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Notas Científicas

In vitro maintenance, under slow-growth conditions, of oil palm germplasm obtained by embryo rescue

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Abstract – The objective of this work was to evaluate the in vitro maintenance of oil palm (*Elaeis guineensis* and *E. oleifera*) accessions under slow-growth conditions. Plants produced by embryo rescue were subject to ½MS culture medium supplemented with the carbohydrates sucrose, mannitol, and sorbitol at 1, 2, and 3% under 20 and 25±2ºC. After 12 months, the temperature of 20°C reduced plant growth. Sucrose is the most appropriate carbohydrate for maintaining the quality of the plants, whereas mannitol and sorbitol result in a reduced plant survival.

Index terms: *Elaeis guineensis*, *Elaeis oleifera*, Arecaceae, plant genetic resources.

Manutenção in vitro, sob condições de crescimento lento, de germoplasma de palma de óleo obtido com o resgate de embriões

Resumo – O objetivo deste trabalho foi avaliar a manutenção in vitro de acessos de óleo de palma (*Elaeis guineenses* e *E. oleifera*) sob condições de crescimento lento. Plantas produzidas pelo resgate de embriões foram submetidas ao meio de cultura ½MS, acrescido dos carboidratos sacarose, manitol e sorbitol nas concentrações de 1, 2 e 3%, em 20 e 25±2ºC. Após 12 meses, a temperatura de 20°C retardou o crescimento das plantas. A sacarose é o carboidrato mais adequado para a manutenção da qualidade das plantas, enquanto o manitol e o sorbitol resultam em menor sobrevivência das plantas.

Termos para indexação: *Elaeis guineensis*, *Elaeis oleifera*, Arecaceae, recursos genéticos vegetais.

Plant species of the genus *Elaeis* produce seeds with intermediary characteristics, i.e., that can withstand partial dehydration, but are cold-sensitive and cannot support prolonged exposure under conventional genebank conditions (Ellis et al., 1990, 1991). Therefore, these plants present serious limitations for ex situ germplasm conservation in the long term. The routine form of conservation is maintaining the collections in the field, which generally requires high costs, specialized labor, and extensive areas. Added to this, is the fact that collections maintained in the field are under constant threat of losses or depredation through exposure to biotic and abiotic factors that compromise the safety of accessions conserved in this way (Engelmann, 2011).

As an alternative and auxiliary measure for plant germplasm conservation in the field, biotechnological strategies based on concepts of in vitro culture of plant cells, tissues, and organs have been developed and improved. These techniques, besides acting as auxiliary measures for the conservation of germplasm in the field, have the potential to overcome some limitations associated with conventional methods of conservation ex situ and to facilitate the exchange of pest-free germplasm (Scherwinski-Pereira et al., 2010). In vitro conservation protocols should promote maximum survival, genetic stability, as well as enable the subculture frequencies of the plant material to be extended.

In general, in vitro germplasm banks are maintained under minimal growth conditions, obtained by slowing down the plant metabolism, which is usually achieved by reducing temperature and light intensity, or by modifying the formulations of the culture media, including dilution of mineral elements, reduction of sugar concentrations, changes in the use of growth regulators, or the addition of osmotically active compounds (Lédo et al., 2007; Engelmann, 2011;

Yun-peng et al., 2012; Lédo et al., 2014; Nogueira et al., 2014).

Currently, in vitro technology for the conservation of oil palm germplasm is focused almost exclusively on the technique of cryopreservation of zygotic embryos (Villa et al., 2007), somatic embryos (Konan et al., 2010), and pollen (Tandon et al., 2007). However, despite its relative effectiveness, cryopreservation requires a highly-specialized structure and labor, and it is a technique aimed at long-term conservation (Sánchez-Chiang & Jiménez, 2010). A complementary alternative is in vitro maintenance of whole plants, for the purpose of making the germplasm available at short and medium terms. However, for this technique to be truly effective, it is necessary to adapt the used methodologies, by determining the best formulation of the culture media, the number of samples to be conserved, the frequency of the subcultures, and the optimal temperature and light conditions for the maintenance of in vitro collections (Sánchez-Chiang & Jiménez, 2010; Scherwinski-Pereira et al., 2010). For that reason, studies on in vitro conservation of whole oil palm plants are still incipient.

The objective of this work was to evaluate the in vitro maintenance of oil palm (*Elaeis guineensis* and *Elaeis oleifera*) accessions under slow-growth conditions.

The materials used in the experiments were obtained from the oil palm germplasm bank, of Embrapa Amazônia Ocidental, located in the municipality of Rio Preto da Eva, in the state of Amazonas, Brazil. To determine slow-growth conditions, the effects both of temperature and of carbohydrate types and concentrations were tested. To study the effects of temperature on in vitro conservation, the following genotypes were tested: three of *E. oleifera*, EOD08, EOD09, and EOD15; three of *E. guineensis* var. *dura*, EGD01, EGD05, and EGD11; and three of *E. guineensis* var. *tenera*, EGT03, EGT05, and EGT11 (Table 1).

The plants used in the experiments were regenerated from zigotic embryos, isolated from mature fruits and germinated on half-strength Murashige and Skoog (½MS) medium, supplemented with biotin, pantothenic acid, 3% sucrose, and 0.25% Phytagel (Sigma, St. Louis, MO, USA) (Luis et al., 2010). The in vitro plants were selected, giving preference to those of similar size (10±1 cm) and with equal numbers of leaves and roots (1 to 2 roots and 2 to 3 expanded leaves). After selection, the roots and leaves of the plants were cut to initial average height of approximately 4±1 cm. Then, the plants were inoculated in ½MS medium, supplemented with 3% sucrose and 0.25% Phytagel (Sigma, St. Louis, MO, USA). The experiment was

Table 1. Survival rate, length of the aerial part (LAP), growth rate, and appearance (color) of the leaves of oil palm (*Elaeis* sp.) accessions after 12 months of in vitro storage at 20 and 25ºC temperature[1].

Origin	Accession	Survival rate (%)		Mean	LAP (cm)		Mean	Growth rate (%)		Mean	Appearance[2]		Mean
		20ºC	25ºC		20ºC	25ºC		20ºC	25ºC		20ºC	25ºC	
						Elaeis guineensis var. *tenera*							
Yangambi	EGT03	100.0	100.0		11.8	22.9		134.9	306.7		2.2	1.5	
Pobé	EGT05	100.0	100.0	100.0a	15.5	19.3	18.6a	167.1	215.8	235.1c	1.5	1.7	1.5a
Congo	EGT11	100.0	100.0		21.0	20.9		245.3	340.7		1.2	1.2	
Mean		100.0	100.0		16.1	21.1		182.4	287.7		1.6	1.5	
						Elaeis guineensis var. *dura*							
Bahia	EGD01	100.0	100.0		15.6	21.3		229.9	360.2		1.9	1.6	
Deli – Dabou	EGD05	100.0	100.0	100.0a	18.2	20.2	17.4a	200.3	305.9	249.7b	1.9	1.8	1.9a
Nigeria	EGD11	100.0	100.0		12.7	16.1		169.6	232.3		1.8	2.2	
Mean		100.0	100.0		15.5	19.2		199.9	299.5		1.9	1.9	
						Elaeis oleifera							
Manicoré – Democracia	EOD08	90.0	90.0		13.7	20.5		261.1	375.0		2.0	1.8	
Moura – E. Caburis	EOD09	100.0	100.0	96.7a	19.2	21.8	17.8a	279.3	319.1	322.7a	1.7	1.5	1.7a
Autazes – Nova Esperança	EOD15	100.0	100.0		10.7	20.5		268.2	431.8		1.8	1.5	
Mean		96.7	96.7		14.6	20.9		269.5	375.3		1.8	1.6	
General mean		98.9A	98.9A		15.4B	20.4A		217.3B	320.8A		1.8A	1.7A	

[1]Means followed by equal letters, lowercase in the columns and uppercase in the lines, within each variable, do not differ by the Scott & Knott test, at 5% probability. [2]Scale of leaf appearance: 1, dark-green leaves; 2, light-green leaves; and 3, yellowish leaves with dark spots or completely necrosed leaves.

maintained simultaneously in growth rooms with temperatures of 20 and 25±2ºC, light intensity of 30 µmol m^{-2} s^{-1}, and photoperiod of 12 hours.

To test the effect of different carbohydrates and concentrations, the EOD08 and EGT11 genotypes of the *E. oleifera* and *E. guineensis* oil palms, respectively, were used. Plants were selected as described in the previous experiment. The plants were inoculated in ½MS medium, supplemented with 0.25% Phytagel and with the carbohydrates sucrose, mannitol, and sorbitol (Sigma, St. Louis, MO, USA) at the concentrations of 1, 2, and 3%. The experiment was maintained in a growth room at 25±2ºC, with 30 µmol m^{-2} s^{-1} light intensity and a 12-hour photoperiod.

For both experiments (temperature and carbohydrates), the plants were inoculated in 25x250-mm test tubes containing 15 mL of culture media. The pH was adjusted to 5.7±0.1, and sterilization was performed by autoclaving at 121ºC for 20 min, under 1.2 atm of pressure. The experiment was conducted in a completely randomized design, with ten replicates per treatment; each treatment consisted of a test tube with one plant. The evaluations were performed over 12 months for the following leaf traits: survival rate, length of the aerial part, growth rate, and color (appearance). For the variable appearance of leaves, the following scale of scores was attributed: 1, dark-green leaves; 2, light-green leaves; and 3, yellowish leaves with dark spots or completely necrosed leaves. The data were subjected to analysis of variance, followed by the Scott & Knott post hoc test, at 5% probability.

Plants maintained at a temperature of 20°C showed a significant reduction in height and growth rate, although their normal development was not affected (Table 1). The obtained results are in accordance with those reported for the conservation of the germplasm of tropical species, which suggest that the most favorable temperature for the maintenance of these species under minimal growth conditions is between 15 and 20°C, providing a reduction in metabolic activity and a decrease in growth (Engelmann, 2011).

At the different temperatures, the survival rate of the oil palm plants remained high (> 95%). Furthermore, no significant differences were observed in the appearance of the plants after 12 months of conservation. Similar results were found for coconut (*Cocos nucifera* L.) plants maintained in vitro at 27±1°C, for which the survival rate was 75% after 18 months of cultivation (García et al., 2008). Lédo et al. (2007) observed, for this same species, the maintenance of 100% plant survival after 365 days of cultivation, and considered in vitro cultivation to be a promising alternative for the conservation of germplasm in palms. The use of low temperatures in the in vitro maintenance of the germplasm of palm species was previously reported only for date palm (*Phoenix dactylifera* L.) (Bekheet, 2011).

In general, media containing sucrose at 3% favor the normal development of the conserved plants and result in plants with better appearance, with the predominance of uniform green leaves throughout the storage process (Table 2). The greatest delay in the growth of the oil palm plants was in the culture media containing mannitol. In spite of the positive result in growth reduction, this carbohydrate resulted in accentuated necrosis of the aerial part and in a consequent reduction in plant survival. Similar results were found in coconut

Table 2. Survival rate, length of the aerial part (LAP), growth rate, and appearance (color) of the leaves of oil palm (*Elaeis* sp.) accessions after 12 months of in vitro storage at 25ºC with different carbohydrates at concentrations of 1, 2, and 3%[1].

Carbohydrate	Survival rate (%)			Mean	LAP (cm)			Mean	Growth rate (%)			Mean	Appearance[2]			Mean
	1%	2%	3%		1%	2%	3%		1%	2%	3%		1%	2%	3%	
							Elaeis oleifera (EOD08)									
Sorbitol	90.0	80.0	100.0	90.0a	10.5bA	11.0bA	12.0cA	11.2c	238.2	245.2	220.6	234.7b	2.6	2.3	1.7	2.20b
Mannitol	90.0	90.0	100.0	93.3a	7.8bA	7.7cA	6.3dA	7.3d	103.2	111.2	113.8	109.4c	2.7	2.7	2.5	2.60a
Sucrose	100.0	100.0	100.0	100.0a	11.9aA	14.2aA	14.4bA	13.5b	273.3	315.1	408.8	332.4a	2.1	1.9	1.7	1.90c
							Elaeis guineensis (EGT11)									
Sorbitol	100.0	100.0	100.0	100.0a	14.6aB	16.2aB	19.7aA	16.9a	236.2	260.1	329.7	275.4b	1.9	2.0	1.5	1.8c
Mannitol	75.0	62.5	100.0	79.1b	7.5bA	6.7cA	7.6dA	7.3d	122.5	105.5	80.6	102.9c	2.6	3.0	2.9	2.8a
Sucrose	87.5	100.0	100.0	95.8a	9.7bC	13.9aB	18.2aA	13.9b	205.2	303.2	352.7	287.1b	2.1	2.2	2.1	2.2b

[1]Means followed by equal letters, lowercase in the columns and uppercase in the lines, within each variable, do not differ by the Scott & Knott test, at 5% probability. [2]Scale of leaf appearance: 1, dark-green leaves; 2, light-green leaves; and 3, yellowish leaves with dark spots or completely necrosed leaves.

(Lédo et al., 2007), in which the addition of mannitol resulted in lower growth of the aerial part and roots, significantly affecting plant survival. In the present study, the use of sorbitol produced intermediate results, but close to those obtained when the oil palm plants were cultivated in sucrose, with high explant survival, predominance of light-green leaves, and low incidence of necrosis.

Therefore, the obtained data show that oil palm plants can be maintained under minimal growth conditions simply by reducing the temperature in the growth room from 25 to 20°C. This method, besides being effective, can provide stability to the conserved materials, given that the occurrence of stress during the conservation period is one of the factors that could induce somaclonal variation. Sucrose is the most appropriate carbohydrate for maintaining the quality of the plants, whereas mannitol and sorbitol result in a reduced plant survival.

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