THE EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION ON *EUTERPE OLERACEA* MART. (AÇAÍ) SEEDLINGS[1]

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ABSTRACT - With the objective of verifying the response of *Euterpe oleracea* seedlings to seven arbuscular mycorrhizal fungi species, an experimental trial was carried out under greenhouse conditions. Seeds of *E. oleracea* were sown in carbonized rice husk. Germinating seeds were initially transferred to plastic cups, containing fumigated Reddish Yellow Quartz Sand and inoculated with arbuscular mycorrhizal fungi. Two months later, seedlings were transferred to 2 kg black plastic bags, containing the same soil without fumigation. Plant growth and mineral nutrients were evaluated nine months after mycorrhizal inoculation. Differential effects were observed among the species tested, with *Scutellispora gilmorei* being the most effective ones in promoting growth and nutrient content of *E. oleracea* seedlings. The increment resulted from inoculation with *S. gilmorei* were 92% in total plant height, 116% in stem diameter, 361% in dry matter production, 191% in N, 664% in P, 46% in K, 562% in Ca, 363% in Mg and 350% in Zn contents, comparing to uninoculated controls. Infected root length was positively correlated to nutrient content and plant growth. It was concluded that growth and nutrient uptake of *E. oleracea* seedlings could be significantly improved by inoculation of effective arbuscular mycorrhizal fungi.

Index terms: plant growth, nutrient content, infected root length.

EFEITOS DA INOCULAÇÃO DE FUNGOS MICORRÍZICOS ARBUSCULARES EM MUDAS DE *EUTERPE OLERACEA* MART. (AÇAÍ)

RESUMO - Com objetivo de verificar a resposta das plântulas de *Euterpe oleracea* Mart. à inoculação de sete espécies de fungos micorrízicos arbusculares, foi realizado um experimento em casa de vegetação. Sementes de *E. oleracea* germinadas em casca de arroz carbonizada foram inicialmente transferidas para copos de plástico contendo Areia Quartzosa Vermelho-Amarela fumigada, e nelas inoculados fungos micorrízicos arbusculares. Dois meses depois, as plântulas foram repassadas para sacos de plástico preto contendo o mesmo solo, sem fumigação. Foram avaliados o crescimento e a nutrição mineral das plantas nove meses após a inoculação. Efeitos diferenciados foram observados entre as sete espécies testadas. *Scutellispora gilmorei* foi a espécie mais efetiva em promover o crescimento das plantas de *E. oleracea* e a absorção de nutrientes por elas. O incremento resultante da inoculação com *S. gilmorei* foi de 92% em altura total da planta, 116% em diâmetro do caule, 361% em produção de matéria seca, 191% em N, 664% em P, 46% em K, 562% em Ca, 363% em Mg e 350% em Zn absorvidos, em comparação com o controle sem inoculação. Houve correlações positivas entre comprimento de raiz infectada, crescimento da planta e nutrientes absorvidos. Os resultados permitiram concluir que plantas de *E. oleracea* quando inoculada com espécie eficiente de fungo micorrízico arbuscular pode ter um aumento significativo no crescimento e absorção de nutrientes.

Termos para indexação: crescimento da planta, nutriente absorvido, comprimento da raiz infectada.

INTRODUCTION

Euterpe oleracea is a native palm tree of Amazon, which grows both in periodically flooded humid area and in dry land. Juice of its fruit is a very

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popular aliment for local people and its palm heart is used for palmetto industries. Due to the promising perspective of the internal and external markets of palmetto, producers are interested in commercial plantation of this palm tree (Nogueira et al., 1995). Although *E. oleracea* is a rustic plant, slow inicial growth and considerable mortality of seedlings have been the main problems to its successful establishment in the field (Bovi et al., 1987).

Benefits from mycorrhizal inoculation were observed in many tree species, with remarks on plant growth, nutrient uptake and seedling´s survival rate (Janos, 1980). Little is known about the mycorrhizal inoculation effects on *E. oleracea*.

The objective of this study was to investigate the response of *E. oleracea* seedlings to inoculation of seven arbuscular mycorrhizal fungi species under greenhouse conditions.

MATERIAL AND METHODS

The experiment was carried out in the research center of Embrapa-CPATU in Belém, State of Pará, Brazil. Seeds of *E. oleracea* were sown in carbonized rice husk. Right after germination, seedlings were transfered to the plastic cups, containing 500 mL of fumigated Reddish Yellow Quartz Sand which showed the following chemical characteristics: pH in water = 5.1; Al = 0.3 $mmol_c/kg$; Ca+Mg = 1.5 $mmol_c/kg$; Ca = 0.9 $mmol_c/kg$; K= 31 mg/kg and P = 7 mg/kg, to ensure the multiplication of the introduced arbuscular mycorrhizal fungi (AMF). The species of AMF used in this study are presented in Table 1. The AMF were previously multiplied in culture pots, containing *Brachiaria decumbens* Stapf as host plant. Inoculation was done by deposition of 30 g of soil inoculum, a mixture of soil + 300-400 spores of AMF + root segments of *Brachiaria decumbens*, right underneath the germinating seeds whereas for the uninoculated controls, the same amount of fumigated soil was used instead. Ten mL of a microbial suspension, which was prepared by filtering 5x of the mixed soil inoculum, were added to all plastic cups. Two months later, all the seedlings were transfered once again to black plastic bags, containing 2 kg of the same soil without fumigation. Throughout the experiment, all the seedlings had received four applications of 10 mL of a complete nutrient solution per bag (Bolly-Jones, 1956). The experimental design was completely randomized with ten repetitions per treatment and one plant per replicate. Nine months after the inoculation with AMF, all the seedlings were evaluated for plant growth, nutrient concentration and content, percentages of infected root length, growth and nutrient increment. At harvest, total plant height (leaf + stem) and stem diameter (at soil level) were taken. The aerial part of the plant was separated from the root at soil level and washed with distilled water. Root system was carefully removed from the soil and washed thoroughly with tap water. A part of the roots was cleared and stained (Phillips & Hayman, 1970) and the percentage of root length colonized by AMF was determined by microscopic observation of 25 root segments of 1cm long per plant (Giovannetti & Mosse, 1980). The shoot and the rest of the root system were then dried at 70^0C until reach the constant dry weight. Nutrient concentrations and contents were determined on dried shoot. Percentages of growth and nutrient increment were calculated by: (value of inoculated plant - value of uninoculated plant)/value of uninoculated plant x 100.

TABLE 1. Tested arbuscular mycorrhizal fungi and their origin.

Arbuscular mycorrhizal fungi	Origin
Scutellispora gilmorei (Walker & Sanders)	Isolated from rhizosphere of Brazilian nut seedling
Acaulospora sp.	Isolated from rhizosphere of black pepper
Gigaspora margarita (Backer & Hall)	Instituto Agronômico de Campinas
Entrophospora colombiana (Spain & Schenck)	Centro International de Agronomia Tropical
Scutellispora heterogama (Walker & Sanders)	Instituto Agronômico de Campinas
Gigaspora sp.	Isolated from rhizosphere of oil palm
Scutellispora sp.	Isolated from rhizosphere of oil palm

Analysis of variance was performed on all data and all treatment means were further separated by Tukey´s test for significant differences at P< 0.05.

RESULTS AND DISCUSSION

Differential effects were observed among the species tested (Table 2). The AMF species, *Scutellispora gilmorei*, *Acaulospora* sp., *Gigaspora margarita* and *Entrophospora colombiana* increased significantly total plant height, stem diameter and dry matter production of *Euterpe oleracea* seedlings in comparison with uninoculated controls. *Scutellispora heterogama*, *Gigaspora* sp., and *Scutellispora* sp. showed no effects on plant growth of *E. oleracea* seedlings. Different growth responses to several tropical AMF species inoculation were also reported on cassava (Sieverding & Howeler, 1985), maize (Diederichs, 1991), urucu (Chu & Kato, 1992) and rubber tree (Ikram et al., 1993). The percentages of root length infected by effective species of AMF were much higher than those of uneffective ones, so did the number of spores recovered from the soil at the end of the experiment. Since the seedlings had been in not fumigated soil for seven months, it was expected to find infected root segments and spores of indigenous AMF from the uninoculated controls, but their effectivenesses were much lower than those introduced effective ones. The same result was observed on eight months old *Bactris gasipaes* seedlings, where the percentages of root colonization were 53% and 13% for the inoculated and uninoculated ones, respectively (Sudo et al., 1996).

S. gilmorei, *Acaulospora* sp. and *G. margarita* increased significantly both P and Ca concentrations and contents of the shoot (Table 3). According to Bovi et al. (1987), application of 1g of single superphosphate per kilogram of soil increased growth of *E. oleracea* seedling in low P content soil. Thus, the increased absorption of P due to mycorrhizal inoculation could certainly contribute to the growth response of *E. oleracea* seedlings. The increased Ca content could be an indirect effect of reduced P concentration in the soil solution which favors the equilibrium of exchangeable and solution form in the direction of solution form (Samuel et al., 1993). Since Ca is a polyphosphate granule constituent in

TABLE 2. Plant height, stem diameter, dry matter production, percentage of infected root length (IRL) of *Euterpe oleracea* seedlings and number of spores of arbuscular mycorrhizal fungi (AMF) recovered from 50 g of soil, nine months after mycorrhizal inoculation[1].

AMF	Plant height	Stem diameter	Dry matter		IRL	Spores
			Aerial part	Root		
	(cm/plant)	(cm/plant)	(g/plant)		(nº%)	(nº/50 g soil)
S. gilmorei	78.86a	1.58a	11.93a	5.17a	50.29a	173.5
Acaulospora sp.	69.86ab	1.47ab	10.34ab	5.86a	35.73b	898.5
G. margarita	64.79b	1.26b	7.88abc	4.59a	18.86c	111.5
E. colombiana	61.93b	1.23b	7.49abc	4.14ab	7.31d	101.5
S. heterogama	47.86c	0.81c	3.70cd	1.51bc	6.40d	13.5
Gigaspora sp.	44.93c	0.77c	2.63d	0.94c	2.29d	9.5
Scutellospora sp.	40.57c	0.72c	2.35d	0.89c	0.40d	9.3
Control	41.07c	0.73c	2.67d	1.04c	0.66d	3.0
CV (%)	14.47	15.21	40.40	53.35	26.42	-

[1] Column means followed by the same letter are not significantly different as determined by Tukey´s test (p=0.05).

TABLE 3. Nutrient concentrations (conc.) and contents (cont.) in dry matter of shoots of *Euterpe oleracea* seedlings, nine months after arbuscular mycorrhizal fungi inoculation[1].

AMF[2]	N		P		K		Ca		Mg		Zn	
	Conc. (mg/g)	Cont. (mg/pl)	Conc. (mg/g)	Cont. (mg/pl)	Conc. (mg/g)	Cont. (mg/pl)	Conc. (mg/g)	Cont. (mg/pl)	Conc. (mg/g)	Cont. mg/pl)	Conc. (μg/g)	Cont. (mg/pl)
Sg	1.10b	128.56a	0.08a	9.86a	0.43b	50.18a	0.59a	71.08a	0.22a	26.31a	38.14b	0.45a
A sp.	1.10b	113.18ab	0.08a	9.81a	0.50b	47.80ab	0.56ab	58.00ab	0.26a	28.24a	44.29ab	0.46a
Gm	1.00b	85.81abc	0.07ab	5.33b	0.55b	37.80bc	0.56ab	43.74b	0.31a	25.26a	57.00a	0.43a
Ec	1.18b	78.31bcd	0.06bc	4.57cb	0.74b	47.42ab	0.52abc	38.95b	0.22a	17.30ab	38.71b	0.29ab
Sh	1.09b	46.37cd	0.05c	1.98cb	1.13a	40.21abc	0.38d	14.87c	0.21a	7.95b	44.14ab	0.17b
G sp.	1.64a	41.33d	0.05c	1.26c	1.27a	32.70c	0.40cd	11.22c	0.20a	5.20b	37.86b	0.10b
S sp.	1.65a	38.72d	0.06bc	1.33c	1.29a	30.17c	0.45bcd	10.44c	0.20a	5.12b	41.14b	0.10b
Control	1.68a	44.40cd	0.05c	1.29c	1.32a	34.21c	0.40cd	10.73c	0.21a	5.68b	37.71b	0.10b
CV (%)	15.28	35.28	15.18	49.79	21.45	16.25	15.85	43.26	28.07	58.69	19.92	49.01

[1] Column means followed by the same letter are not significantly different as determined by Tukey´s test (p=0.05).

[2] Sg=*Scutellispora gilmorei*; A=*Acaulospora* sp.; Gm=*Gigaspora margarita*; Ec=*Entrophospora colombiana*; Sh=*Scutellispora heterogama*; G=*Gigaspora* sp.; S=*Scutellispora* sp.; Control=uninoculated control.

hyphae (Vander Zaag et al., 1979) which can function in the transference of P from hyphae to plant by stimulating the production of alkaline phosphatase the increased uptake of Ca can indirectly contribute to the growth response of *E. oleracea* seedlings. Better growth response due to significant increase in uptake of P and Ca was also observed in the vegetatively propagated *Theobroma cacao* L. (Chulan & Martin, 1992). N and K concentrations were reduced in these treatments. The reduction of N concentration apparently results from the dilution effect found in a larger plant, since the N content of these treatments was superior to the uninoculated controls (Jarrel & Beverly, 1981). The reduced K concentration could be attributed to the significant increase in Ca concentration. Both Ca and Mg compete with K for entry into plants. When the absorption of either one or both increases, uptake of K reduces (Samuel et al., 1993). Thus, the availability of K is more dependent on its relative concentration to that of Ca and Mg than on the total quantity of K present in the soil. No difference was observed in Mg concentration among treatments and the highest Zn concentration was found in seedlings inoculated with *G. margarita*. However, Mg and Zn contents were significantly increased by inoculation with *S. gilmorei, Acauloapora* sp. or *G. margarita*.

According to the results (Tables 2 and 3), the effectiveness that was determined by growth and nutrient increments of seven species of AMF tested can be arranged in the following order: *S. gilmorei* > *Acaulospora* sp. >*G. margarita* > *E. colombiana* > *S. heterogama* > *Gigaspora* sp. > *Scutellospora* sp. The increments resulted from inoculation with *S. gilmorei* were 92% in plant height, 116% in stem diameter, 361% in dry matter production, 191% in N content, 664% in P content, 46% in K content, 562% in Ca content, 363% in Mg content and 350% in Zn content. The species of *S. gilmorei*, *Acaulospora* sp. and *Gigaspora* sp. had been tested on hybrid seedlings of oil palm (Dura x Pisifera). The greatest increments of 47% in plant height and 133% in leaf area were found in six months old seedlings inoculated with *S. gilmorei* while the greatest increase of 124% in dry matter production resulted

from the inoculation of *Gigaspora* sp. Both *Gigaspora* sp. and *Acaulospora* sp. increased P content of seedlings by 369% and 351%, respectively (Chu, 1997). Significant growth increment was also observed in eight months old hybrid seedlings of palm (*E. oleracea* x *E. edulis)* inoculated with *Glomus clarum* or with *Acaulospora scrobiculata* (Sudo et al., 1996). Inoculation with mixed inoculum of *Glomus clarum*, *Glomus etunicatum* and *G. margarita* increased 45% in plant height, 22% in stem diameter, 126% in dry matter production, 180% in leaf area, 70% in P content, 80% in K content and 159% in N content of *B. gasipaes* seedling (Sudo et al., 1996). Since mycorrhizal infection is universal in plant kingdom (Gerdemann, 1968), the effect of inoculation certainly depends on the right host-AMF combination, the native microbial population and the soil conditions.

The genetic heterogeneity makes *Euterpe* a genus difficult to work with, due to the large variation in growth response within replicates. A large number of plants are usually recommended to be used in the field experiment to diminish the heterogeneity effect (Bovi et al., 1987). Although more than 40% of the variation coefficient was found in dry matter production and nutrient content under this greenhouse trial, significant differences between treatments had been obtained.

Significant positive correlation were found between growth response and percentage of infected root length; growth response and nutrient content; nutrient content and percentage of infected root length. N and K concentrations were negatively correlated with growth response and percentage of infected root length (Table 4). Significant negative correlation between dry matter of aerial part and K concentration was also observed in coffee seedling inoculated with AMF (Saggin-Júnior et al., 1994).

The results show that it is possible to obtain the optimum size of *E. oleracea* seedling (50-100 cm) for better survival rate in a shorter period of time, inoculating with effective mycorrhizal fungi. Still the effectiveness of *S. gilmorei*, *Acaulospora* sp. and *Gigaspora* sp. needs to be tested under field conditions.

TABLE 4. Linear correlation among plant (P.) height, stem (S.) diameter, dry matter of aerial part (DMAP), nutrient concentration and content and percentage of infected root length (IRL) of *Euterpe oleracea* seedlings in soil inoculated with different arbuscular mycorrhizal fungi[1].

Variables	P. height	S. diameter	DMAP	N conc.	N cont.	P conc.	P cont.	K conc.	K cont.	Ca conc.	Ca cont.	Mg cont.	Zn cont.	IRL
P. height		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
S. diameter	0.94		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DMAP	0.95	0.93		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N conc.	-0.65	-0.62	-0.63		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N cont.	0.90	0.94	0.96	-0.46		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P conc.	0.63	0.72	0.65	-0.48	0.62		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P cont.	0.88	0.89	0.93	-0.57	0.90	0.76		0.000	0.000	0.000	0.000	0.000	0.000	0.000
K conc.	-0.92	-0.91	-0.92	0.67	-0.88	-0.60	-0.85		0.000	0.000	0.000	0.000	0.000	0.000
K cont.	0.66	0.65	0.70	-0.59	0.68	0.48	0.66	-0.61		0.000	0.000	0.000	0.000	0.002
Ca conc.	0.65	0.71	0.62	-0.52	0.55	0.60	0.64	-0.66	0.49		0.000	0.000	0.000	0.000
Ca cont.	0.95	0.94	0.98	-0.63	0.93	0.68	0.94	-0.91	0.67	0.73		0.000	0.000	0.000
Mg cont.	0.87	0.87	0.92	-0.56	0.90	0.57	0.86	-0.88	0.56	0.56	0.89		0.000	0.000
Zn cont.	0.89	0.86	0.91	-0.64	0.85	0.64	0.85	-0.88	0.62	0.58	0.89	0.90		0.000
IRL	0.79	0.76	0.73	-0.50	0.69	0.60	0.69	-0.75	0.40	0.61	0.77	0.70	0.72	

[1] The correlations were estimated by 80 observations; the probabilities of correlation obtained by Student t test are above the diagonal and the correlation coefficients (r) are under the diagonal.

CONCLUSIONS

1. *Euterpe oleracea* is a mycorrhizal dependent plant and the inoculation of effective arbuscular mycorrhizal fungi could increase significantly its growth and nutrient uptake.

2. *Scutellispora gilmorei* and *Acaulospora* sp. are the promising arbuscular mycorrhiza species to be used in the seedling formation of *Euterpe oleracea* under greenhouse conditions.

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