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01-144- *IN VITRO* CULTURE OF CUPUASSU ZYGOTIC EMBRYOS (*Theobroma grandiflorum* (Willd. ex Spreng.) Schum.).

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ABSTRACT

The increase of the economic potential of native fruitful species of the Amazon has been growing in the last years. Among the several species stands out the cupuassu (*Theobroma grandiflorum* (Willd.ex Spreng.) Schum.), tends in view the several possibilities of commercial exploitation. The several techniques of tissue cultures, if integrated in programs of genetic improvement, they become a valuable instrument for the fast cloning of genotypes. This work had as objective to establish protocol for the *in vitro* production of seedlings starting from the conversion of cupuassu zygotic embryos. The research experiments were conducted in laboratory; mature fruits were collected from of cupuassu plants at Embrapa Amazônia Oriental, Belém-PA, Brazil. The mature zygotic embryos were excised from mature seeds surface-disinfected in ethanol at 70% for two minutes followed by immersion in solution of sodium hypochloride at 2% for 20 minutes. The mature embryo zygotics were excised under aseptic conditions and cultivated in two substrates: agar and vermiculite, in the absence and presence of MS e ½ MS medium. After the fourth week of inoculation, it was verified the growth of the epicotyl axis and the first pair's of leaves formation. The substrate agar at 0,6% promotes larger percentage of conversion of embryos (100%) when compared with the vermiculite (83.33%). The seedlings in agar, in the absence of medium of culture, present larger growth of the aerial part (13.56 cm), and the addition of ½ MS and MS medium don't provide an increase in the length of the aerial part in both substrates. The best acting of the seedlings cultivated in agar at 0.6% in the absence of culture medium is well evidenced, however the vermiculite also show to be a substratum adapted besides presenting a low cost.

INTRODUCTION

The increase of the economic potential of native fruitful species of the Amazon has been growing in the last years. Among the several species stands out the cupuassu (*Theobroma grandiflorum* (Willd.ex Spreng.) Schum.), tends in view the several possibilities of commercial exploitation. The largest potential of exploration of the culture is the pulp for the production of ice creams, sweet, jellies, nectar, liqueurs, stewed fruits, sweet, cakes, cookies, yogurt and juices. Of the seeds it can be obtained the chocolate and also to extract a fat similar to the cocoa butter (Venturieri, 1993). The importance of the culture of embryos gave him due to discovery that could be cultivated separately of the maternal tissue and of reservation, when aseptic and nutritional conditions were supplied. Embryos have been used as explants for the clone propagation and organogenesis (Hu and Ferreira, 1998). *In vitro* embryo culture may provide a means to select genotypes with desired characteristics in a short period of time (Llano-Agudelo et al., 1995). This work had as objective to establish protocol for the *in vitro* production of seedlings starting from the conversion of cupuassu zygotic embryos.

MATERIAL AND METHODS

Mature fruits were collected from of cupuassu plants at Embrapa Amazônia Oriental, Belém-PA, Brazil. The mature zygotic embryos were excised from mature seeds surface-disinfected in 70% ethanol for two min followed by immersion in solution of 2% sodium hypochloride for 20 min and rinsed in sterilized distilled water. The mature embryo zygotics were excised and aseptically and cultivated in two conditions: solid medium (6 g.l⁻¹ agar) and vermiculite, in the absence and presence of 30 ml MS medium (Murashige and Skoog, 1962) and 30 ml of a half MS medium, supplemented with 30 g.l⁻¹ sucrose. The MS medium was adjusted to pH 5.8 and all the treatments were submitted the sterilization in autoclave for 120°C for 15 min. Cultures were incubated at 26 ± 2°C, for a light/dark cycle, 16/8 hr, photonic flux ca. 52 µmol.m⁻².s⁻¹. At the end of 45d of culture, the following parameters were evaluated: percentage of germination, percentage of abnormal plantlets, fresh weighs the aerial biomass (g), the roots (g) and the cotyledons (g) and length the stem (cm). The results were analyzed as a randomized design, in a 2 x 3 factorial scheme, with five replications.

Each experimental unit was constituted of 10 flasks, contains a zygotic embryo each. The Tukey test with a significance level of 5% was used for statistical comparisons.

RESULTS AND DISCUSSION

After the fourth week of inoculation, it was verified the growth of the epicotyl axis and the first pair's of leaves formation. There was significant effect of the interaction "substratum x culture medium" for the fresh weights the aerial biomass and the cotyledons. Significant interaction was not detected for the percentage of germination, fresh weights the roots and length the stem. However there was significant effect of the substratum for the percentage of germination, and the substratum and culture medium for the fresh weight the cotyledons and length of the stem. The solid medium (6 g.l⁻¹ agar) promotes larger percentage of conversion of embryos (100%) when compared with the vermiculite (83.33%), (Table I). This result not coincides with the results of Lopes (2000), that obtained larger germination the mogno seeds (*Swietenia macrophylla*) in vermiculite. The seedlings in agar, in the absence of culture medium, present larger growth of the stem (13.56 cm), and the addition of ½ MS and MS medium don't provide an increase in the length of the stem in both substrates (Table I). The best acting of the seedlings cultivated in agar at 6.0 g/l in the absence of culture medium is well evidenced, however the vermiculite also show to be a substratum adapted besides presenting a low cost.

CONCLUSIONS

- the *in vitro* conversion of cupuassu zygotic embryos, isolated of seeds in complete stadium of physiologic maturation, it originates complete and normal plantlets;
- the solid medium and vermiculite are appropriate for the culture of cupuassu embryos;
- it is not necessary the presence of MS medium for the conversion of mature embryos and for the initial growth of plantlets cultivated *in vitro*.

Table I. Effect of solid medium and vermiculite, in the presence or absence the MS medium, on growth of cupuassu embryos cultured *in vitro*.

	% germination			Average
	absence MS	½ MS	MS	
Solid medium	100.0	100.0	100.0	100.00a
Vermiculite	90.0	90.0	70.0	83.33b
Average	95.0	95.0	85.0	
	Fresh weight – aerial biomass (g)			Average
	absence MS	½ MS	MS	
Solid medium	1.31Ab	1.58Aa	1.20Aa	1.37
Vermiculite	1.90Aa	0.93Bb	0.96Ba	1.26
Average	1.61A	1.26AB	1.08B	
	Fresh weight- roots (g)			Average
	absence MS	½ MS	MS	
Solid medium	1.14	1.25	1.10	1.33
Vermiculite	1.58	1.13	1.30	1.17
Average	1.36	1.19	1.20	
	Fresh weight- cotyledons (g)			Average
	absence MS	½ MS	MS	
Solid medium	7.00	6.15	6.71	6.62a
Vermiculite	6.22	5.07	5.23	5.51b
Average	6.61A	5.61AB	5.97B	
	Length – stem (cm)			Average
	absence MS	½ MS	MS	
Solid medium	14.03	14.45	12.20	13.56a
Vermiculite	14.25	9.70	8.80	10.92b
Average	14.14A	12.08AB	10.50B	

Averages proceeded by lower cases, in the column, and for letters capital, in the line, are not significantly different at the level of 5% for the test of Tukey.

REFERENCES

- HU, C.Y.; FERREIRA, A.G. Cultura de embriões. In: TORRES, A.C.; CALDAS, L.S.; BUSO, J.A. (eds.). **Cultura de tecidos e transformação genética de plantas**. Brasília: EMBRAPA-SPI/ EMBRAPA-CNPq, 1998. v.1, p.371-393.
- LLANO-AGUDELO, B.E.; GONZALEZ-ROSAS, H.; SALAZAR-GARCIA S. *In vitro* culture of mature avocado embryos. **Fruits**, Paris, v.50, n.1, p.59-64, jan-fév. 1995.
- LOPES, S.C. **Micropropagação do mogno** (*Swietenia macrophylla* King). Pelotas: Universidade Federal de Pelotas, 2000. 54p.

MURASHIGE, T.; SKOOG, F. A. revised medium for rapid growth and bioassays with tobacco tissue cultures. **Physiologia Plantarum**, Copenhagen, v.15, n.3, p.473-497, 1962.

VENTURIERI, G.A. **Cupuaçu**: a espécie, sua cultura, usos e processamento. Belém: Clube do Cupu, 1993. 108p.