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Accelerated *in vitro* propagation of elite oil palm genotypes (*Elaeis guineensis* Jacq.) by substituting cytokinin with putrescine

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Clonal multiplication of oil palm enables the formation of commercial plantations with higher yield, which is possible through somatic embryogenesis. However, due to different modifications made in the existing protocols to avoid the appearance of the “mantled flower”, the success of this technique has been limited. Thus, this study aimed to apply a cloning protocol, using somatic embryogenesis, to 32 elite oil palm genotypes from a commercial plantation on which cytokinin was substituted with putrescine. All tested genotypes responded positively to callus induction. Moreover, the percentages of responses were differentiated: 65.62% of these genotypes produced embryogenic lines, and 40.62% out of that percentage presented lines with moderate or high multiplication capacity, which is the main factor that enabled the obtaining of clones to form a commercial clonal plantation. The somatic embryogenesis was efficient, making this protocol applicable at a commercial scale, since it allows the obtaining of clones up to 360 days. However, future evaluations on these clones are needed to investigate the appearance of the “mantled flower”.

Key words: Cloning, polyamines, tissue culture, commercial plantation.

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is an oleaginous palm of African origin with a great economic importance, both for food industry and for non-food industry (Ganand, 2014). The oil extracted from this palm is nowadays the most produced and consumed in the world, responsible for 30% of the world production, being the main raw matter for oleo chemical industry (Mielke, 2013). The

global dependency on oil palm tends to increase in the coming years and it is forecasted that in the year 2020, at least 78 million of tons of oil extracted from this palm would be demanded by consumers in the entire world (Mielke, 2013) since, besides the interest developed by the food industry, oil palm is becoming the most important raw matter in the biofuel industry (Ganand,

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2014). Studies have indicated that until 2035, these yields need to increase even more, and that would only be possible by improving the productive chain (Ganand, 2014). Among these efforts, plant breeding is fundamental, since it allows the introduction of superior cultivars in commercial plantations, hence contributing to the increment of productivity. However, oil palm breeding is limited due to long periods and evaluation costs needed to reach the desired cultivar (Rance et al., 2001). Selection cycle that includes evaluation, phenotypic selection and the crossing between the selected families to form a new population requires approximately 19 years (Wong and Bernardo, 2008).

Besides the long selection period, oil palm presents high heterozygosity, especially when the propagation of the plant is performed by seeding, having as result formation of heterogeneous plantations, promoting non-uniformity in production and difficulties in agronomical practices (Srisawat and Kanchanapom, 2005). The development of commercial plantations of elite clones can offer advantages such as harvest uniformity, simplification in management practices and optimization of the oil production (Malike et al., 2012), plus multiplication of the superior genotypes in a short period of time.

For clonal multiplication of oil palm, the somatic embryogenesis has been the most used method (Konan et al., 2006; Ong-Abdullah et al., 2015; Ooi et al., 2013; Palanyandy et al., 2013), and it has already been tested from different explants, such as immature leaves of young plants, immature inflorescences and zygotic embryos (Balzon et al., 2013; Yusnita and Hapsoro, 2011; Rajesh et al., 2003; Scherwinsky-Pereira et al., 2010; Thuzar et al., 2011). It is important to highlight that details for clonal multiplication of oil palm are not published, since researches are conducted by companies with commercial interests, not interested in providing their protocols (Scherwinsky-Pereira et al., 2010).

Other aspect to be considered in micropropagation of oil palm is the appearance of the “mantled flower”, a floral abnormality that is present in plants obtained by *in vitro* culture (Alwee et al., 2006) severely affecting the productivity potential. The causes of the “mantled” are unknown and thus currently it has been considered an epigenetic variation since cytokinin stimulates the hypomethylation of the DNA, which results in a homeotic transformation of the floral organs (Ong-Abdullah et al., 2015).

The use of cytokinin (Eeuwens et al., 2002; Jones et al., 1995; Roowi et al., 2010) and the combination of high concentrations of cytokinin and low concentrations of auxins in the culture medium (Smulders and Klerk, 2011), have been pointed as the main causes of this abnormality when the plants are micropropagated. Modifications in the composition of the culture medium to control the “mantled” have been well-succeeded (Eeuwens et al., 2002). However, these modifications lead to reduction of

the success of the somatic embryogenesis technique (Kushairi et al., 2010).

According to Khan et al. (1992) and Galston and Kaur-Sawhney (1995), polyamines can substitute cytokinins effectively in the *in vitro* culture. The polyamine “putrescine” is related to the high efficiency of somatic embryogenesis and is correlated with active cell division (Kackar and Shekhawat, 2007). The polyamines minimize the frequency of DNA methylations (Brooks et al., 2010) and are essential in many cell processes, from transcription to membrane fluidity (Baron and Stasolla, 2008). The utilization of polyamine instead of cytokinin in the somatic embryogenesis in oil palm was described by Rajesh et al. (2003). However, these authors used zygotic embryos as explants. Moreover, studies using immature leaves from adult plants as source of explants, and also, that aim to produce clones to establish a commercial plantation are not yet of public domain. This work aimed to demonstrate the efficiency of putrescine in somatic embryogenesis among selected 32 elite genotypes of oil palm, as well as to identify the potential of these genotypes to obtain clones of commercial plantation.

MATERIALS AND METHODS

Selection of genotypes for cloning

To implement the experiment, thirty-two genotypes of oil palm (*E. guineensis* Jacq.) ‘Tenera’ hybrids dura (D) x psífera (P) were selected from Agropalma S.A.’s commercial plantation (Table 1), located in the city of Tailândia, Pará, Brazil. The selected materials are elites from different research centers and show considerable genetic variation. The individual selection of elite plants to be cloned was performed systematically and accurately considering yield, vegetative and plant health aspects.

Considering productivity aspects, the elite plant to be cloned should provide: good yields as fresh fruit bunch production (200 to 250 kg), good number of fresh fruit bunches, oil potential in the fruit bunch (28%), average weight of fresh fruit bunch and annual production of at least 10 tons of oil per ha per year. Plants showing slow growth (0.25 - 0.50 m per year) were considered as a good vegetative aspect for selection. For the vegetative aspects, plants that showed slow growth (0.25 were 0.50 m per year) were considered. Finally, for phytosanitary aspects, free arrays of diseases and symptoms of nutritional deficiency were considered (Corrêa et al., 2015).

Referencing of apical meristem, removal of the palmito, obtaining and preparation of the foliar explants

For extraction of the explants, the posterior growth of the matrix plant was not compromised before the cloning process of the selected genotypes; the stipe region was determined to cut the palmito. Three adult plants were used in pretesting (Figure 1A), performing a longitudinal cut in the stipe and measurements to reference the meristem region (Figure 1B), so that the same meristem region could be preserved safely at the moment of extraction. The palmito extraction in all donor elite plants was based on this height measurement reference (Figure 1C). After extraction, each palmito was sent to the Laboratory of Vegetal Cell and Tissue

Table 1. “Tenera” hybrids of oil palm (*E. guineensis* Jacq.) selected as explants donors to clone through somatic embryogenesis using putrescine.

Tenera hybrids	Company supplier of seeds	Number of accessed matrixes	Age (years)
Deli x Ekona	ASD	2	9
Deli x Ghana	ASD	3	11
Deli x La Mé	Murrin Corporation	9	9-26
Deli x La Mé	Embrapa Manaus	4	11-13
Deli x Yangambi	Univanich Palm Oil	8	10-27
Deli x Avros	ASD	2	13
Kigoma	ASD	2	13
Deli x Dami	Dami Las Flores	2	14-15



Figure 1. Apical meristem referencing procedures for safe palmetto extraction and obtaining of leaf explants to induce somatic embryogenesis in oil palm. A: Cutting of an adult plant oil palm to perform stem slitting and measurements, in order to secure palm extraction without compromising the apical meristem. B: Referencing safety for cutting palm hearts, where the arrow indicates the region of apical meristem. C: Palm extraction based on measurements done by a professional. D: Removal of external palm leaf layers of protection for leaf explant preparation. E- F: Immature leaflets individually separated for the induction of somatic embryogenesis in oil palm

Culture at the Federal University of Viçosa, in Viçosa/MG, Brazil. After 72 h of material removal in the laboratory, the palmitos were sanitized externally with 70% alcohol and external leaf layers were carefully removed (Figure 1D), being the immature leaves separated in seven layers: Leaf- 1 (most external) to Leaf- 7 (most internal) (Figure 1E and F) (Corley and Tinker, 2003). Next, the leaflets of each leaf were detached from the rachis, with 1/3 of the leaflet apex being eliminated. The leaflets were then submitted to disinfection with sodium hypochlorite (1% of active chlorine) for 20 min and rinsed successively eight times in sterile deionized water in laminar flow chamber. After disinfection, the leaflets were sectioned transversally into 1 cm segments of length, which were used as

explants to induce somatic embryogenesis.

Induction of callus formation

The basal medium (MB) used in this work was composed of salts and vitamins Y3 (Eeuwens, 1978), 30 g L⁻¹ of sucrose, 1 g L⁻¹ of casein hydrolyzed, 100 mg L⁻¹ of myo-inositol, 100 mg L⁻¹ of arginine, 100 mg L⁻¹ of asparagine, 100 mg L⁻¹ of glutamine and jellied with 2.5 g L⁻¹ of Phytigel® (Sigma, USA). The pH was 5.7 ± 0.1 and the mediums were autoclaved for 20 min at 121°C and 1.5 atm. For callus induction, the culture medium consisted of MB,

supplemented with 800 μM of 2,4-dichlorophenoxyacetic acid (2,4-D) and 3 g L^{-1} of active charcoal (Sigma, USA) (Corrêa et al., 2015). The explants were inoculated into Petri dishes of polystyrene (90 x 15 mm) containing 30 mL of this culture medium. Five explants were inoculated per dish and 500 dishes were sealed with PVC film (Rolopac[®]) and kept in the growing room, at a temperature of $27 \pm 1^\circ\text{C}$ in absence of light for 90 days. After the incubation period, the percentage of explants that formed callus on each genotype was evaluated.

Multiplication of the callus and obtaining of embryogenic lines

The callus obtained from each genotype was inoculated into multiplication medium. This medium constituted of MB added with 9 μM of 2,4-D and 1000 μM of putrescine (Corrêa et al., 2015). After inoculation of the callus, the dishes were sealed with PVC film (Rolopac[®]) and kept in the growing room, at a temperature of $27 \pm 1^\circ\text{C}$ in darkness. After 60 days, the callus that formed embryogenic lines were separated. These were subdivided and subcultivated in the same multiplication medium.

The lines considered embryogenic presented granular aspect and yellow coloration, having the capacity of multiplication classified as null, low, moderate or high.

Criteria for evaluation of embryogenic lines

The genotypes that did not produce embryogenic lines were classified as "null". The embryogenic lines that increased up to two times the original size, having multiplication difficulties, were classified as "low", and the genotype that formed this type of line was classified as a material producing embryogenic masses with "low" multiplication capacity. The genotype that produced embryogenic masses with "moderate" multiplication capacity were those in which the obtained lines increased up to three times their original size, when conditioned into the multiplication medium. Finally, the genotypes that presented embryogenic lines with "high" multiplication capacity were those in which the lines increased five times or more from the original size.

Obtaining of somatic embryos

After the multiplication stage, the embryogenic lines were conditioned in regeneration medium. The regeneration medium was constituted of MB with addition of 0.1 μM 2,4-D, active charcoal (Sigma, USA) and 1000 μM of putrescine. After inoculating embryogenic lines in this media, the dishes were sealed with PVC film (Rolopac[®]) and kept in the growing room, at a temperature of $27 \pm 1^\circ\text{C}$ in absence of light for 60 days.

Germination of somatic embryos

The culture medium for germination constituted of MB supplemented with 0.54 μM of NAA and 1000 μM of putrescine (Corrêa et al., 2015). The obtained somatic embryos in the previous stage were individualized and inoculated into test tubes (150 x 25 mm) containing 10 ml of the germination medium and after 30 days, were put into jars to complete germination. The test tubes and/or jars were kept in the growing room, at a temperature of $27 \pm 2^\circ\text{C}$ with a photoperiod of 16 h/day and irradiance of $\pm 40 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by tubular lamps of LED (18W, Arapecalluminação LED). From the 60th day, counting of the number of obtained plants was registered in the process. Only plants with complete root and

shoot development were considered in that counting.

Acclimatization of clonal seedlings

For the acclimatization, the plantlets' roots were rinsed with regular water to remove the excess of culture medium. Next, the plantlets were inoculated into test tubes (150 x 25 mm) containing 10 mL of nutritive solution $\frac{1}{2}$ strength (macro- and micronutrients Y3 reduced to half) for a period of seven days. These were kept in the growing room, at a temperature of $27 \pm 2^\circ\text{C}$ with a photoperiod of 16 h/day and irradiance of $\pm 40 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by tubular lamps of LED (18W, Arapecalluminação LED).

After this period, the plantlets were transferred to plastic tubes of 63 mm in diameter and 130 mm tall, filled with 180 cm^3 of substrate Plantimax[®], with addition of 8.0 kg of simple superphosphate per m^3 of substrate. Next, the plantlets were transferred to the greenhouse and kept in intermittent misting chamber, controlled by a temporizer with fixed irrigation interval.

The programming used for the mist was an irrigation of ten seconds every 20 min in the first 15 days. After this period, the mist occurred at every hour, with irrigation time of 20 s. The irrigation nozzle used had a flow rate of 120 mL/min. The maximum temperature reached in the interior of this system was 35°C . A Thermo-reflective blanket (Aluminet[®]) with 50% of light restriction provided the shading of the mist chamber. The plantlets were supplied weekly with 20 mL of $\frac{1}{2}$ force nutritive solution. After 90 days of acclimatization, the clonal seedlings were sent to Agropalma S.A.

In the company, the seedlings were transplanted to plastic bags with a capacity of 15 L, dimension of 25 cm in diameter and 33 cm tall, containing a composed substrate of 60% soil and 40% composting material (industrial residues of oil palm). The seedlings were kept in acclimatization in pre-nursery protected with 50% shade from 30 days. After 30 days, seedlings were transferred to open nursery to complete their development and, subsequently, to implantation of the clonal garden.

RESULTS

Referencing of the apical meristem

The study of referencing of the apical meristem of the adult palm performed for secure extraction of the palmito indicated that cutting of the palmito must be done minimally at 30 cm height of the last open inflorescence, regardless if the plant is male or female. Being extracted at this height, apical meristem was preserved and protection was continued, ensuring the resumption of the plant growth matrix.

Contamination index

The contamination index in all genotypes was less than 5%, indicating that the use of immature leaflets from the palmito region as explants is an excellent alternative for induction of somatic embryogenesis in oil palm, since tissues are protected by external foliar layers. Moreover, disinfection methodology was also efficient, allowing the success of *in vitro* establishment of all selected elite genotypes.

Table 2. Rate of callogenesis after 90 days of *in vitro* culture in induction medium for the 32 elite oil palm genotypes (*E. guineensis* Jacq.) selected for cloning through somatic embryogenesis using putrescine.

Genotype	Genetic background	Callogenesis (%)
A-01	Deli x Ekona	5.35
A-02	Deli x Ekona	7.15
A-03	Deli x La Mé	7.98
A-04	Deli x La Mé	52.05
A-05	Deli x La Mé	35.15
A-06	Deli x La Mé	21.76
A-07	Deli x Ghana	16.78
A-08	Deli x Ghana	4.77
A-09	Deli x Yangambi	5.68
A-10	Deli x Yangambi	3.91
A-11	Deli x Dami	4
A-12	Deli x Dami	1
A-13	Deli x La Mé	2.77
A-14	Deli x La Mé	4.12
A-15	Deli x Avros	6.2
A-16	Deli x Avros	5.88
A-17	Kigoma	9.53
A-18	Kigoma	2.92
A-19	Deli x La Mé (Embrapa)	1.61
A-20	Deli x La Mé (Embrapa)	1
A-21	Deli x La Mé	2.61
A-22	Deli x La Mé	3.47
A-23	Deli x Yangambi	6.09
A-24	Deli x Yangambi	0.62
A-25	Deli x Yangambi	6.18
A-26	Deli x Yangambi	1.11
A-27	Deli x Yangambi	0.28
A-28	Deli x Yangambi	10.04
A-29	Deli x La Mé (Embrapa)	1.14
A-30	Deli x Ghana	4.58
A-31	Deli x La Mé	17.21
A-32	Deli x La Mé (Embrapa)	4.62

Induction of callus formation

At 90 days after induction of somatic embryogenesis, it was observed that all 32 elite genotypes selected responded to callogenesis induction.

Percentages of response of evaluated genotypes varied. Some presented only 1% of explants with developed callus and others reached up to 52.05% (Table 2).

Characteristics of the developed callus were similar in all the genotypes, occurring generally in the extremities of the foliar explant at the region where it was cut and selected. All developed callus presented nodular aspect with globular structures and beige to light yellow coloration (Figure 2A).

Embryogenic lines

The formation of embryogenic lines, main factor to guarantee the production of oil palm clones through somatic embryogenesis, occurred in 65.62% of the elite genotypes tested (Table 3). It was observed that even in those genotypes in which the response to callogenesis was low (e.g., genotype A-20 with 1% of formed callus) formation of embryogenic lines was presented (Table 2). Although, the formation of embryogenic lines has occurred in most genotypes submitted to induction of somatic embryogenesis, the continuous capacity of proliferation showed differentiated responses. Among the genotypes that formed embryogenic lines, 40.62% showed these structures with a multiplication capacity classified as low or null (Table 3).

Embryogenic lines could be observed between the 30th and 60th day of inoculation of the callus in multiplication medium. These can be viewed without the aid of magnifying glasses, since they have different characteristics as compared to those callus that originated them. The callus that gave origin to the embryogenic lines had mucilaginous aspect, light brown to beige coloration. The lines were friable, showing granular aspect and yellow coloration (Figure 2B). Even after the 10th consecutive subculture, all the embryogenic lines (even those with low multiplication capacity) did not lose their quality and potential to regenerate plants.

Formation of somatic embryos

After 60 days in regeneration medium, the embryogenic lines were differentiated, forming somatic embryos that eventually completed their development and maturation. The somatic embryos had white coloration and hardly adhered one to the other (Figure 2C). Moreover, with the aid of a scalpel, these could be separated, subdivided and transferred to germination medium. At 20 days in the germination medium, somatic embryos was elongated and changed coloration to whitish green (Figure 2D). Between the 30th and 60th culture day in this medium, the somatic embryos turned into complete plantlets, with shoot and root (Figure 2E).

Acclimatization of the clonal seedlings and commercial pre-nursery establishment

After 90 day in the germination medium, 92% of the plantlets were acclimatized successfully demonstrating that the method used for this purpose was efficient. The process of acclimatization was completed at 90 days, when plants reached a height of approximately 25 cm and 4 expanded leaves. A total of 158 ramets (plants) of each elite genotype was sent to the company, which were established in pre-nursery (Figure 2F) with this plantation already established (Figure 2G).

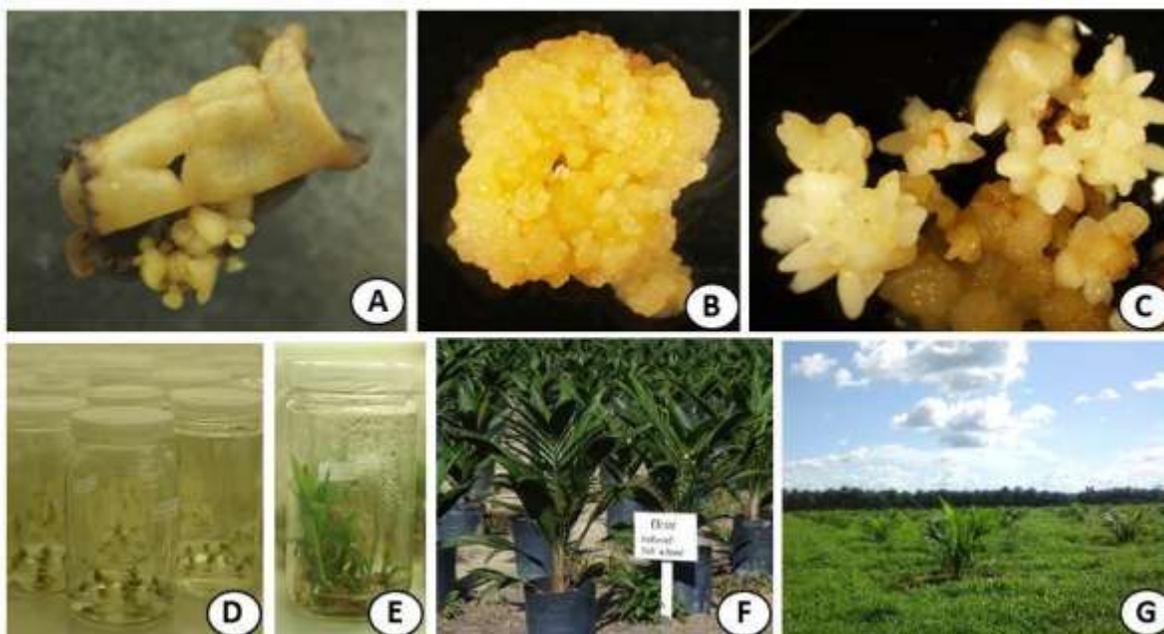


Figure 2. Somatic embryogenesis from leaf explants of the oil palm. A: Callus with nodular-defined globular structures with beige to light yellow color formed at the end of leaf explants after 90 days of cultivation *in vitro* through induction. B: Embryogenic lineage with nodular structures and yellowing after 60 days of *in vitro* culture in multiplication medium. C: Somatic embryos with white color adhered to each other, obtained after 60 days of *in vitro* culture of embryogenic lines in regeneration medium. D: Somatic embryos in germination medium, with stretching and color changing. E: Seedlings obtained after 60 days of *in vitro* culture in germination medium. F: First Brazilian oil palm clone obtained after 12 months of somatic embryogenesis induction in adult plant oil palm. G: Genotype clones A-02 established in commercial plantations. Bars: A, B and C equal to 3 mm; D and E equal to 1.5 cm.

Table 3. Classification of the 32 oil palm genotypes (*E. guineensis* Jacq.) selected for cloning through somatic embryogenesis using putrescine, for the capacity of formation and multiplication of embryogenic lines.

Multiplication capacity of embryogenic lines	Percentage of genotypes (%)	Genotypes
Null	34.37	A-01; A-03; A-06; A-11; A-12; A-19; A-23; A-24; A-25; A-27; A-32
Low	25	A-05; A-07; A-09; A-14; A-22; A-26; A-29; A-13
Moderate	12.5	A-04; A-18; A-30; A-31
High	28.12	A-02; A-08; A-10; A-15; A-16; A-17; A-20; A-21; A-28

*Null: genotypes that have not formed embryogenic lines. Low: genotypes formed embryogenic lines that increased twice the initial size. Moderate: genotypes that showed embryogenic lines that increased up to three times the initial size. High: genotypes that formed embryogenic lines with multiplication capacity by up to five times the initial size.

The required time for somatic embryogenesis in oil palm using procedures proposed here from obtaining of the foliar explants to acclimatization of the plants, was up to 360 days, and the putrescine was a promissory alternative to substitute the cytokinin, ensuring the success of obtaining oil palm clones in reduced time. The first Brazilian clones of oil palm from adult plants were obtained with success in this work. There is still need to evaluate the performance of these materials in the field, for the “mantled flower” emergence.

DISCUSSION

Tissue culture through somatic embryogenesis is an attractive alternative to massively propagate elite genotypes of oil palm (Ooi et al., 2013). Nevertheless, the efficiency of plant regeneration is strongly associated with the chosen type of explant. The most used explants for induction of somatic embryogenesis are immature leaflets, which are more responsive and offer less risks of contamination. These structures are found to be

protected from microorganisms contact, and are wrapped by old leaves inside the palmito, thus not requiring severe disinfection methods that could cause damage to the explants (Corley and Tinker, 2003). This has been proven by the low index of contamination observed in the tested genotypes. In this study, the extraction of immature leaves caused no damage to the donor plants, opposite to what is described in the literature (Corley and Tinker, 2003), being the donor plants completely healthy and recovered after months of the extraction.

The doses of 2,4-D used to induce somatic embryogenesis in this work (800 μM) was high as compared to those described by other authors, who recommend concentrations of 2,4-D that vary from 10 to 450 μM (Yusnita and Hapsoro, 2011; Scherwinski-Pereira et al., 2010; Rajesh et al., 2003;). In general, in oil palm, high auxin concentrations are always used in the presence of activated charcoal (AC) (Yusnita and Hapsoro, 2011). In this work, this high concentration of auxin with AC was proved efficient for inducing embryogenesis in oil palm, leading to formation of callus in all tested genotypes.

The percentage of explants that formed callus varied among crossing groups as well among genotypes of the same group. Silva et al. (2012) and Thawaro and Techato (2009) considered that the response to induction of somatic embryogenesis in oil palm is a characteristic dependent on the genotype. The response can also vary among explants of the same genotype, and are related to the physiological state of the involved. According to Ooi et al. (2012), the sensibility of tissues to the induction of somatic embryogenesis is deeply related to the endogenous level of auxin present in the explants. The endogenous level of auxin can be increased from exogenous application of 2,4-D, thus stimulating the totipotency of cells (Gueye et al., 2009).

The formation of embryogenic lines, assured cloning of 65.62% of the tested genotypes. Moreover, a great part of these genotypes (40.62%) produced lines with moderate and high multiplication capacity. This allows this method to be used for cloning at a commercial scale of these genotypes. As compared to reports made by Soh et al. (2011) and Kushairi et al. (2010) where low efficiency of the cloning process with success rates of 3 to 6% was mentioned, the adopted cloning process in this work can be considered efficient. Kushairi et al. (2010) claimed that the rate of somatic embryogenesis in oil palm is low due to modifications made to the cloning protocols in order to avoid the appearance of the "mantled flower", a floral abnormality that occurs in plants from the *in vitro* culture, affecting severally the productivity potential due to the production of parthenocarpic fruits (Corley et al., 1986).

The main modification in micropropagation protocols of oil palm adopted to control "mantled flower" was the reduction or even elimination of cytokinin as multiplication inductor in culture medium (Eeuwens et al., 2002; Jaligot

et al., 2002; Jaligot et al., 2011) believing that cytokinin stimulates the hypomethylation of DNA, which results in a homeotic transformation of the floral organs. As negative consequence of this, the "mantled flower" appears in micropropagated plants of oil palm (Eeuwens et al., 2002; Ong-Abdullah et al., 2015). In the protocol used in this work, cytokinin was successfully substituted with the polyamine "putrescine". In others palms, such as coconut, Rajesh and Karun (2014) suggested an important role for polyamines in somatic embryogenesis. Polyamines in plants can serve as intracellular mediators of hormonal activity (Redha and Suleman, 2014) and are involved in diverse physiological processes in plants, including embryogenesis and organogenesis (Alcázar et al., 2010), protection of DNA against damages caused by reactive oxygen species (ROS) (Ha et al., 1998), and in the control of DNA methylation (Brooks et al., 2010).

Polyamines play a stabilizing role on nucleic acids by binding to phosphate groups, and in particular, they form complexes with DNA, this binding leads to stabilization of the nucleic acid structure or changes in their conformation (Hou et al., 2001).

Besides the use of putrescine to substitute cytokinin which is successful in all the stages of somatic embryogenesis in oil palm, the proposed protocol was efficient in relation to the necessary time for production of clonal seedlings (up to 360 days). As compared to literature descriptions, this time is considerably shorter. Wong et al. (1999) reported that 12 months is the time spent just to obtain callus. In more recent works, Soh et al. (2011) reported that after modifications of the actual protocols to avoid appearing of "mantled flower", these became ineffective in relation to the necessary time to produce clones. For these authors, the time spent from explant preparation to the obtaining of clones is 29 months. When a liquid system is used, this time is reduced to 18 months, which is still longer than the time described in this work.

From the results of this work, it was not possible to state that putrescine will inhibit the appearance of "mantled flower" from the obtained clones, since this demands years of field evaluation. However, it is possible to assure that putrescine was efficient for substituting cytokinin, in function of the multiplication of embryogenic lines, regeneration of somatic embryos and germination and obtaining of the plants in reasonably short time. In this context, conduction of phenotypic analysis for the appearance or not of the "mantled flower" is suggested, once clones have reached productive age.

Conclusion

The proposed protocol is efficient for the procedure of extracting foliar explants, not compromising the growing of the matrix plant. The response to somatic embryogenesis in oil palm can be considered genotype-

dependent, where a great part of the tested genotypes showed responsiveness to the used technique. Putrescine is a promising alternative for substituting cytokinin and guarantees the success in obtaining clones of oil palm in reduced time. Then, this protocol is recommended for propagation of oil palm clones at a commercial scale. The first Brazilian clones of oil palm from adult plants were obtained successfully in this work. Yet, it is necessary to evaluate the performance of these materials in field, with regards to the appearance of the "mantled flower".

Conflict of interest

The authors have not declared any conflict of interest

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