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Callus Induction from Zygotic Embryos of Coconut MATAG F2

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Authors' contributions

This work was carried out in collaboration between all authors. Author ARZ were involved in overall planning, conceived the idea and designed the experiments. Author GSK made a major contribution to conducting experiments. Author NA made a major contribution to conducting experiments. Author MSSF made a major contribution to conducting experiments. Author OAN made a minor contribution to conducting experiments. All authors read and approved the final manuscript.

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ABSTRACT

The effect of 2,4-D (in concentrations of 5.0, 10.0, 20.0 and 40.0 mg/L) applied in combination with BAP (1.0, 10.0 and 20.0 mg/L) on callus induction in coconut MATAG F2 zygotic embryos cultured on Murashige and Skoog (MS) medium was investigated. The effects of IBA, TDZ and NAA combined with 2,4-D were also tested. The best callus formation (20%) was obtained on MS medium supplemented with 2,4-D at 10.0 mg/L. The induced calli were yellowish in colour and structurally compact. Different portions of the zygotic embryo were also compared for callus induction when used as explants and cultured on MS medium supplemented 10.0 mg/L 2,4-D. The incidence of callus formation (up to 83%) was highest from the middle portion of the embryos.

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Keywords: 2,4-D; plant growth regulator; MATAG coconut; zygotic embryo; callus induction.

1. INTRODUCTION

Coconut palms (*Cocos nucifera* L) are grown in more than 90 countries of the world, yielding a total production of 61 million tonnes of coconuts per year. Tropical Asia accounts for most of the world's production, with 73% coming from Indonesia, Philippines and India (FAOSTAT) [1]. Coconut is known for its numerous traditional uses, ranging from food to cosmetics [2], earning the palm the moniker 'the tree of life'. Coconut cultivation faces a number of major problems that reduce its productivity and competitiveness. These difficulties are being addressed in field and laboratory research, with in vitro technology featuring prominently in the latter. Over the past 60 years, much attention has been directed towards developing and improving protocols for (i) embryo culture; (ii) clonal propagation via somatic embryogenesis; (iii) homozygote production via anther culture; (iv) germplasm conservation via cryopreservation; and (v) genetic transformation [3]. Basic in vitro techniques are the common feature in these areas of research. While somatic embryogenesis of the coconut palm has been intensively studied, a protocol that enables efficient regeneration of plants has not been perfected to enable its production on a large scale [4]. This is partly attributed to difficulties encountered in the initiation of callus that serves as a starting material for plantlet regeneration. In general, callus induction through the in vitro technique is dependent not only on the plant species but also on experimental variables such as light, type of explants, explants age, hormone used, and type of medium [5-7]. A medium containing 2.0 mg/L BA + 2.0 mg/L2,4-D,or 2.0 mg/L 2,4-D on its own has been reported to give the best response in callus proliferation in potato [8]. Abd Elaleem et al. [9] reported that callus formation was best achieved with 3.0 mg/L 2,4-D or 2.0 mg/L 2,4-D +2.0 mg/L BA. Nevertheless, the exact growth regulator requirements could vary between different cultivars of coconut. This paper presents the results of a study carried out to determine the most suitable concentrations of 2,4-D and other plant growth regulators (BAP, IBA, TDZ and NAA) for *in vitro* callus production of coconut MATAG F2.

2. MATERIALS AND METHODS

2.1 Plant Materials and Aseptic Culture

Inoculation of zygotic embryos (Fig. 1a) was conducted in the laminar flow that was cleaned with 70% ethanol before commencing work. Scalpel blades and forceps were first immersed in 70% ethanol and flamed on the Bunsen burner before they were used to extract the zygotic embryos of MATAG coconuts. The excised embryos were sterilized by immersion in 50% ethanol for 2 min, followed by 1% Vircon for 30 min. Further sterilization using Clorox (sodium hypochlorite, 5.2% with two drops of Tween 20, a polysorbate surfactant, added as a detergent and emulsifier). The embryos were immersed in 50% Clorox for 40 min, followed by immersion in 10% Clorox for 20 min. After rinsing three times in sterile distilled water, the embryos were cultured on the specified media and incubated at 26 ± 2℃ in the dark. The cultured embryos were then observed after 2 months and the percentage of callus induction recorded. The cultured embryos were then observed after 3 months and the percentage of callus induction was recorded.

2.2 Effect of Plant Growth Regulators on Callus Induction

Sterilized zygotic embryos were placed in 150 mL flasks containing 40 mL of Y3 medium together with 2,4-D in concentrations of 0, 5.0, 10.0, 20.0 and 40.0 mg/L, either on their own or in combination with BAP (1.0, 10.0 and 20.0 mg/L) as listed in Table 1. The MS medium was supplemented with 0.1 g arginine, 0.1 g asparagine, 0.1g glutamine, 3.0% sucrose and 0.3% gelrite agar for gelling, A total of 20flasks containing 5 zygotic embryos each were used for each treatment. Medium sterilization was performed by autoclaving at 121℃ for 20 min. The medium pH was adjusted to5.7-5.8 before adding agar. Observations were recorded at monthly intervals. Results were expressed as the percentage of callus induction after 3 months of culture. Using results from the aforementioned experiment as a guide, a similar experiment was repeated using two selected different combinations of 2,4-D (10.0 and 20.0 mg/L)and another plant growth regulator, IBA, TDZ or NAA, at concentrations of 1.0 and 10.0 mg/L. Observations were recorded at monthly intervals. Results were expressed as the percentage of callus induction after 3 months of culture.

2.3 Propensity of Different Portions of the Zygotic Embryo to Callus Induction

Another experiment was conducted to examine the propensity of different portions of the zygotic

embryo to callus initiation. Zygotic embryos were each cut into three segments i.e. the top, middle, and bottom segments. The zygotic embryo segments were then transferred onto the fresh medium containing 10.0 mg/L 2,4-D. The percentage frequency of callus formation was determined.

2.4 Statistical Analyses

All statistical analyses were performed using SPSS software. The experiment followed a completely randomized design, using 20 flasks (150 mL) for each treatment. Each flask contained 5test samples and all experiments were repeated twice. The means and standard errors (indicated as ±values) were calculated for the treatment responses.

3. RESULTS AND DISCUSSION

3.1 2,4-D and Callus Induction in Zygotic Embryos

Whole embryos (Fig. 1a) were cultured on Y3 medium containing different concentration of 2,4-D and BAP. As shown in Table 1, there are differences in the callus induction data arising from the variations of the plant growth media used with respect to percentage of callus initiation. The highest callus induction in embryos was obtained in treatment with 20 mg/L 2,4-D (30%) but some browning was observed (Fig. 1c). A treatment 10 mg/L 2,4-D (20% of callus initiation) showed the best for callus initiation with no browning. The callus was compact, translucent & yellowish after 3 months of incubation. Result showed that application of higher 2,4-D at 40 mg/L resulted loose, more browning and darker to the derived callus (Fig. 1e). Combination of low 2,4-D (5 mg/L) and BAP (10 mg/L) was found to be effective on single shoot structure inanition. There was root structure and some callus also observed (Fig. 1d). The positive effect of 2,4-D in promoting the callus was also detected by Tahir et al. [10]. Their statement that callus development was directly related to the presence of 2,4-D in most plant Species in plant tissue culture work. It was observed that highest percentage of Sugarcane (*Saccharum officinarum*) callus formation at 3.5 mg/L 2,4-D. According to Qin et al. [11] and Xing et al. [12], this was considered as a process of dedifferentiation of organized tissue. Thus, there was optimum Callus induction with increase in 2,4-D concentration [12]. According to Yusnita& Haisoro [13] that work with oil palm, it was shown that increasing of 2,4-D concentrations from 1-4 mg/L led to the increasing percentage of callus formation, but higher concentration of 2,4-D up to 10 mg/L resulting decreasing percentage of callus formation after 7 months of culture.

3.2 Effect of the Plant Growth Regulators IBA, TDZ and NAA on Callus Induction

Two different concentrations of 2,4-D (10.0 mg/L and 20.0 mg/L) in combination with the plant hormones IBA, TDZ or NAA at 1 and 10 mg/L were used in MS medium to observe callus induction using zygotic embryos as explants. The rate of callus formation varied in different treatments used as shown in Fig. 2. Callus induction was 30% in the presence of the auxin 2, 4-D (10.0 mg/L) and IBA (10 mg/L). However, the callus obtained was watery and soft. Initiation of callus that was yellowish and compact was obtained with 2,4-D at 10.0 mg/L alone, confirming the earlier observation (Fig. 1b). Callus induction in treatment of NAA (10.0 mg/L) and 2, 4-D (10.0 mg/L) showed at 2% (Fig. 2). The results obtained in this study were broadly similar to those reported by Perez et al. [14] where callus was induced in immature zygotic embryos of *Carica papaya* L. Maradol using 2,4-D at a concentration of 15 mg/L. Bett et al. [15] found that an increase in 2,4-D levels enhanced the percentage callus induction of sweet potato. Their finding showed that the highest rate of callus induction was recorded in all varieties for both leaf and stem explants at high concentrations of 2,4-D. Previous studies by Liu and Cantliffe [16] and Jarret et al. [17] showed that 2,4-D at 3.0 mg/L was effective in inducing somatic embryos, although no callus was produced with a similarly low concentration in the present study.

3.3 Propensity of Different Portions of the Zygotic Embryo to Callus Induction

Zygotic embryos were cut into three portions, i.e. the top, middle and bottom segments. These explants were then cultured on the medium containing 10 mg/L 2,4-D and observed for callus induction. The embryo portions showed different rates of callus induction, with embryos from middle segment performing the best (Fig. 3). In experiments that compared callus formation in node, leaf and roots, Savita et al. [18] observed that nodal segments were the best for callus induction. Furthermore, lower concentration of

2,4-D (1 mg/L) was sufficient to induce callus in 96% of cultures from nodal segments whereas a higher concentration of 2,4-D (4 mg/L) was required for 98% callus induction in leaves.

Table 1. Effect of 2,4-D and BAP on callus initiation in whole zygotic embryos of MATAG F2

Treatments (mg/l)		Callus initiation (%)	Remarks
2,4-D	BAP		
0	0	0	No callus, explants browning
5	1	0	Embryos break
5	10	0	Single shoots structure produced
5	20	0	Embryos break
10	0	20	Compact callus, translucent & yellowish
10	1	20	Compact callus, translucent & yellowish
10	10	10	Compact callus, translucent & yellowish
10	20	5	Compact callus, translucent & browning
20	0	30	Compact callus, with some browning
20	1	20	Compact callus, with some browning
20	10	10	Compact callus, with some browning
20	20	0	Soft callus, browning
40	0	1	Loose, compact callus, all calli brown
40	1	5	Loose, compact callus, all calli brown
40	10	0	Loose, compact callus, all calli brown
40	20	0	Loose, compact callus, all calli brown

Data collected after 3 months of culture

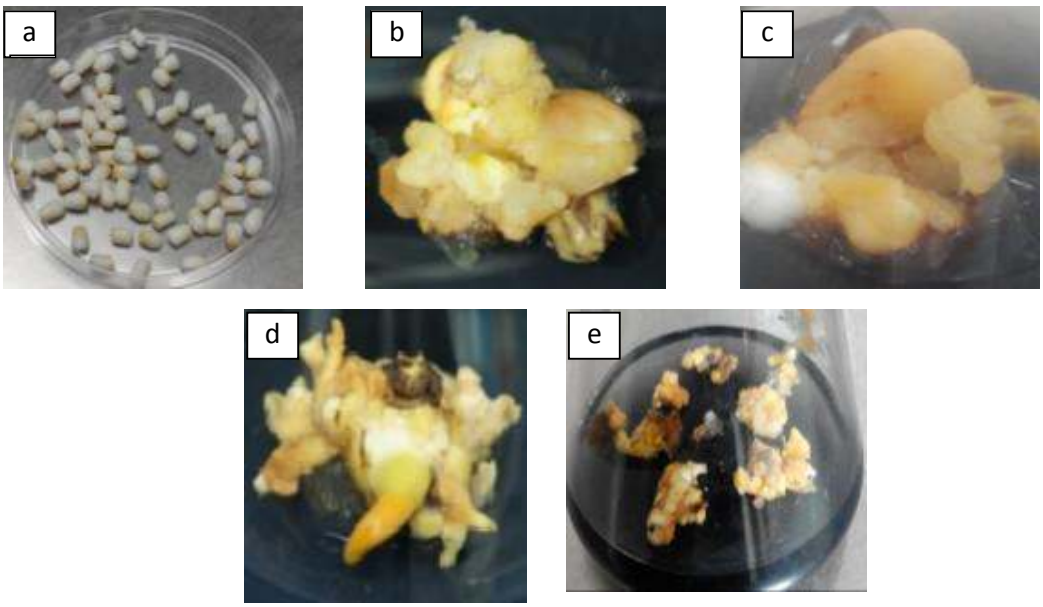

Fig. 1. Callus initiation and development in whole embryos cultured in MS medium containing 2,4-D and BAP. (a) Whole embryos after removing the endosperm. (b) Callus initiated on medium containing 10 mg/L 2,4-D. (c) Callus initiated on medium containing 20 mg/L 2,4-D. (d) Single shoot structure with root shown on medium containing 2,4D+BAP. (e) Loose callus with marked browning developed on medium with high 2,4-D concentration (40 mg/L)

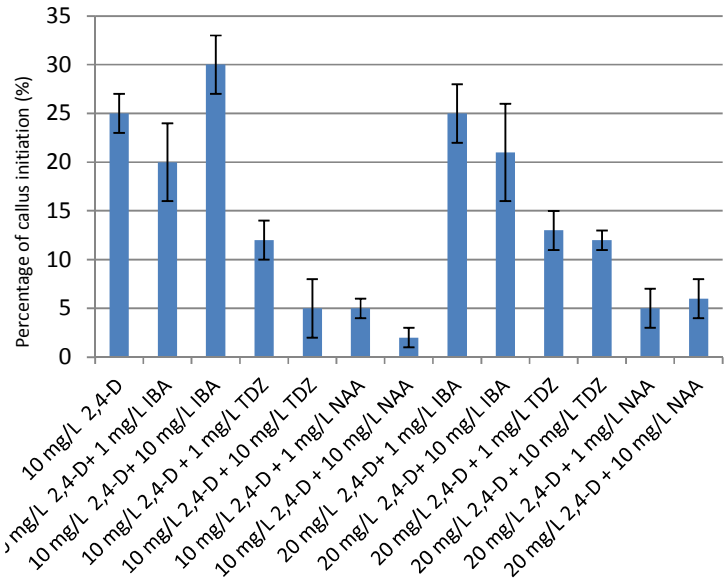

Fig. 2. Effect of 2,4-D at concentrations of 10.0 mg/L and 20.0 mg/L combined with various plant various growth regulators on callus induction in zygotic embryos

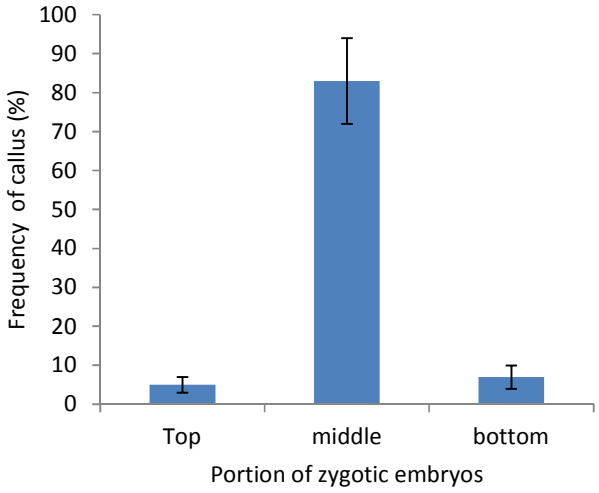

Fig. 3. Effect of different portions of the zygotic embryos on callus induction

4. CONCLUSION

The highest rate of callus induction in coconut MATAG F2 zygotic embryos cultured in MS medium was observed in the presence of 10.0 mg/L2,4-D, with the middle portion of the embryo giving the best response.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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