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INITIAL DIRECT ORGANOGENESIS OF TWO GENOTYPES CHRYSANTHEMUM (*Dendrathema grandiflora*) IN THE ABSENCE OF GROWTH REGULATORS ON MS MEDIUM

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ABSTRACT

Shoot multiplication of chrysanthemum (*Dendrathema grandiflora)* plantlets was obtained by treating nodal segments of chrysanthemum explants. Explants were cultured onto Murashige and Skoog (MS) media with different concentrations cytokinin (BAP), and in the absence of growth regulators. Sterilization of the explants was obtained, by treating with 1.0% $HgCl_2$ for three minutes plus 2-3 drops of tween-20 and then rinsed twice with distilled water. The effect of different concentrations of BAP on the shoot proliferation of chrysanthemum explants were checked. The parameters under study were: number of days to shoot initiation, shoot initiation percentage, number of shoots per explants, shoot length and root per plant. Results showed that the addition of BAP in the MS media were able to induce shoots from the explants, but in the absence of growth regulators, microshoots and roots were able to grow as well as those treatments. The results demonstrated that there were no statistically significantly differences on the all parameters of initial direct organogenesis of two genotypes chrysanthemum (*Dendrathema grandiflora*) which has been investigated.

Keywords: chrysanthemum, tissue culture, BAP.

INTRODUCTION

Chrysanthemum is high popularity and demand flowering herb in Indonesia. It belongs to the family Compositeae (Asteraceae). It is attractive ornamental plant, which becomes one of the first commercial targets for micropropagation.

Tissue culture can be utilized for the large-scale production of Chrysanthemum. Although the tissue culture of chrysanthemum has been widely explored, several unexplored topics remain, and there is always the constant search for reducing the cost of raising tissue cultured plants. The technique has already been employed to study large scale propagation of *C. morifolium* through different regeneration pathways (Shatnawi *et al*., 2010, Nalini, 2012, Keresa, *et al.*, 2012).

Micropropagation using axillary shoot proliferation from nodal and shoot tip culture is the most popularity technique on Chrysanthemum tissue culture propagation. Widiastoety (1987) placed three types of explant (shoot tips, axillary buds and leaf pieces) in a modified MS medium. Amin *et al.* (1997) demonstrated that axillary and adventitious bud multiplication of chrysanthemum was possible from the nodal, shoot tip and petiole explants.

In tissue culture, the use of plant growth regulators influences different plant processes comprising mostly of growth, shoot initiation, rooting etc. (Hobbie, 1998). Therefore, many kinds of plant growth regulators have been used with the aim to induce shoot initiation and root formation. Synthetic growth regulators that have been found most reliable in stimulating shoot initiation are the auxins i.e., indole acetic acid (IAA), naphthalene acetic acid (NAA) and indole butyric acid (IBA) (Keresa *et al*., 2012; Lindiro *et al.,* 2013) and the cytokinin i.e., Benzylaminopurine (BAP) (Shatnawi *et al*., 2010).

Gul (2001) reported that in chrysanthemum, maximum shoot regeneration was observed from stem nodal segments at 0.5mg/l BAP. Park *et al.* (2002) reported that the most effective medium for chrysanthemum shoot regeneration was a MS basal medium supplemented with 10 µM NAA and 5.0 µM BAP. Karim *et al.* (2003) reported that maximum frequency of explants produced axillary shoot and the highest number of shoots per explant were obtained when MS medium fortified with 1.0 mg/l BAP.

The use of plant growth regulators on tissue culture of chrysanthemum is well explored, but the growth of explants in the absence of growth regulators is less undertaken. Therefore, the present study was attempted to determine a suitable protocol and to find out the effect of plant growth regulators, combination and Chrysanthemum Genotype 211 on initial direct organogenesis of two genotypes Chrysanthemum (*Dendrathema grandiflora*) in MS Medium.

METHODS

The study was carried out from April to October, 2015 at the plant tissue culture laboratory, Faculty of Agriculture, UPN Veteran East Java University, Surabaya, Indonesia. Two genotypes of chrysanthemum, namely 211 and 213 obtained from plants cultivated by Condido Agro Laboratory, Pasuruan, Indonesia. They were used to provide starting nodal explants for the study. After 30 days, nodal cuttings were collected from different growing chrysanthemum genotypes.

Nodal cuttings were washed in running tap water for 15 min, washed for 5 min in 15% (v/v) commercial liquid detergent, rinsed three times in sterile distilled water, given a 10-sec dip in 70% ethanol and then transferred to 0.2% HgCl2 containing two drops of

Tween-20 for 2 min. Then, entire nodal cuttings were rinsed thoroughly three times in sterile distilled water, followed by immersing in a sterilization solution containing 20% and 1% sodium hypochlorite solution for 100 mL distilled water. After 5 min, the cutting nodes were rinsed three to four times with sterile distilled water. The shoot fragments were sterilized by sinking in Na hypoclorite 3% for 7-8 minutes. The explants were rinsed several times with distilled sterile water.

The medium used for *in vitro* culture of chrysanthemum shoots was Murashige and Skoog (MS) medium supplemented with 30 g/L sugar and 5.5 g/L agar. Explants consisting in single-node stem segments were cultured on MS medium with different sets of growth regulators, with different concentrations, alone and with combinations. The description is as follows:
Different concentrations of Cytokinin (BAP):

C1 = 0.0 mg/l
C2 = 0.5 mg/l
C3 = 1.0 mg/l
C4 = 2.0 mg/l

Inoculation was carried out in a sterile laminar airflow hood. Single nodal cuttings were inoculated into a bottle containing 40 ml of the sterile media under evaluation. These were incubated at 22 ± 20 ^{0}C under fluorescent lamps for four weeks for regeneration of microshoot. The shoots regenerated from the nodal segments were excised and transferred to another MS medium for root initiation.

The experiment was set up in a completely randomized design. The effect of media composition on shoot proliferation was monitored for 60 days, each treatment with 4 replicates containing three inoculated microshoots per medium per bottle. Data from rooting experiments were collected from 10 replicates, each containing three plantlets per medium per bottle. ANOVA and LSD test at $p<0.05$ were used for statistical analyses. Analyses were performed using SPSS version 16.0

RESULT AND DISCUSSIONS

The nodal of chrysanthemum were cultured in media without growth regulators, and with varying concentrations of BAP (a cytokinin) plant growth regulators. Effects of those treatments were tested. The data regarding the effect of different concentrations of BAP on the regeneration of chrysanthemum plantlets genotype 211 and 213 using single nodal as explants were given in Table-1 and Table-2.

Table-1. Effect of different concentration of BAP on the regeneration of Chrysanthemum Genotype 211.

Treatments	No. New shoot	Shoot initiation (days)	Shoot initiation (%)	Length of shoots (cm)	Root per explant
A1(0.0 ppm)	21.54 a	6.60 a	65.20 a	2.1 b	0.00
A2 (0.5 ppm)	20.37 a	6.70 a	76.08 b	2.0 b	0.00
A3 (1.0 ppm)	21.45 a	7.10 a	67.47 a	1.8 ab	0.00
A4 (2.0 ppm)	21.98 a	6.90 a	62.51 a	1.7 a	0.00
LSD (p<0.05)	9.0	1.1	12.2	0.2	0.0

At least one similar letter shows not significant difference in 5% level according to Duncan's Multiple Range Test

Table-2. Effect of different concentration of BAP on the regeneration of Chrysanthemum Genotype 213.

Treatments	No. New shoot	Shoot initiation (days)	Shoot initiation (%)	Length of shoots (cm)	Root per explant
A1(0.0 ppm)	2.15 a	7.00 a	55.55 a	8.1 b	6.51 b
A2 (0.5 ppm)	1.13 a	7.10 a	56.00 a	8.7 c	5.12 a
A3 (1.0 ppm)	2.45 a	6.90 a	57.41 a	8.8 c	5.04 a
A4 (2.0 ppm)	1.98 a	6.90 a	52.58 a	7.7 a	6.90 b
LSD (p<0.05)	2.0	1.1	12.2	0.2	0.4

At least one similar letter shows not significant difference in 5% level according to Duncan's Multiple Range Test

Figure-1. *In vitro* micropropagation of chrysanthemum on the absence of growth regulators medium, cultivar 213 (A), and cultivar 211 (B). Growth observed after 60 days.

Figure-2. *In vitro* micropropagation of chrysanthemum (A) on the absence of growth regulators medium after 14 days, and (B) on 1.5 ppm BAP after 60 days.

The earliest shoot initiation of Chrysanthemum Genotype 211 (6.6 days) was recorded in A0 (0.0 ppm BAP), followed by concentration of BAP used i.e. A2 (0.5 ppm BAP) which took 6.7 days to initiate shoots. Both these treatments were statistically at par with each other. A4 (1.5 ppm BAP) took 6.9 days to initiate shoots while the maximum number of days (7.10) was required with A4 (2 ppm BAP) to initiate shoots. Treatments requiring few days to shoot initiation are desirable for early emergence of shoots. Thus in the absence of growth regulators proven its value by taking fewer days for shoot initiation.

Not only the data trend performed by Chrysanthemum Genotype 211, but also by Chrysanthemum Genotype 213. In Table-2, T1 (0.0 ppm BAP) presented the fewer days for shoot initiation. All the treatments were statistically similar on the days for shoot initiation.

Statistically analyzed data recorded on percentage of shoot initiation is presented in Table 1 and Table 2. The highly significant data revealed the superiority of A2 (0.5 ppm BAP) over all the other treatments on Chrysanthemum Genotype 211. But the treatments were statistically similar on percentage of shoot initiation of Chrysanthemum Genotype 213. These results are not also similar to those as obtained by Karim *et al.* (2002) who described 1.0 mg/l BAP as the best BAP concentration as it had produced 91% of shoot initiation in chrysanthemum while using shoot tips as explant.

A statistically nonsignificant data on number of new shoots per explant revealed outstanding performance of Table-1 and Table-2. A4 (2 ppm BAP) produced maximum number of shoots per explant 21.98 and A3 (1 ppm BAP) produced 2.45 of Chrysanthemum Genotype 211 and 213 respectively. The fact that was nonsignificant regarding the average length of shoots and root per explant, is presented in the Table-1 and Table-2.

It could be inferred from above results that many parameters excelled on the 1.0 ppm BAP. Those doze is the optimum concentration for the better performance of this particular hormone. But deviation from this normal concentration of the growth regulator showed many results. The fact that higher dozes fail to manifest their effect could be attributed to an obnoxious effect at higher concentration, whereas, the absence of growth regulators not indicated poor performance.

BAP belongs to the cytokinin group and it has been reported that BAP is the most effective plant growth regulator in relation to shoot proliferation (Ilahi *et al*., 2007; Nahid *et al*., 2007; Rahayu and Prayogi, 2013; Lindiro *et al*., 2013; Jaime and da Silva, 2014). Vantu (2005) also reported that the agar solidified MS medium containing 2 mg/l BAP and 0,002 mg/l NAA was optimum for rapid mass production of *Chrysanthemum morifolium* plantlets, but these can be rooted on MS medium in the absence of growth regulators. In the low consentration of BAP (0.1 mg/L) combination with GA3 (0.5 mg/L), Keresa *et al*. (2012) reported that proliferation rate of 3.2 new microshoots per one inoculated was achieved. BAP at 1.0 and 1.5 mg/L promoted organogenesis of the callus to produce shoot as much as 10% (Kasli, 2009)

CONCLUSIONS

The results demonstrated that in the low concentration of BAP there were no statistically

significantly differences on the all parameter of initial direct organogenesis of two genotypes chrysanthemum (*Dendrathema grandiflora*) which has been investigated.

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