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In vitro germination of zygotic embryos of hybrid BRS Manicoré (*E. guineensis* X *E. oleifera*)

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ABSTRACT

The interspecific oil palm hybrid BRS Manicoré (*E. guineensis* x *E. oleifera*) has superior agronomic characteristics. However, the germination rate is low (30%) and the process is slow when the seeds are sown in a conventional form. The purpose of this study was to optimize the *in vitro* germination of zygotic embryos of this hybrid comparing seed lots. The viability of zygotic embryos was evaluated by the tetrazolium test (0.075%) for 4 h. The embryos were cultured on MS and Y3 culture media, with and without the addition of NaH_2PO_4, as well as on MS, MS½ and N6 medium. In MS medium containing NaH_2PO_4, the germination rate was increased from 40 to 70% in comparison with the medium without sodium phosphate. The comparison between the culture media MS, MS ½, N6 and Y3 showed that 75% of zygotic embryos cultured in the Y3 medium formed whole plants (with roots and shoots defined), a higher percentage than embryos cultured on MS, MS ½ and N6 media (46, 35 and 17% respectively). In the same Y3 culture medium, the embryos were larger (36% ≥ 2 cm and 30% ≥ 5 cm) than in the other media. Results obtained by the tetrazolium test were similar to those of germination, showing the effect of the genotype of each seed lot. For the germination and development of plantlets it is essential to add NaH_2PO_4 to a culture medium containing no phosphate or with a low phosphate concentration.

Key words: interspecific hybrid, sodium phosphate, tetrazolium, Y3.

INTRODUCTION

The hybrid palm BRS Manicoré is the result of a cross between two species of palm trees, the African one (*Elaeis guineensis*) and caiaué (*Elaeis oleifera*) that occurs in the Tropical Moist Amazon (Collares 2011). This interspecific hybrid stands out from the other cultivars produced, because it has high production capacity, around 30 ton/bunch/ha, is immune to fatal yellowing disease (Cunha et al. 2009) and is smaller than the African oil palm, which facilitates manual labor. Moreover, it produces more unsaturated oil, that is clearer, more uniform and contains less saturated fatty acids than the oil of the African palm (Ramos et al. 2006).

However, one of the difficulties of this culture is the availability of seedlings on a large scale, as palms have a single meristem and propagation

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by conventional methods is impossible. It is therefore produced exclusively from seeds (Kiem 1958), which have slow, irregular and often low germination (Meerow 1991). In some cases, embryos abort within the seed, preventing their development (Alves et al. 2011). Thus, only 30% of the seeds germinate, which complicates the conventional propagation of palms of economic interest (Angelo et al. 2007).

The *in vitro* culture of zygotic embryos can be a useful tool to reduce the germination time and provide higher rates of embryos developed into plants (Lima 2013), as the *in vitro* culture allows the removal of physical barriers such as hard endocarp and facilitates the supply of nutrients to the developing embryo (Pádua et al. 2014). *In vitro* germination can overcome this problem by embryo rescue. In this study we optimized the *in vitro* germination of zygotic embryos of BRS Manicoré hybrid palm comparing various culture media and various seed lots.

MATERIALS AND METHODS

Plant Material

The research was conducted in the Laboratory of Micropropagation of the Department of Botany - UFPR, Curitiba, Brazil. The seeds were obtained from mother plants of the hybrid BRS Manicoré (*E. guineensis* x *E. oleifera*) after controlled crossing and were supplied by EMBRAPA - Western Amazonia. The seeds were collected 150 days after pollination. The hard endocarps were removed and kernels washed with mild soap and running water. They were then immersed in 70% ethanol for 5 min followed by 20 min in commercial bleach (10% active chlorine v/v) containing 1% Tween-20 and rinsed four times with sterile distilled water. The embryos were isolated from the seeds and then disinfected with commercial bleach (2% active chlorine) for 5 min and finally rinsed three times in sterile distilled water.

Tetrazolium Test of Seed Viability

The zygotic embryos were isolated and placed on moistened Germitest paper, packed in plastic bags and placed in an oven at 30°C for 16 h. They were then kept in individual plastic containers (4 replicates of 25 embryos for each seed lot), embedded in a tetrazolium solution 0.075% (w/v), pH 6.5 ± 0.1, and placed in a B.O.D at 40°C for 4 h in the dark. After this period, the embryos were washed with running tap water on a sieve in order to remove excess salt (Maquiné et al. 2014).

The embryos were evaluated under a stereoscopic microscope by observing the degree of coloration of the main parts: tigelo and haustorium and the embryos were classified as either vigorous (capable of germinating) or non-vigorous. This classification followed staining patterns of oil palm seeds based on classes established by Maquiné et al. (2014) and modified by Lima (2013). Class 1: Viables with high vigor, tigelo and haustorium with homogeneous red or pink color (Figure 1c); Class 2: Viables with medium vigor, tigelo with homogeneous staining and haustorium with lack of staining (Figures 1d and e); Class 3: Viables with low vigor, tigelo and haustorium areas without staining (Figures 1f and g); Class 4: unviable and dead, tigelo and haustorium without staining (Figures 1h and i). The viability of embryos was calculated as the mean vigor of classes 1 and 2.

Germination *in vitro* of Zygotic Embryos

Embryos of lots CS 428, CS 735, CS 736, CS 1139, 1477 CS, CS 1681 were inoculated in test tubes of 15 cm in height and 2.5 cm in diameter, containing 15 ml of culture media. In a first experiment, four culture media were used: 1) salts and vitamins of Murashige and Skoog (1962) (MS), 2) half strength MS medium, with half concentration of salts and full vitamin formulation (MS1/2) with 500 $mg.L^{-1}$ of cysteine, 3) Y3 medium (Eeuwens 1976), 4) and N6 medium (Chu et al. 1975) with 100 $mg.L^{-}$

^{1} of myo-inositol. In the second experiment MS medium plus NaH_2PO_4 (0.17 g/L^{-1}) and the same media without NaH_2PO_4 were compared. The same comparison was made in the case of Y3 culture medium. All media were supplemented with 2 g L^{-1} activated charcoal (AC), 30 g L^{-1} sucrose and solidified with 6 g L^{-1} agar (Vetec). The pH of the culture media was adjusted to 5.8 with NaOH 0.1 N or HCl 0.1 N and the media were autoclaved at 120°C for 20 min. Activated charcoal was added along with agar, after pH adjustment.

After inoculation, the cultures were maintained in the dark for 30 d at 25 ± 2°C during the day and 21 ± 2°C overnight, and transferred under fluorescent light (white light) with irradiance of 40 µmol. $m^{-2}.s^{-1}$ and photoperiod of 16 h during four weeks. After 30 d the percentages of viable embryos, embryos without response and abnormal embryos were evaluated. Crooked and swollen embryos showing a ligule and primary root were considered germinated. Atrophied, rootless and shootless embryos were considered as abnormal. After 45 days the shoot length was measured. The plantlets with shoots and roots were transferred to 56 cm^3 polyethylene tubes containing vermiculite as substrate. After planting, they remained in a greenhouse with artificial lighting (light intensity of 13 µmol.$m^{-2}.s^{-1}$, 12 hours photoperiod and temperature of 24 ± 5°C during the day and 20 ± 5°C overnight) automatically irrigated for five minutes every six hours .

The experimental design was entirely randomized with one embryo per tube and 50 repetitions (tubes) per treatment. The experiment in which four culture media were compared (MS, MS ½, N6, Y3 and MS with and without sodium phosphate) was carried out using two lots of seeds whereas three lots were used for the comparison between Y3 media with and without phosphate. Data were subjected to Bartlett´s test in order to verify the homogeneity of variances and then the means were compared by Tukey´s test at 5% probability.

RESULTS

Zygotic embryos of oil palm hybrid Manicoré were classified according to the four classes of vigor and viability, based on the intensity, uniformity and location of color patterns of the two parts of the embryo (tigelo and haustorium). Classes 1 and 2 (Figures 1c, d and e) correspond to viable and vigorous embryos that can turn into plantlets with root and developed leaf sheaths. Class 3 (Figures 1f and g) encompasses viable but weak embryos, that are alive but unlikely to produce a plant. Class 4 (Figures 1h and i) corresponds to unviable embryos which can be dead or unable to give plantlets with roots, because of a lack of metabolic activity in the tigelo portion, where the embryonic axis is located.

As shown in Table I, the viability of the embryos varies with the lot of seeds, which means that it can be influenced by the genotype of each lot. Among six lots, only three (CS 428, CS 1477 and CS 1681) had most of the embryos in class 1

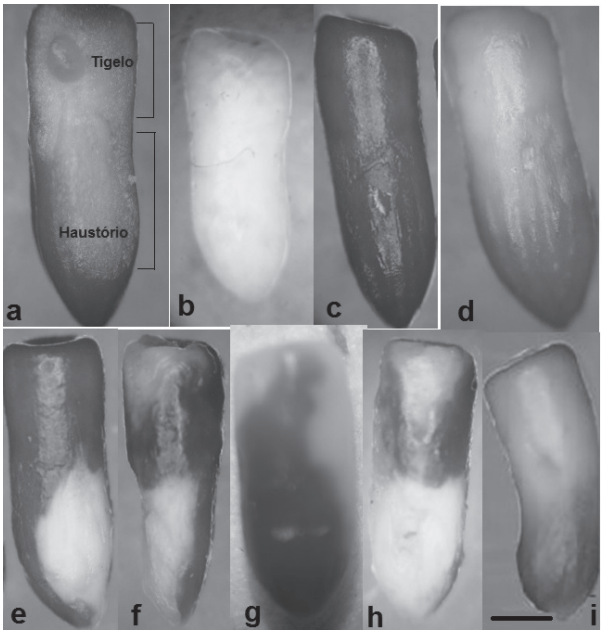

Figure 1 - Representation of color classes: **a**) Longitudinal section of the embryo highlighting the location of tigelo and haustorium. **b**) Zygotic embryo without tetrazolium treatment. **c**) Zygotic embryo with tetrazolium staining; **d**) and **e**) Class 2; **f**) and **g**) Class 3; **h**) and **i**) Class 4.Bar = 1.5 cm for all figures.

according to the tetrazolium test, in other words, embryos that have the ability to produce well-developed plantlets.

Lot CS 736 shows most of the embryos in Class 2 (Table I). They are viable with medium vigor, but still have the capacity to produce well-developed plantlets. The viability of the embryos is related to the germination and development of normal plantlets (with roots and shoots) (Figure 2i). These viable embryos cultured in suitable culture media successfully germinated.

Different culture media were compared for lots CS 428 and 1139 which presented 96 and 73% of viable embryos (Table I). Germination rates of 59 and 90% were observed, respectively, for these lots in Y3 culture medium (Table II) whilst the results obtained in other culture media were lower. The overall average for germination rate in all culture media (MS, MS 1/2, N6, Y3 and MS with and without phosphate) was 37.5% for CS 428 and 49.5% for CS 1139 (Table II).

Relating the average viability of the embryos of three lots of seeds (735, 1477 and 1681) with germination rates in two Y3 culture media (with and without phosphate), embryos viability of 46, 76.25 and 92.50%, respectively, and mean germination rates of 33.6, 68.6 and 91.6% were observed (Table II). In both Y3 culture media, lot CS 1681 had the highest percentages of viability and germination, with 92.5 and 91.6% respectively (Table II).

In the first two days of culture, zygotic embryos did not show changes in their morphology in the tested media (MS, MS½, N6 and Y3). In the first week of culture only enlargement and swelling were observed (Figure 2a). Embryos cultured for 10 d were curved (Figure 2b) and after 15 d some

TABLE I
Classification of lots of seeds of hybrid oil palm according to vigor determined by tetrazolium test.

Lots	CS 428 (%)	CS 735 (%)	CS 736 (%)	CS 1477 (%)	CS 1681 (%)	CS 1139 (%)	General rate (%)
High vigor	92.0 a	25.0 ab	6.00 b	55.0 a	72.50 a	43.00 a	48.91 a
Medium vigor	4.0 b	21.0 ab	30.0 a	21.25 b	20.00 b	30.0 ab	21.00 ab
Low vigor	4.0 b	15.00 b	27.0 ab	10.25 b	6.25 bc	20.0 bc	13.75 b
Unviable or dead	1.0 b	39.0 a	37.0 a	14.00 b	1.25 c	7.50 c	16.62 b
DMS	8.57	22.35	23.65	29.67	13.93	18.27	30.70
CV%	16.17	42.58	45.05	56.23	26.55	34.64	75.73

Values followed by the same letter do not differ significantly by Tukey´s test at 5% probability.

TABLE II
Viability and germination rate of embryos of five lots of hybrid oil palm seeds in several culture media.**

Lots of seeds	CS 428 (%)	CS 1139 (%)	CS 735 (%)	CS 1477 (%)	CS 1681 (%)
Viability (TTC)*	96.00	73.00	46.00	76.25	92.50
		Germination rate (%)			
MS	42.0	50.0			
MS1/2	38.0	32.0			
N6	18.0	16.0			
Y3	59.0	90.0	38.4	79.0	88.8
Y3 + NaH_2PO_4	-----	-----	28.9	58.3	94.4
MS	31.7	49.0			
MS + NaH_2PO_4	38.4	60.0			
Overall average of all treatments (%)	37.5	49.5	33.6	68.6	91.6

*by tetrazolium test. Sum of the means of classes 1 and 2. **Zygotic embryos with leaf primordia and primary root, observed after 30 days.

embryos remained curved and presented a plumular hook with ligule and others only root primordia (Figure 2c). On the twentieth day the foliar and root primordia appeared in all of them and a decrease of the haustorium size was observed. At this stage, nutritional supplementation was provided by the culture medium, and the haustorium was no longer needed to supply nutrients to the embryo (Figure 2d). After 30 days, the plantlets already had shoots and roots (Figures 2 and f) and after 50 days they were acclimatized. In all culture media some explants did not respond and some others developed abnormally (Table III).

The zygotic embryos germinated in all the culture media, but in the Y3 culture medium the germination process was better (75% of embryos with the first leaf and primary root) and the embryos developed into complete plants, in other words, with defined root and shoots, unlike the embryos cultured in the other media (Figure 2i and Table III).

When comparing the development of the embryos on MS medium supplemented with NaH_2PO_4 and those on MS medium without NaH_2PO_4 we observed 67.5% of germination in the first medium after 15 days whereas those on the second medium showed a high percentage of explants with no response (Table IV and Figure 2h). On the thirtieth day, the development continued to be better (49.2% of plantlets with leaf primordia and root) in the MS medium supplemented with NaH_2PO_4 (Table IV).

TABLE III
Behavior of hybrid oil palm zygotic embryos (BRS Manicore) (lots CS 428 and CS 1139) cultivated *in vitro* in different culture media, evaluated after 15 and 30 days.

	15 days	30 days	30 days	30 days
Culture media	Curved with haustorium (%)	Leaf primordia and root 1ª (%)	Non-responding explants (%)	Abnormal explants (%)
MS	33.5 a	46.0 ab	20.0 ab	1.0 a
MS1/2	30.0 a	35.0 ab	30.5 ab	5.5 a
N6	13.5 a	17.0 b	65.0 a	5.5 a
Y3	19.5 a	75.0 a	5.0 b	1.5 a
DMS	75.74	45.00	49.42	18.38
CV%	77.09	25.90	40.00	133.00

Numbers followed by the same letter do not differ statistically each other by Tukey´s test at 5% of probability.

TABLE IV
Behavior of zygotic embryos of hybrid oil palm (BRS Manicoré) cultured *in vitro* in ms and Y3 culture media with or without sodium phosphate addition, evaluated after 15 and 30 days.

Treatments	15 days	30 days	30 days	30 days
Culture media	Curved with haustorium (%)	Leaf primordia and root 1ª (%)	Non-responding explants (%)	Abnormal explants (%)
MS	40.0 b	40.3 a	20.0 a	28.2 a
MS+NaH_2PO_4	67.5 a	49.2 a	27.9 a	20.0 a
DMS	24.0	59.5	114	41.2
CV%	10.4	30.9	110	39.6
Y3	53.0 a	59.1 a	21.2 a	11.3 a
Y3+NaH_2PO_4	55.7 a	58.6 a	14.5 a	21.5 a
DMS	55.2	48.5	27.2	17.1
CV%	58.7	47.6	88.2	60.3

Numbers followed by the same letter do not differ statistically by Tukey´s test at 5% of probability. Curved at 30 days data not shown. Mean of CS 428 and CS 1139 lots for MS media; mean of CS 735, 1139 and 1681 for Y3 media.

In Y3 culture medium a higher percentage of embryos developed into complete plantlets (roots and shoots) than in MS medium (Table III), so, this medium was used again, with or without supplementation of NaH_2PO_4. However, there were no statistical differences between the rate of developing embryos in both Y3 media (Table IV). In both treatments embryos developed normally after 15 and 30 days of culture.

After 45 days of culture, the size of the plants ranged among MS, MS½, N6 and Y3 media (Figure 2i): 36% of plantlets cultured in the Y3 media

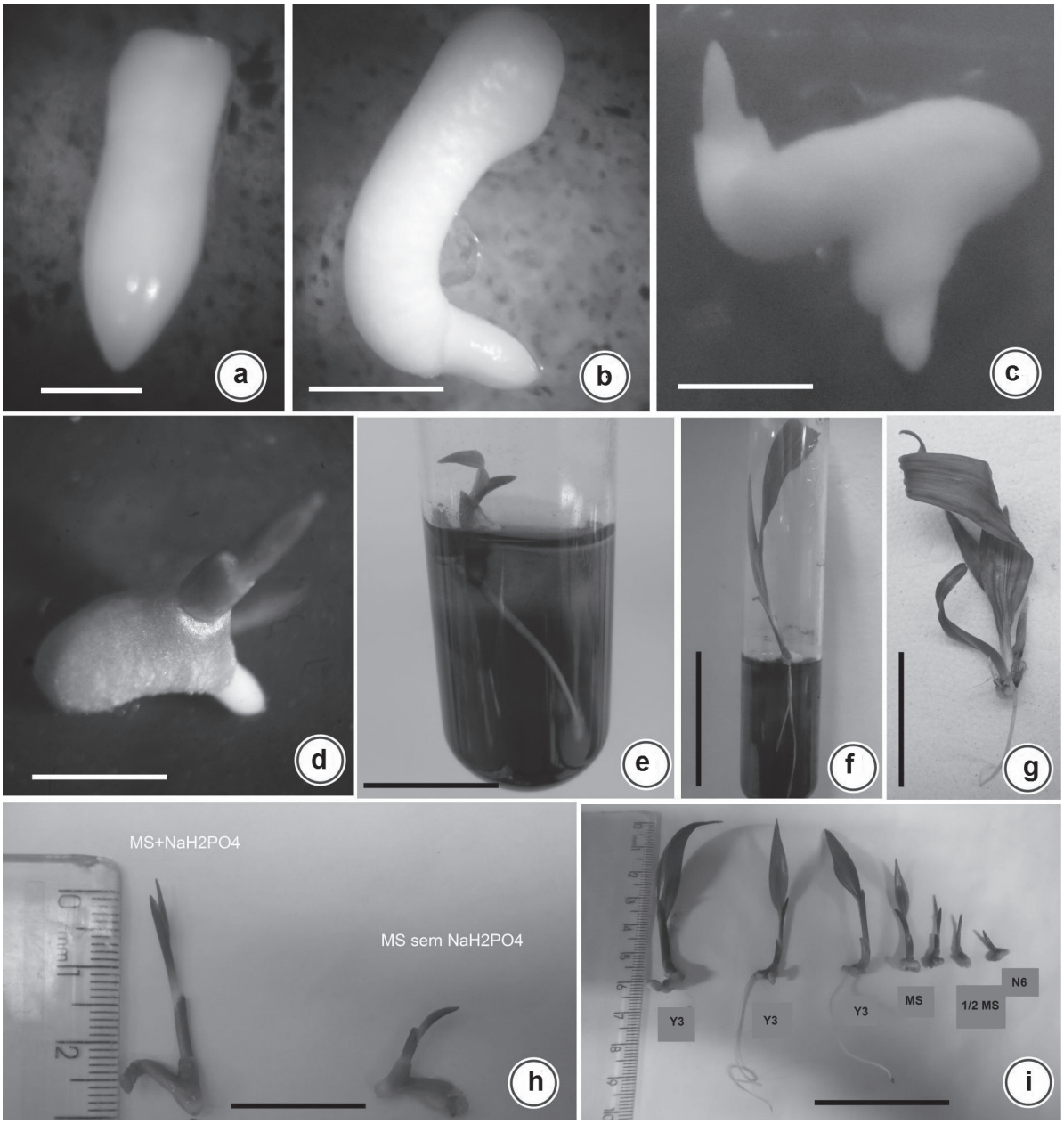

Figure 2 - *In vitro* germination of zygotic embryos of hybrid BRS Manicoré. **a**) Swollen embryo after 5 days. **b**) Bending process after 10 days. **c**) Embryo with ligule, plumule and haustorium after 15 days. **d**) Plantlets with foliar and primary root after 20 days. **e**) Root and leaf after 30 days. **f**) 45 days of culture. **g**) Root and leaf after 50 days. **h**) Plantlets on MS medium with or without NaH_2PO_4 after 40 days. **i**) Influence of culture media in the development of plantlets after 35 days. BARS: A = 1.5 cm B to I= 2 cm.

TABLE V
Size of plantlets developed from BRS Manicoré oil palm zygotic embryos CS 428 and CS 1139 lots cultivated *in vitro* in different culture media and observed after 45 days.

Culture media	Plantlets 1- 2 cm (%)	Plantlets 2 - 6 cm (%)	Non-responding explants (%)	Abnormal explants (%)
MS	20.0 a	0.5 b	45.0 a	15.0 ab
MS1/2	27.4 a	3.2 b	41.9 a	24.1 ab
N6	17.7 a	0.5 b	44.0 a	35.2 a
Y3	36.0 a	30.0 a	2.0 b	7.0 b
DMS	38.5	9.4	24.8	24.6

Numbers followed by the same letter do not differ statistically by Tukey´s test at 5% probability. Values less than 1 cm not shown.

reached a height of 1 to 2 cm and 30% reached 2 to 6 cm. The MS, MS ½ and N6 media showed 20, 27.4 and 17.7% of plantlets with 1 to 2 cm respectively (Table V and Figure 2i).

DISCUSSION

The tetrazolium test can be used to evaluate the viability of oil palm hybrid embryos. The TTC test is based on the activity of dehydrogenase enzymes which act on respiratory processes of tissues, where the release of hydrogen ions occurs and 2, 3, 5 - triphenyl tetrazolium chloride reacts with H^+ to form a red, insoluble substance called formazan (Delouche et al. 1976). In the present study, lots submitted to the TTC test showed different rates of viability and, among the six lots, only three (CS 428, 1477 and 1681) had embryos with a uniform color. This indicates that they are able to give rise to well-developed plantlets. This result is similar to that obtained by Lima (2013): 93% of seeds were viable as the staining of the oil palm embryos (*Elaeis guineensis*) by the TTC test was uniform.

The germination rate of the seeds is not always related to the viability of each lot since zygotic embryos of CS 1681, when cultured in a Y3 culture medium with and without phosphate showed 92% viability and a germination rate of 88.8%. This shows that, while 92% are viable, only 88.8% were able to germinate. Zygotic embryos (ZE) from seeds of CS 428 and 1139 had a viability of 96 and 73% according to TTC test, while 59 and 90% germinated in Y3 culture medium. This may be because the seeds of these lots were viable, but ZE of CS 1139 had more vigour to develop than those of CS 428. Other authors also consider that the TTC test can be used for rapid assessment of the viability of oil palm seeds. For example Mok (1972) reported a mean value of embryo viability of 98.6% and a mean rate of *ex vitro* germination of 95%. Murugesan et al. (2002) also found a correlation between viability and germination rate: oil palm embryos cultured in MS medium supplemented with IAA and kinetin reached a germination rate of 93% while the percentage of viability of these embryos varied between 88 and 92%, at TTC concentrations of 0.5% and 0.75%, respectively.

Zygotic embryos began their development after two days in the culture media. This delay can be explained by the fact that when the explants are put into a nutrient media, there may be an initial leakage of ions from damaged cells, especially Na^+, Ca^{2+}, K^+, Mg^{2+} (Soares 2011), so that the concentration in the plant tissues actually decreases. After this period, cells begin active absorption and their internal concentration rises slowly.

The addition of sodium phosphate in MS culture medium is critical for *in vitro* germination. It was observed that when the culture medium contained NaH_2PO_4, 49% of the embryos germinated after 30 d of culture, whilst in NaH_2PO_4-free MS medium there was a high percentage of non-responding explants. In another study (Cardoso et al. 2010), the addition of sodium phosphate to MS and MS½ media was also critical for converting oil

palm zygotic embryos into plantlets and enabled plantlets with a larger stem and root to be obtained. When adding 0.17 g/L^{-1} NaH_2PO_4 to MS media, these authors obtained an 85.18% germination rate for seeds of hybrid CN 470.

Phosphate is the way phosphorus is absorbed by plant cells from the culture media and this process is more rapid than for other ions (George et al. 2008). According to these authors, the phosphate concentration in MS medium is insufficient for some species and this concentration is reduced to zero in a few weeks. Despite the MS medium contains 170 mg L^{-1} KH_2PO_4 the presence of a higher amount of phosphate is essential in most *in vitro* cultures due to the role of phosphorus in energy metabolism and regulation of enzymatic processes, being differentiation of shoot one of the consequences of phosphorus addition to the culture medium (Santiago et al. 2001). It also influences height, stem thickness and root size (Barcelos et al. 2001). Furthermore, phosphorus is part of the nucleotides, forming units of nucleic acids such as DNA and RNA, directly involved in the process of protein synthesis (George et al. 2008).

In the present study, Y3 culture medium was the most suitable for the full development of the plantlet into shoot and primary root. With regard to the optimization of *in vitro* germination, in Y3 culture medium the germination rate was higher (75%) than in other media. This could be due to the composition of this medium which contains nutrients similar to what the plant naturally needs, including sodium phosphate (320 mg/L) that is not present in MS media, having only potassium phosphate (170 mg/L). The concentration of phosphate ions is therefore of great importance for the development of palm plants. The mixture of nutrients such as urea, triple superphosphate, potassium chloride and magnesium sulfate used for natural seed germination enables plants to develop well (Barcelos et al. 2001).

On the other hand, some salts and organic substances of Y3 medium are different of those present in MS medium and some elements have different concentrations when compared to MS medium. It is therefore difficult to assign the better results obtained on Y3 medium only to phosphorus concentration. In other surveys of oil palms or their hybrids, MS, MS ½ and Y3 media were adequate for *in vitro* germination of zygotic embryos without statistical differences between the media, with 90% of germination in MS ½ and Y3 media, and 85% in MS medium (Chourykaew and Kanchanapoom 1996). The results of germination of the lots CS 428 and CS 1139 obtained in the present study showed that in MS culture medium the germination rates were low (42 and 50%, respectively) when compared to the rates obtained in Y3 medium (59 and 90%, respectively). However, Pádua et al. (2014) found that embryos of Manicoré seeds collected 100 days after anthesis presented a germination rate of 88% in MS or Y3 medium.

This work showed that the culture medium suitable for germination can vary according to the genotype of each lot of seed of oil palm hybrid BRS Manicoré. Similarly, Alves et al. (2011) showed that oil palm embryo development varies with the genotype since 37.93% of CJ 2141 embryos, grown on MS medium supplemented with phosphate, developed roots and shoot, meanwhile there was no embryonic development in the case of CJ 502.

CONCLUSIONS

The tetrazolium test can be used to evaluate the embryos viability. The germination rate of the seed lots is related with their viability which varies for each lot of seeds.

The addition of NaH_2PO_4 to the culture media is essential for the germination of the oil palm hybrid, especially in the media which do not contain sodium phosphate or have a low phosphate concentration.

The Y3 culture medium is better for the full plantlet development into shoot and primary root. This is due to the composition of this medium that contains all the salts and vitamins that oil palm plantlets need to develop.

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RESUMO

O híbrido interespecífico BRS Manicoré (*E. guineensis* x *E. oleifera*), tem características agronômicas superiores. Porém, a taxa de germinação é baixa (30%) e o processo demorado quando as sementes são semeadas de forma convencional. O objetivo deste trabalho foi otimizar a germinação *in vitro* de embriões zigóticos desse híbrido comparando os lotes de sementes. A viabilidade dos embriões zigóticos foi avaliada pelo teste de tetrazólio (0,075%) durante 4 horas. Os embriões foram cultivados nos meios de cultura MS e Y3, com e sem a adição de NaH_2PO_4, assim como MS, MS ½ e N6. No meio MS contendo NaH_2PO_4, a taxa de germinação é aumentada de 40 a 70% em comparação com o meio sem fosfato. A comparação entre os meios de cultura MS, MS ½, N6 e Y3 mostraram que 75% dos embriões zigóticos cultivados no meio Y3 formaram plantas completas (raiz e parte aérea definida), uma porcentagem maior que dos embriões cultivados nos meios MS, MS ½ e N6 (46; 35 e 17% respectivamente). No mesmo meio de cultura Y3, os embriões apresentaram tamanho maior (36% ≥ 2 cm e 30% ≥ 5 cm) que nos outros meios. Resultados obtidos pelo teste de tetrazólio foram semelhantes aos de germinação, mostrando o efeito do genótipo de cada lote de sementes. A adição de NaH_2PO_4 é fundamental para a germinação e o desenvolvimento das plântulas nos meios de cultura que não contêm fosfato ou com baixa concentração de fosfato.

Palavras-chave: híbrido interespecífico, fosfato de sódio, tetrazólio, Y3.

REFERENCES

ALVES SAO, LEMOS OF DE, FILHO BGS DOS AND SILVA AL. 2011. *In vitro* protocol optimization for development of interspecific hybrids of oil palm (*Elaeis oleifera* (H.B.K) Cortés x E. *guineensis* Jacq). J Biotec Biodiversity 2(3): 1-6.

ANGELO PCS DA, MORAES LAC, SOUSA NR, LOPES R AND CUNHA RNV DA. 2007. Resgate de embriões híbridos de dendezeiro x caiaué. Comunicado Técnico nº 265 – Embrapa, Manaus, AM.

BARCELOS E, RODRIGUES MRL, CUNHA RNV DA AND SANTOS JA DOS. 2001. Produção de mudas de dendezeiro na Amazônia. Circular Técnica: ISSN 1517-2449. Embrapa Amazônia Ocidental, Manaus, AM.

CARDOSO JN DE, MIRANDA VS, LEMOS OF DE AND COELHO IL. 2010. Obtenção de plântulas de híbridos de dendezeiro por cultivo *in vitro*. Rev Ci Agra 53(2): 177-181.

CHOURYKAEW B AND KANCHANAPOOM K. 1996. *In vitro* culture of embryos and callus of oil palm (*Elaeis guineensis* Jacq.). J Sci Soc 22: 1-12.

CHU CC, WANG CC, SUN CC, HSU C, YIN KC, CHU CY AND BI FY. 1975. Establishment of an Efficient Medium for Anther Culture of Rice through Comparative Experiments on The Nitrogen Sources. Sci Si 18: 659.

COLLARES D. 2011. Produção de cultivares e sementes de dendê na Embrapa. Agroenergia em Revista 2: 30-31.

CUNHA RNV, LOPES R, ROCHA RNC, LIMA WAA, TEIXEIRA PC, BARCELOS E AND RODRIGUES MR. 2009. Domesticação e melhoramento do caiaué. In: Borém A; Lopes MT and Clement CR (Eds), Domesticação e melhoramento: espécies amazônicas. Embrapa, p. 275-296.

DELOUCHE JC, STILL TW, RASPET M AND LIENHARD M. 1976. O teste de tetrazólio para viabilidade da semente. Brasília, p. 55-103.

EEUWENS CJ. 1976. Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palms (*Cocos nucifera* L.) and culture *in vitro*. Physiol Plant 36: 23-28.

GEORGE EF, HALL AM AND KLERK GJ DE. 2008. The components of Plant Tissue Culture Media I: Macro- and Micro-Nutrients. In: Plant propagation by tissue culture. The Technology. 3rd ed., p. 65-87.

KIEM SC. 1958. Propagation of palms. Principes 2: 133-138.

LIMA SCS DE. 2013. Teste de germinação *in vitro* e de viabilidade em embriões zigóticos de dendê (*Elaeis guineensis)*. Embrapa - Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq. Manaus, AM.

MAQUINÉ TM, CYSNE AQ, LIMA WAA DE, ABREU SC, GREEN M AND RIOS S DE A. 2014. Germination of seeds of interspecific hybrid caiaué x oil palm submitted to the mechanical depulping. Amer J Pl Sci 5: 2965-2972.

MEEROW AW. 1991. Palm seed germination. Florida: Cooperative Extension Service, 10 p.

MOK KC. 1972. The tetrazolium test for evaluating the viability of oil palm (*Elaeis guineensis* Jacq.) seed. Proceedings of the International Seed Testing Association 37: 771-777.

MURASHIGE T AND SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-497.

MURUGESAN P, VANANGAMUDI K AND UMARANI R. 2002. Evaluation of viability of oil palm (*Elaeis guineensis* Jacq.) seeds by tetrazolium test and comparison with germination and *in vitro* culture results. Proceedings of Placrosym XV, p. 246-250.

PÁDUA MS, PAIVA LV, PIRES MF, SILVA LGT DA, CASTRO EM DE AND STEIN VC. 2014. Influence of culture medium and age of zygotic embryos on *in vitro* germination of *Elaeis guineensis* Jacq. Afr J Biotechnol 13(14): 1515-1523.

RAMOS EJA, VEIGA AS AND JUNIOR JF. 2006. Potencial produtivo de híbridos interespecíficos entre dendezeiro e caiauzeiro nas Condições do Nordeste Paraense. Documento 247. Embrapa Amazônia Oriental. Belém, PA.

SANTIAGO EJA, PAIVA R, PAIVA PDO, SANTANA JRF AND GOMES GAC. 2001. Meios de cultura: Cultura de tecidos. Paiva e Paiva, UFLA 3: 22-35.

SOARES JDR. 2011. Germinação de embriões e crescimento inicial *in vitro* de macaúba. Cienc Rural 41(5): 773-778.