Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. **1(8),** 70-72, August **(2012)** *Res.J.Recent Sci.*

Short Communication

The effects of IBA and 2ip on callogenesis and shoot formatting of *Cymbidium orchid* var "Red Tiffani"

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Available online at: www.isca.in
Received 18th April 2012, revised 25th April 2012, accepted 4th May 2012

Abstract

Cymbidium orchid var "Red Tiffani" is one commercial orchid flower that propagated with micropropagation method. For study on effect of IBA and 2ip on micropropagation of Cymbidium orchid var "Red tiffani", four Concentrations of IBA (0, 1, 2 and 4 mg l^{-1}) and four Concentration of 2ip (0, 1, 2 and 4 mg l^{-1}) in three replications were examined. Explants selected on young leaf and after sterilization, for callus induction transport in MS medium with IBA and 2ip. After 5 week, calli transport in new same medium for shoot induction. In this study traits such as days of callus initiation, percent of callus, number of shoot per explants, shoots fresh weight, shoot length were examined. Result showed that the IBA and 2ip effect of callus and shoot traits respectively.

Keywords: Plant regulators, leaf explants, MS medium, callus

Introduction

The Orchidaceace is the main plant family and second largest cut flowers and potted floricultural crop[1,2]. This family has around 20000 species[3]. Three groups of orchid are epiphyte orchids, terrestrial orchids and saprophytic orchids[4,5]. There are many commercially cultivars that often they are hybrids with different colors[2]. Many orchids obtain several years to flower[6]. Orchids propagated in two ways include seed[7-9], vegetative[10]. Propagation with seed is difficult, for this reason vegetative propagation is more used. Today, for propagation orchid, micropropagation is very used[11-13]. Tissue culture is useful way to obtain biologically active constituents those play important role[14]. Also *Cymbidium orchid* is commercial orchid that propagated with micropropagation method. One of the methods of micropropagation of cymbidium orchid is applied of leaf explants[7]. *Cymbidium orchid* var "Red Tiffani" are grown commercially in Iran.

Many researchers have been done with application IBA and 2ip on the leaf micropropagation. Chen and Chang examined four auxins (IAA, IBA, NAA and 2, 4-D) and five cytokinins (2iP, zeatin, kinetin, BA and TDZ) on direct somatic embryogenesis on leaf explants of a *sympodial orchid* Oncidium "Gower Ramsey". They found that embryo formation on leaf explants was retarded by auxins, but promoted by cytokinins[15]. Gow et al, with effect of auxins and cytokinins on amount of direct embryo formation on different leaf locations of two *Phalaenopsis*, found that applied of IBA and cytokinins are useful for improved explant position highly affected embryogenic competence of leaf cells[16]. The results of Jahan showed that high frequency of calli was obtained from leaf of *Anthurium andraeanum* L.[17]. Gopalakrishnan et al, examined BAP and NAA on leaf explants of *Plumbago*. They found that best rooting become in MS supplemented with 5.38 µM IBA[18]. The objectives of this research are finding suitable concentration of IBA and 2ip for callus induction and shoot formatting of *Cymbidium orchid* leaf explants.

Material and Methods

Plant material obtained *Cymbidium orchid* var "Red Tiffani" were selected. The leaf explants used as plant material. The explants must be taken before the leaf tips differentiate fully and don't have an ability to form callus. For sterilization, leaf explants were surface sterilized for 1 minute in 70% (v/v) ethanol and flooded in gentamicin solution for 20 minutes then flooded in 5% (v/v) sodium hypochloride for 10 minutes. Leaves were rinsed in sterile distilled water. Sterile leaf explants were sectioned to about 0.5 cm. MS medium with IBA and 2ip was used for callus induction. Five leaf explants from *Cymbidium orchid* var "Red Tiffani" were transplanted onto the petri dishes containing callus induction medium. This medium consisted of half-strength MS basal salt and vitamins supplemented with 30 g/l sucrose, 6 g/l agar and plant regulators (IBA: 0, 1, 2 and 4 mgl^{-1} and 2ip: 0, 1, 2 and 4 mgl^{-1}) (Table-1). The pH of the medium was adjusted to 5.8. Then petri dishes autoclaves at 121ºC. All cultures were placed in the dark at 27°C for five weeks for callus initiation. In this stage, traits such as days of callus initiation, percent of callus were calculated. After calli formed in medium, explants with calli transferred to same pervious media. After two weeks of transferred of calli, traits such as number of shoot per explants, shoots fresh weight, shoot length were calculated. Data were statistically analyzed using SPSS. ANOVA was used to calculate statistical significance, and mean ± SD (standard deviation) differing significantly were determined using Duncan at $P < 0.05$ level.

Results and Discussion

After 35 days, best and fast callus initiation was observed in S4 (MS + 0 mgl^{-1} IBA + 4 mgl^{-1} 2ip) with 26 days. Low callus induction was recorded in S11 (MS + 2 mgl^{-1} IBA + 2 mgl^{-1} 2ip) with 32 days (table-2). Plant hormones are most important physiological factors that affected on callus initiation in micropropagation. Juan et al, reported that in callus induction of *Astragalus membranaceus*, cytokinin with auxine play a crucial role [19].

Also best percent of callus was observed in S4 (MS + 0 mgl^{-1} IBA + 4 mgl^{-1} 2ip). Low percent of callus was recorded in S11 (MS + 2 mgl^{-1} IBA + 2 mgl^{-1} 2ip) (table-2). Callus can be obtained from leaf explants using medium containing auxin. Desired secondary metabolite can be manipulated as conditions required from the callus[20]. Behra et al, consistent a protocol for induction of callus of plantlets through *in vitro* culture of sugarcane (*Saccharum officinarum*)[21]. Our result showed that the maximum number of shoot per explants obtained from S15 (MS + 4 mgl^{-1} IBA + 2 mgl^{-1} 2ip) with 4.4 and minimum of this trait in S2 (MS + 0 mgl^{-1} IBA + 1 mgl^{-1} 2ip) with 2.1 number of shoot per explants. Cytokinin is required in optimal quantity for shoot proliferation in many plants. Also low concentration of auxin along with cytokinin increases the rate of shoot multiplication[22]. Yew et al, found that 2iP was the best for shoot elongation which was measured in length of shoot[23].

The maximum and minimum shoots fresh weight (16.1 and 11 mg) were obtained in medium containing S8 (MS + 1 mgl^{-1} IBA + 4 mgl^{-1} 2ip) and S1 (MS + 0 mgl^{-1} IBA + 0 mgl^{-1} 2ip) respectively. The maximum and minimum shoot length (12 and 8 mm) were obtained in medium containing S8 (MS + 1 mgl^{-1} IBA + 4 mgl^{-1} 2ip) and S1 (MS + 0 mgl^{-1} IBA + 0 mgl^{-1} 2ip) respectively. Guang-jie et al, found that 2-ip concentration affected shoot height with increasing concentrations. They believe that concentration of 2-ip is suitable for propagation *in vitro* as well as for induction of adventitious shoot regeneration[24]. 2ip exhibited significant influence on the shoot length[25]. A positive effect of cytokinins on orchid development was reported by Rasmussen[26]. Cytokinin formulations were earlier shown to be critical for shoot elongation of many plant species[27]

Conclusion

Micropropagation of plants has become a significant technique to reproduce and make the availability of orchids that is otherwise difficult to propagate traditionally by seed or vegetative. This research showed that the choosing an appropriate concentration of IBA and 2ip was effective on traits of micropropagation of *Cymbidium orchid*.

Table-1
List of treatment in experiment

Treatment	Medium	Treatment	medium
S1	MS + 0 mgl^{-1} IBA + 0 mgl^{-1} 2ip	S9	MS + 2 mgl^{-1} IBA + 0 mgl^{-1} 2ip
S2	MS + 0 mgl^{-1} IBA + 1 mgl^{-1} 2ip	S10	MS + 2 mgl^{-1} IBA + 1 mgl^{-1} 2ip
S3	MS + 0 mgl^{-1} IBA + 2 mgl^{-1} 2ip	S11	MS + 2 mgl^{-1} IBA + 2 mgl^{-1} 2ip
S4	MS + 0 mgl^{-1} IBA + 4 mgl^{-1} 2ip	S12	MS + 2 mgl^{-1} IBA + 4 mgl^{-1} 2ip
S5	MS + 1 mgl^{-1} IBA + 0 mgl^{-1} 2ip	S13	MS + 4 mgl^{-1} IBA + 0 mgl^{-1} 2ip
S6	MS + 1 mgl^{-1} IBA + 1 mgl^{-1} 2ip	S14	MS + 4 mgl^{-1} IBA + 1 mgl^{-1} 2ip
S7	MS + 1 mgl^{-1} IBA + 2 mgl^{-1} 2ip	S15	MS + 4 mgl^{-1} IBA + 2 mgl^{-1} 2ip
S8	MS + 1 mgl^{-1} IBA + 4 mgl^{-1} 2ip	S16	MS + 4 mgl^{-1} IBA + 4 mgl^{-1} 2ip

Table-2
Mean comparison of different concentrations of IBA and 2ip on evaluated traits

Treatment	days of callus initiation	percent of callus (%)	Number of shoot per explants	shoots fresh weight (mg)	Shoot length (mm)
MS + 0 mgl^{-1} IBA + 0 mgl^{-1} 2ip	32 a	70 c	2.1 c	11.0	8.00 d
MS + 0 mgl^{-1} IBA + 1 mgl^{-1} 2ip	28 b	70 c	2.1 c	14.0	10.2 c
MS + 0 mgl^{-1} IBA + 2 mgl^{-1} 2ip	28 b	80 b	3.3 b	14.0	10.2 c
MS + 0 mgl^{-1} IBA + 4 mgl^{-1} 2ip	26 c	100 a	3.2 c	15.0	11.0 b
MS + 1 mgl^{-1} IBA + 0 mgl^{-1} 2ip	30 a	90 a	3.3 b	11.2	8.20 d
MS + 1 mgl^{-1} IBA + 1 mgl^{-1} 2ip	29 b	90 a	2.4 b	15.3	11.0 b
MS + 1 mgl^{-1} IBA + 2 mgl^{-1} 2ip	27 c	70 c	2.4 b	15.3	11.8 a
MS + 1 mgl^{-1} IBA + 4 mgl^{-1} 2ip	28 b	80 b	2.4 b	16.1	12.0 a
MS + 2 mgl^{-1} IBA + 0 mgl^{-1} 2ip	30 a	70 c	3.2 c	12.3	9.50 c
MS + 2 mgl^{-1} IBA + 1 mgl^{-1} 2ip	30 a	70 c	3.2 c	14.1	10.2 c
MS + 2 mgl^{-1} IBA + 2 mgl^{-1} 2ip	32 a	60 c	4.2 a	15.2	11.0 b
MS + 2 mgl^{-1} IBA + 4 mgl^{-1} 2ip	28 b	80 b	2.2 c	15.2	11.0 b
MS + 4 mgl^{-1} IBA + 0 mgl^{-1} 2ip	29 b	80 b	2.2 c	14.1	10.4 b
MS + 4 mgl^{-1} IBA + 1 mgl^{-1} 2ip	29 b	70 c	2.2 c	11.3	8.20 d
MS + 4 mgl^{-1} IBA + 2 mgl^{-1} 2ip	30 a	80 b	4.4 a	11.2	8.20 d
MS + 4 mgl^{-1} IBA + 4 mgl^{-1} 2ip	30 a	70 c	4.2 a	11.4	8.30 d
Values in each row followed by the same letter are not significantly different by Duncan in $P < 0.05$					

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