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MICROPROPAGATION OF *CALATHEA MEDALLION* AN ORNAMENTAL PLANT

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Abstract

An *n vitro* arranged propagation was formed for micropropagation in specified lovely ornamental plant, *Calathea medallion*. In the present study, the outcome of numerous sterilization techniques on axillary shoot buds obtained from the explants which were sprouted from rhizomes giving the highest Survivor percentage 63.3% when treated with of 30% (v/v) NaOCl + 70% (v/v) ethanol + 1 g/l (w/v) $HgCl_2$. Also the media supplemented with different concentrations of plant growth regulators on induction of shoots were examined, the highest number of multiple shoots 21.2 shoot/plant was obtained on MS basal Medium supplemented with 1, 5 mg/L 6-Benzylaminopurine (BAP) + 1,5 mg/L 1-Naphthaleneacetic acid (NAA) + 30 g/L sucrose + 6 g/L plant agar and was used as multiplication medium. Test the effects of activated charcoal on the maximum roots induction 5.86 root/plant was recorded on MS medium supplemented with 1.5 mg/L Naphthalene acetic acid (NAA) + 1.5 mg/L indole butyric acid (IBA). The plantlets were successfully acclimatized under greenhouse condition.

Key words : *Calathea medallion*, Micropropagation, growth regulators.

Introduction

Calathea medallion is one of the important ornamental plants of Marantaceae family; it produces very attractive inflorescence and has been used in landscaping especially for screening and indoor plant.

The other *Calathea* sp. has also been used widely in horticultural industry due to its attractive foliage colors and variegation patterns (Yang and Yeh 2008), showing variation in its vase life of the inflorescence like the other exotic tropical ornamentals such as *Heliconia* sp., *Strelitzia* sp. and *Alpinia* sp. (Paull and Chantrachit 2001).

Calathea was traditionally propagated through seeds and by vegetative techniques using the rhizome cuttings and micro propagation such *Calathea ornata Koern*, *Calathea orbifolia (Linden) Kennedy*, and *Maranta leuconeura cv. Kerchoviana* (Yang and Yeh 2008; Podwyszy1997; Ebrahim and Ibrahim 2000).

In vitro shoot multiplication of *Maranta leuconeura E. Moor. 'Kerchoviana'* had been occurred when the shoot tip explants were cultured on Murashige and Skoog (MS) medium supplemented with 5 mg/L^{-1} BAP (Ebrahim and Ibrahim 2000).

A modified MS medium containing 2.5 mg/L^{-1} BAP and 2.5 mg/L^{-1} kin resulted in the most recorded of *In vitro* shoot multiplication of *Calathea ornate Koern. 'Sanderiana'* (Podwyszyn´ska 1997).

Ex Vitro acclimatization remains a critical stage for young tissue cultured plants during which they have to cope with different stresses and to adapt to the environmental changes.

The special conditions during *In vitro* culture cause morph physiological disorders of plantlets that resulted in poor survival during acclimatization (Hazarika 2006). Malfunctioning of stomata and poor development of the photosynthetic apparatus are the major constraints for successful acclimatization (Wetzstein and Sommer 1982; Preece and Sutter 1991).

Materials and Methods

Plant material active growing shoots were excised from adult *Calathea medallion* plants and used as donor

plants supplying the initial explants lateral buds (2-4 mm).They were isolated from the cuttings which were purchased from Pico Modern Agriculture Company greenhouses.

The explants were washed with detergent under running tap water to remove any soil particles attached to the shoot buds.

The external leaves were removed and the shoot buds were trimmed down until the size ranged from (2.5 to 3.0 cm).

The explants were then rinsed under running tap water for 30 min. Followed by surface sterilizing with three different treatments.

Culture media The Murashige and Skoog (1962) culture media (MS) had been used at full strength supplemented with different concentration of hormones as cytokinins (KIN, BAP and NAA) either alone or in combinations to investigate its effect on the multiplication stage of Calathea. The medium was prepared using stock solutions of the mined salts and vitamins (stored frozen) in distilled water. After addition of sucrose, necessary additions of growth regulators and other additives were made from stock solutions stored below 5°C and the pH was adjusted to 5.7 with NaOH or HC1. For preparation of solid medium, agar or microagar was added and melted on electric heater before dispensing into the sterilized culture vessels.

These experiments were carried out at the tissue culture lab of Pico Modern Agriculture Company, Mansoriya, Giza, Egypt.

Surface sterilization suitable explants (360) were collected, divided into (3) groups and washed with detergent under running tap water to remove any soil particles attached to the shoot buds, under aseptic conditions, previously prepared surface sterilized with three different treatments as followed:

- Treatment (1)

  30% NaOCl → 30% NaOCl → 70% ethanol
- Treatment (2.A)

  30% NaOCl → 70% ethanol → 100 mg/l $HgCl_2$

  Treatment (2.B)

  30% NaOCl → 70% ethanol → 1 g/l HgCl2
- Treatment (3)

  30% NaOCl → 70% ethanol → 300 mg/l $HgCl_2$.

The treated explants were placed under aseptic conditions inside the culture cabinet (laminar air flow), in tubes containing 10 ml of MS basal medium.

This stage contained three treatments and each treatment consisted of twenty tubes as replicates and each tube contained one explants. These tubes were incubated for six weeks in growth room at 25±2°C and 16 hr. illumination of 2000 LUX (white fluorescent lamps). Contamination and survival percentages were recorded.

Culture containers glass containers light transmittance 85%, capacity 350 ml culture vessels, were autoclaved and used for culture process. The test used tubes were with the dimension of 150 mm x 25 mm. All empty glassware and water for surface sterilization of explants were autoclaved at the temperature of 121°C and pressure of 1.5 kg cm-2 for 20 min.

Culture incubation conditions cultures were incubated at the temperature of (25±2°C). Under illumination with cool daylight fluorescent tubes 2000 LUX with photoperiod of 8/16 L/D hr. They were normally maintained for 4 weeks of growth before subculturing onto fresh media. According to the growth response and emergency problems, the cultures were usually evaluated after each culture on a particular media.

Transfer of plants to soil plantlets of *Calathea medallion* were shifted from the rooting medium to soil after washing the roots with water to remove the medium traces. They were individually transplanted to plugs (around 2*5 cm) containing autoclaved mixture of peat moss + perlite (2:1) as the transplanting medium. Initially the plantlets were covered with polyethylene film, which will be gradually eliminated in two weeks, then placed in a shaded greenhouse for completing their acclimatization, as they were transferred in to 10 cm plastic pots.

Layout of the experiments and statistical analysis

The following data had been recorded:

- Survival percentage.
- Callus formation.
- Number of shoots / explant (plantlet).
- Number of leaves / explant (plantlet).
- Plant length.
- Number of roots / explant (plantlet).
- Root length.
- Number of buds / explant (plantlet).

Results and Discussion

Culture establishment

Although several workers (Gill and Gill, 1994) had suggested frequent transfer of explants to fresh medium for the establishment of cultures.

In vitro Multiplication of shoots through subculturing

The data in table 1 revealed that all the studied criteria

had been significantly affected with the different culture media containing different supplemented with various concentrations and combinations of hormones (KIN & BAP). The data pertaining to the effect of BA and NAA alone or in all possible combinations on shoot multiplication.

Plant length

Table 1: Effect of different concentrations of KIN and BAP and their combinations on plant growth in multiplication stage of *Calathea medallion*.

Number of plants full formation	Root length (C.M)	Number of roots	Number of buds	Number of leaves	Plant length (C.M)	Treatment mg/lMS +1 mg/l NAA + 30 g/l sucrose +6 g/l agar
4.5	5.9	3.85	2.73	2.36	4.63	1,5KIN
4.46	5.8	3.88	3.13	2.28	4.93	2KIN
5.2	6.53	4.43	4.48	2.86	6.44	2,5KIN
4.36	6.708	4.3	7.13	2.11	3.625	1,5BAP
4.88	6.01	4.16	7.68	2.23	3.98	2BAP
5.85	6.78	4.91	10.9	2.63	4.45	2,5BAP
4.85	6.35	4.76	9.9	2.25	5.18	,5KIN + ,5BAP
4.96	4.575	4.33	7.98	2.71	4.7	,5KIN + 1BAP
5.31	4.48	4.6	6.16	2.65	3.89	,5KIN + 1.5BAP
5.63	5.81	4.66	8.08	2.55	4.99	1KIN + ,5BAP
5.55	7.15	5.26	12.9	2.45	5.9	1KIN + 1BAP
7.46	7.908	5.6	12.6	2.86	6.18	1KIN + 1,5BAP
7.08	6.55	3.58	12.55	2.55	5.29	1,5KIN + ,5BAP
9.11	7.15	5.08	15.8	2.5	6.22	1,5KIN + 1BAP
7.01	8.24	5.86	21.2	2.78	6.56	1,5KIN + 1,5BAP
0.799	0.648	0.753	1.1995	0.258	0.54	L.S.D at 5%

The data in table 1 show that the culture media affected significantly on this character.

The tallest (longest) plantlet (6.56 cm) had been measured on the medium supplemented with high level of KIN (1.5 mg/L) and BAP (1.5 mg/L), followed by (6.44 cm) for medium supplemented with (1.5 mg/L) KIN.

Whereas the shortest plantlets (3.63 cm) were measured at (1.5 mg/L BAP).

The concentration of the cytokinins in the culture media resulted in taller explants than using each of them alone.

This might be explained that the combined action of the two cytokinins, i.e. KIN. And BAP affected positively on cell division and cell elongation, resulting in taller plantlets.

Using each of them alone showed limited effect on one of these criteria or the anatomical characteristic, of the plant cell.

Number of leaves

The illustrated data in table 1 revealed that the culture media supplemented with different plant growth regulators affected significantly on the number of leaves / plantlet. The greatest mean number (2.86) had been recorded for the plantlet produced on culture media supplemented with 1 mg/L KIN + 1.5 mg/L BAP.

Number of buds

The data revealed that the culture media supplemented with different plant growth regulators affected significantly on the number of buds / plantlet. The greatest mean value for number of buds (21.2) had been recorded for the plantlets on culture media containing 1.5 mg/L KIN + 1.5 mg/L BAP.

Number of roots

The data revealed that the culture media supplemented with different plant growth regulators affected significantly on the number of roots / plantlet. The greatest mean value for number of roots (5.86) had been recorded for the plantlet obtained on media containing 1.5 mg/L KIN + 1.5 mg/L BAP.

Root length

The data in table 1 show that culture media affected significantly on this character the most effective medium was that containing high level of KIN, whereas the medium containing low level of KIN resulted in the shortest roots.

Culturing the explants of Calathea medallion on MS at full strength supplemented with 1.5 mg/L KIN + 1.5 mg/L BAP resulted in the tallest roots (8.24cm) followed by (7.9 cm) resulted from culturing on medium supplemented with 1 mg/L KIN + 1.5 mg/L BAP. On the other hand the shortest roots (4.48 cm) were measured on medium supplemented with 0.5 mg/L KIN + 1.5 mg/L BAP.

Effect of KIN

The application of KIN individually in the culture media had a significant positive effect on the rate of shoot multiplication. The highest value for plant length (6.44 cm), number of leaves (2.86), shoot multiplication (4.48) had been obtained on medium supplemented with (KIN 2, 5 mg/L), which was significantly higher than the values obtained on any other KIN concentration.

Similar result showed that NAA had been widely

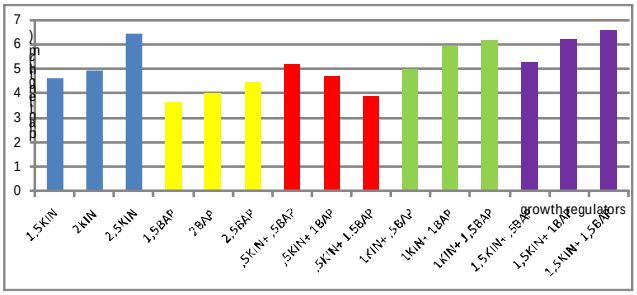

Fig. 1: Effect of different concentrations of NAA and BAP and their combinations on plant length.

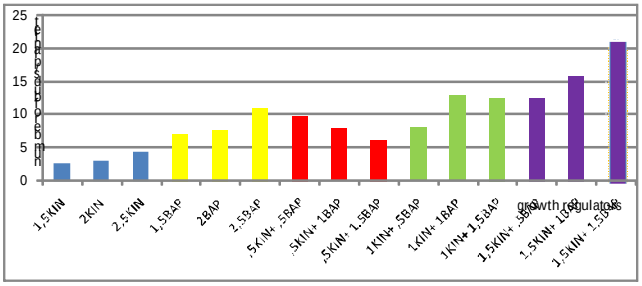

Fig. 3: Effect of different concentrations of NAA and BAP and their combinations on the number of buds / plantlet

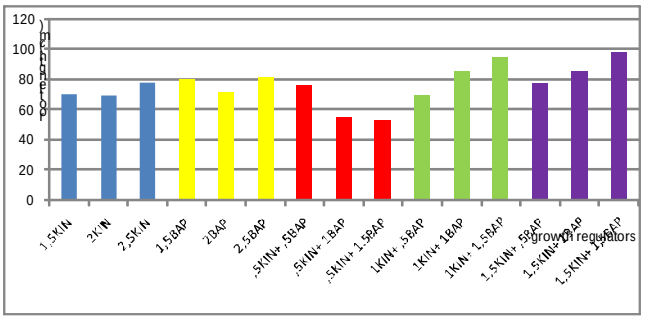

Fig. 5: Effect of different concentrations of NAA and BAP and their combinations on root length.

used for regeneration (Zhang *et al.*, 2006; Torabi- Giglou and Hajieghrari 2008; Khawar *et al.*, 2008).

Effect of BAP

The different concentrations of BAP affected a significantly on *In vitro* shoot multiplication. The highest value for plant length (4.45 cm), number of leaves (2.63), shoot multiplication (10.9) had been obtained on a medium supplemented with (2, 5 mg/L).

According to the literature BAP (6-benzylaminopurine) is a naturally occurring cytokinin Nandi 1986 BAP showed a strong effect with respect to multiplication of shoots.

(Kavyashree, 2009), (Bhau and Wakhlu, 2003) observed that high concentration of BAP decreased shoot multiplication rate of mulberry. Our findings corroborated with the findings of multiple shoot observed from 1.0 to

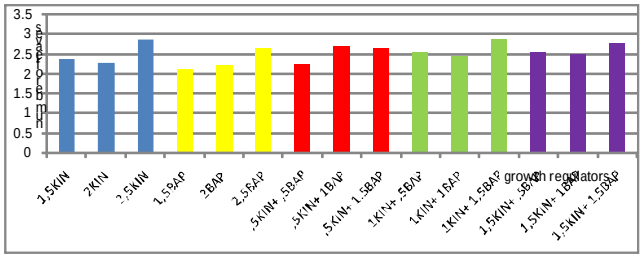

Fig. 2 : Effect of different concentrations of NAA and BAP and their combinations on number of leaves.

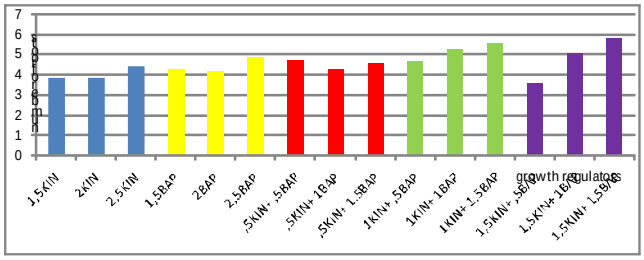

Fig. 4: Effect of different concentrations of NAA and BAP and their combinations on number of roots.

2.0 mg/L BAP (MS media) and maximum response as well as healthy shoot had been observed at 1 mg/l BAP (Ranganathan 2012).

Effect of the interaction between KIN and BAP

The interactions between KIN and BAP affected significantly on the rate of shoot multiplication cultures. When the two were incorporated together in MS medium at various concentrations, the rate of shoot multiplication was significantly enhanced. The combination of 1, 5 mg/l KIN + 1, 5 mg/l BAP gave the highest rate of shoot multiplication (21.2), plant length (6.56 cm), number of leaves (2.78).

The application of BAP was more effective as compared to KIN (Shende *et al.*, 2012).

Similar results were published on shoot induction with the earlier report on *Azadirachta indica* (Arora *et al.*, 2010), *Sida cordifolia* (Sivanesan and Jeong, 2007), *Morus alba* (Balakrishnan *et al.*, 2009) and *Podophyllum hexandrum* (Chakraborty *et al.*, 2010). Mehta et al. 2012, reported that the best shooting response was observed on MS media containing 0.5 mg/L BAP +2.0 mg/L Kin. The rate of *Zingiber* species bud multiplication was significantly different according to the various concentrations and combinations of growth regulators used.

Although explants showed a fair response to individual cytokinins used, the combinations of (BAP and Kin) were found to be ideal for shoot multiplication (Das *et al.*, 2013) Similar results were found by (Anish *et al.*, 2008) in *Bosenbergia pulcherrima*, a threatened ginger.

Persistence of explants in culture media containing higher concentration of cytokinins suppressed shoot elongation (Das *et al.*, 2013) such results are in contrary to that had been reported by other researchers, who used rather high concentrations of plant growth regulators for the multiple shoot formation for some of the Zingiberaceae species (Khatun *et al.*, 2003; Chan and Thong 2004; Bharalee *et al.*, 2005; Sultana *et al.*, 2009). Sanatombi and Sanatombi 2017 reported that BAP, KIN and NAA were found to be the best PGR combinations for micropropagation of *Zingiber montanum*, as well for Kaempferia (Chirangini *et al.*, 2005). NAA combined with BAP promoted regeneration from the callus (Viu *et al.*, 2009).

Effect of auxins at rooting stage

Variable effect of auxin on rooting was observed when NAA was incorporated at different concentrations into the MS solid medium. The highest values were obtained from that culture on medium supplemented with (0 or, 25 or, 5 or 1mg/L NAA). The highest number of roots per shoot (3.48) and longest root length (6.08) was produced on medium supplemented with 1mg/L NAA.

Inductions of few roots were reported MS basal medium half strength without any hormones, however with 0.53 ¼M NAA, improved rooting with their length (Wala and Jasrai, 2003). The greatest number of rooting root and longest were recorded on MS + 0.25 mg/l NAA and 0.50 mg/l IAA alone in the present protocol (Shende *et al.*, 2012). Similar results on root formation were obtained in *Portulaca grandiflora*. Hook (Jain and Bashir, 2010). In contrast, high concentration of NAA (2.00 mg/L) had been required to produce best rooting and root length were reported in Ginger (Kambaska and Santilata, 2009), Similar result was obtained by (Murthy *et al.*, 2010) in *Ceropegia spiralis* L. Using of activated charcoal in the rooting media facilitated rooting, as reported by Komalivalli and Rao 2000; Priyadarshini *et al.*, 2007.

***Ex vitro* Plantlets acclimatization and survival hardening**

Fig. 6: plant full formation.

Plantlets were individually planted in plugs (around 2*5 cm) containing autoclaved mixture of peat moss + perlite (2:1) as the transplanting medium. Initially the plantlets were covered with polyethylene film for two

weeks then placed in a shaded greenhouse for completing their acclimatization, as they were transferred to 10 cm plastic pots. The survival percentage was 95%.

The combination of peat and soil was reported as an effective medium for acclimatization of several micropropagated plantlets (Van Huylenbroeck *et al.*, 1998; Yaacob *et al.*, 2013; Sharma *et al.*, 2014). Peat has traditionally been used in most potted, ornamental plants (Tullio *et al.*, 2012) since it possesses good physical and chemical characteristics, stable structure, and favourable application (Kang *et al.*, 2005).

The optimum ratio of soil and vermiculite or perlite could enhance the growth rate for several species (Kadleèek *et al.*, 2001). Intermediate shade level at 50% or 60% was suitable for acclimatization and propagation of several plantlets or plant seedlings

Table 2 : Effect of different concentrations of NAA (0.0 , 0,25 , 0,5 , 1.0 mg/L) on root growth.

Number of plants full formation	Number of buds	Number of roots	Number of leaves	Root length (C.M)	Plant length (C.M)	Treatment mg/1 ms+30 g/1 Sucrose+6 g/1 agar +1 g/1 activated charcoal	
2.06	0.05	2.05	1.08	3.01	2.21	1	0 NAA
2.11	0.05	2.9	1.41	4.17	3.34	2	,25 NAA
2.08	0.05	3.26	1.4	5.61	4.52	3	,5 NAA
2.08	0.13	3.48	1.81	6.08	4.63	4	1 NAA
0.05	0.09	0.24	0.13	0.34	0.27	L.S.D at 5%	

(Hercílio *et al.*, 2005; Farzinebrahimi *et al.*, 2013).

Humidity was maintained by covering it with rigid plastic cover and frequently spraying of water (Jasrai *et al.*, 2003). Similar process of maintaining humidity was practiced for hardening of banana (Jasrai *et al.*, 2000).

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