca	Mg	Na	Cl	Cu	Fe	Mn	Zn
					ROOT							
0	Myc^-	0.92	0.17	0.41	2.20	0.42	0.35	0.47	28	4889	502	175
	Myc^+	1.95	0.16	0.32	2.20	0.41	0.21	0.27	33	1762	318	73
68.4	Myc^-	1.52	0.17	0.66	2.30	0.53	1.44	2.50	56	5022	605	203
	Myc^+	2.71	0.17	0.61	2.30	0.51	1.70	1.80	45	2657	311	104
136.8	Myc^-	1.72	0.23	1.25	1.70	0.60	1.96	2.86	55	4410	741	221
	Myc^+	3.55	0.16	1.26	1.70	0.70	3.37	4.31	35	2097	567	111
					STEM							
0	Myc^-	1.86	0.08	2.13	1.40	0.18	0.24	0.62	15	146	81	80
	Myc^+	1.75	0.08	1.90	1.40	0.21	0.21	0.66	16	132	59	39
68.4	Myc^-	2.27	0.12	1.15	2.10	0.38	3.63	3.82	18	197	99	108
	Myc^+	2.09	0.11	1.35	1.90	0.26	2.60	4.08	15	129	66	39
136.8	Myc^-	2.28	0.15	1.54	2.30	0.40	4.31	5.58	17	342	138	157
	Myc^+	3.30	0.14	1.43	2.50	0.54	4.54	6.51	14	212	75	59
					LEAF							
0	Myc^-	4.81	0.14	1.89	2.10	0.38	0.22	0.30	17	265	154	71
	Myc^+	4.04	0.20	2.06	2.80	0.40	0.21	0.21	28	262	135	63
68.4	Myc^-	4.21	0.14	1.93	3.00	0.47	0.51	3.13	14	295	225	94
	Myc^+	3.68	0.17	1.96	3.30	0.47	0.54	3.38	22	264	202	69
136.8	Myc^-	4.23	0.16	2.22	3.40	0.51	1.30	4.56	15	347	273	119
	Myc^+	3.80	0.16	2.44	3.60	0.49	1.34	5.18	25	267	224	82

non-mycorrhizal plants of those clones, although no plant could survive more than 40 d. Nevertheless, mycorrhizal plants of clones 05 and 50 presented a nearly 92% of survival at day 35, significantly higher than non-mycorrhizal plants.

Mycorrhizal cassava clones exhibited higher biomass production under saline and non-saline soil conditions (Tables 4 and 5). Among the clones used, SOM-1 was the clone with highest growth potential at all culture conditions. Only in the roots this effect was not so clear. Such results are indicative of the practical importance of this symbiosis for cassava growth and development under osmotic stress. Here, mycorrhizal colonization efficiently contracted the osmotic stress caused by 136.8 mM of NaCl. The inhibitory effect of salt on biomass production would indicate the outset of ionic toxicity. According to Niu et al (1997), at the inhibiting NaCl levels water stress cannot be compensated

with ions from the growth medium and plant cells start to use as osmotic cellular compounds causing growth depression.

There were no significant differences in the nutrient contents due to AM inoculation (Table 8). Nevertheless, since mycorrhizal plants presented higher dry weight than non-mycorrhizal, the absolute contents of each nutrient were also higher, indicating that AM colonization improved nutrient uptake by the plants as reported elsewhere (Azcón and Barea, 1997), even under salt stress.

As far as we know, water stress is the primary stress prior to the outset of any ion specific effect. Differences in the salt tolerance response to severe salinity (136.8 mM) of the cassava clones may have been due to its particular sensitivity to water limitation. The most resistant clone (SOM-1) showed less reduction in leaf water content than the other two clones, both in mycorrhizal and non-mycorrhizal situation (Table 6).

Sodium and Cl contents increased with the salt treatments although no differences were found between mycorrhizal and non mycorrhizal plants.

Mycorrhizal was efficient in promoting aerial growth as can be seen from the shoot/root ratio observed (Table 7) which decreased as a result of increasing salinity of the medium. The promotion of aerial growth was more important at the moderate salt level.

Clone SOM-1 resulted the most salt tolerant clone and this natural salt tolerance could be related to the origin of this clone (coming from an arid, saline desert zone in Somalia) while clones 05 and 50 are from a rainy area in Ivory Coast. As this clone showed no differences in Na and Cl contents in respect to the other clones it seems that SOM-1, together with less leaf water content reduction, had better resistance to those accumulations. Nevertheless, *G. intraradices* improved salt tolerance and promoted the plant development in all the clones at whatever salinity level.

Such fungal behavior makes *G. intraradices* an appropriated fungus to be used for inoculation of cassava clones under osmotic stress conditions.

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