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Full Length Article

Effect of Different Auxins on the Regeneration Capability of Chrysanthemum Leaf Discs

KASHIF WASEEM[1], MUHAMMAD QASIM KHAN†, JAFFAR JASKANI‡, MUHAMMAD SALEEM JILANI AND MUHAMMAD SOHAIL KHAN¶
Department of Horticulture, Faculty of Agriculture, Gomal University D.I. Khan, Pakistan
†*Faculty of Agriculture, Gomal University D.I. Khan, Pakistan*
‡*Institute of Horticultural Sciences, Agricultural University Faisalabad, Pakistan*
¶*Horticulture Section, Agricultural Research Institute (ARI), Ratta Kulachi D.I. Khan, Pakistan*
[1]Corresponding author's e-mail: waseem_hort@yahoo.com

ABSTRACT

A set of experiments were carried out to optimize the best auxin for the regeneration of chrysanthemum plantlets. Explants were inoculated in MS media supplemented with three different auxins (IAA, NAA & IBA) with their varied concentrations including a control treatment to check the direct and indirect organogenesis. Lowest concentration of IAA (0.1 mg L^{-1}) showed its superiority over all the other treatments as it gave 70% shoot initiation, 2.2 shoots per explant, 3.0 cm long shoots with 8.1 leaves and 3.5 nodes per shoot, respectively. As far as different concentrations of NAA was concerned, it was evident from the results that the lowest concentration (0.5 mg L^{-1} NAA) showed the best response towards the regeneration of chrysanthemum plantlets as, it produced 70% shoot initiation, highest shoots per explant (2.0), shoot length (2.6 cm), maximum leaves (5.3) and nodes per shoot (3.1). Whereas, amongst the various IBA concentrations the best response towards regeneration was observed in MS media supplemented with 0.3 mg L^{-1} IBA, as it yielded the maximum 56.7% shoot initiation, shoots per explant (1.9), shoot length (2.4 cm), leaves per shoot (4.7) and 3.0 nodes per shoot.

Key Words: Chrysanthemum; *Dendranthema morifolium*; IAA; NAA; IBA; Regeneration; *In vitro*; Leaf discs
Abbreviations: IAA = Indole Acetic Acid; IBA = Indole Butyric Acid; NAA = Naphthalene Acetic Acid

INTRODUCTION

Chrysanthemum, commonly known as gul-e-daudi or autumn queen, belongs to the family Compositae (Arora, 1990). It is highly attractive and charming short day plant, which behaves both as an annual as well as, perennial flowering herb. It is a showy flower and recently its popularity has increased not only for their outstanding aesthetic beauty, but also for their good potential as cut flowers to many countries of the world (Erler & Siegmund, 1986). The commercial cultivars are usually propagated vegetatively through terminal cuttings and root suckers. These propagation methods not only take longer time to flower, but are also not useful for large scale production. Regeneration through *in vitro* culture has become now a viable alternative to the conventional propagation methods. Levin *et al.* (1988) utilized tissue culture technique for large scale production of chrysanthemum. The formation of healthy shoots and its higher rates of multiplication is one of the prerequisite of an economically viable micro-propagation protocol. It is possible now to obtain a large number of plants from one explant through *in vitro* propagation (Bajaj *et al.*, 1992). Dejong *et al.* (1993) reported that explants from leaves of *in vitro* grown chrysanthemum (*Dendranthema grandiflora* Tzvel.) cultivars regenerated adventitious shoots without an intermediate callus phase.

Chen *et al.* (1985) cultured young leaves from 8 chrysanthemum cultivars on Murashige and Skoog (MS) medium supplemented with BAP at 3 - 5 mg L^{-1} + NAA at 2 mg L^{-1} and found it best for bud induction. Urban *et al.* (1994) reported regeneration of shoots from the leaf segments of Iridon and Helka cultivars of chrysanthemum. Mityushkina *et al.* (1995) regenerated adventitious shoots from leaf explants of 32 of 40 Chrysanthemum morifolium cultivars tested, while Kim and Kim (1998) regenerated shoots directly from internodes and leaf segments of D. indicum and D. zawadskii on basal media supplemented with 3.0 mg L^{-1} BAP and 0.2 mg L^{-1} IAA. Oka *et al.* (1999) reported that adventitious buds were mainly formed at the cut ends of primary leaves of garland chrysanthemum after 6- 9 days culture on MS medium supplemented with 0.1 mg L^{-1} BAP + 0.1 mg L^{-1} NAA. Datta *et al.* (2002) stated that leaf explants of chrysanthemum differentiated shoot buds in

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the presence of BA and IAA. The formation of healthy shoots and its higher rates of multiplication is one of the pre-requisite of an economically viable micro-propagation protocol. Therefore, present study was undertaken to develop a protocol for the large scale clonal propagation of Chrysanthemum using leaf discs as explant treating with different concentrations of different auxins.

MATERIALS AND METHODS

The experiments regarding the regeneration of chrysanthemum Plantlets, using leaf disc as explants, as affected by different concentrations of auxins viz. IAA, NAA and IBA were conducted at the "Plant Tissue Culture Laboratory; Institute of Horticultural Sciences, University of Agriculture, Faisalabad in completely randomized design (CRD) with three replications. To check the effect of different auxins on the regeneration of chrysanthemum plantlets, standard MS media (Murashige & Skoog, 1962) with varied concentrations of IAA (Control, 0.1, 0.3 & 0.5 mg L^{-1} IAA), NAA (Control, 0.5, 1.0 & 1.5 mg L^{-1} NAA) and IBA (Control, 0.3, 0.7 & 1.0 mg L^{-1} IBA) were used.

Preparation of explants. The explant material was collected from six months old Chrysanthemum plants, grown at the floriculture garden of the "Institute of Horticultural Sciences". The collected material was brought to the laboratory and washed thoroughly with running tap water for 30 min. After the washing, leaves 0.5 cm 2 in size were prepared (Datta *et al.*, 2002), from the stock plants, by using sterilized forceps and scalpel.

Sterilization of plant material. The excised explants were dipped in 70% ethanol for 60 seconds. After pretreatment with ethanol, the explants were rinsed with double distilled water twice, so as to lower the toxic affect of ethanol. Leaf discs were then surface sterilized with 1.0% mercurric chloride ($HgCl_2$) for 3 min (Ilahi *et al.*, 2007). After the surface sterilization of explants, Mercuric Chloride was removed and the explants were rinsed with double distilled water thrice, so as to lower the toxic affects of $HgCl_2$.

Culture of explants. Explants were cultured on solidified MS media with agar and its pH was adjusted to 5.7 before autoclaving at 121 C for 30 min. On cooling of the media, explants were cultured in Murashige and Skoog (1962) media containing different concentrations of auxins. One explant in each tube (15 x 2.5 cm) containing 10 mL media was placed. The cultured tubes were incubated for sixteen hours daily light of fluorescent Philip, white tubes with intensified 1000 LUX at 25 C.

For the direct regeneration of chrysanthemum leaf discs, data was recorded for different parameters including shoot initiation percentage, average number of shoots per explant, average shoot length (cm), average number of leaves per shoot and average number of nodes per shoot and callus induction.

Statistics. Recorded data were analyzed statistically using analysis of variance technique (ANOVA) and means were compared by Duncan's multiple range test (Steel & Torrie, 1984).

RESULTS AND DISCUSSION

Effect of different concentration of IAA on the regeneration of chrysanthemum plantlets from leaf disc explants. The lowest concentration of IAA (0.1 mg L^{-1}) showed its superiority over all the other treatments regarding the direct organogenesis of chrysanthemum plantlets as maximum shoot initiation (70%) and number of shoots per explant (2.2) was recorded in (0.1 mg L^{-1} IAA) followed by (0.3 mg L^{-1}) and (0.5 mg L^{-1}) with 43.3 and 36.7% shoot initiation and 1.9 and 1.7 shoots per explant, respectively. (0.0 mg L^{-1} IAA) treatment produced the minimum (26.7%) shoot initiation and shoots per explant (1.4) as shown in Table I. These results are in conformity with Bhattacharya *et al.* (1990), who reported 2.15 shoots per explant for 0.1 mg L^{-1} IAA in the MS medium for chrysanthemum plantlets. Similarly, the longest shoots (3.0 cm) were also recorded in (0.1 mg L^{-1}) followed by (0.3 mg L^{-1}) and (0.5 mg L^{-1}) with 2.5 and 2.0 cm long shoots, respectively. Minimum shoot length (1.5 cm) was exhibited by (0.0 mg L^{-1}IAA).

The data on average number of leaves and nodes per shoot revealed a parallel situation with that of average number of shoots per explant, as (0.1 mg L^{-1}) produced maximum leaves (8.1) and nodes (3.5) per shoot over the other treatments (Fig. 1). Control (0.0 mg L^{-1} IAA) produced the minimum leaves (2.5) and nodes (2.2) per shoot. Other treatments i.e., (0.3 mg L^{-1}), (0.5 mg L^{-1}) produced 6.4, 4.6 leaves, 3.0 and 2.6 nodes per shoot, respectively as shown in Table I. This trend was being observed due to the fact that as (0.1 mg L^{-1}) produced the maximum length of shoots, it was obvious that the same treatment would produce maximum leaves per shoot and vice versa. The results revealed that the lowest concentration of IAA (0.1 mg L^{-1}) exhibited the best response towards the regeneration of chrysanthemum and as the concentration of IAA increased, the response towards the regeneration decreased. Choi *et al.* (2002) also reported that there was a tendency to increase the regeneration frequency with decreasing IAA concentration using leaf disc explant in cucumber. These results are also in accordance to the findings of Rout *et al.* (1997), who concluded that shoot bud regeneration increased linearly with a decrease in IAA concentration in leaf discs of chrysanthemum. These results suggest an inhibitory effect of auxin at higher concentration, but low levels of auxin secure essential for regeneration of shoot bud.

Although some callus formation was observed, when the leaf discs were treated with higher concentrations of IAA (0.5 & 0.3 mg/L^{-1}), but both produced weak calluses with green and small yellow pale color, respectively (Fig. 2). None of these was embryogenic or organogenic and showed mortality later on 0.0 and 0.1 mg L^{-1} IAA was

Table I. Effect of different concentration of IAA on the regeneration of chrysanthemum from leaf disc explants

Treatments (IAA mg L^{-1})	Shoot initiation %	shoots explant^{-1}	Length of shoots (cm)	Av. leaves shoot^{-1}	Av. nodes shoot^{-1}	Callus formation
0.0	26.7 C	1.4 C	1.5 D	2.5 D	2.2 C	-
0.1	70.0 A	2.2 A	3.0 A	8.1 A	3.5 A	-
0.3	43.3 B	1.9 B	2.5 B	6.4 B	3.0 B	+
0.5	36.7 B	1.7 B	2.0 C	4.6 C	2.6C	+
LSD (P<.05)	**9.4**	**0.2**	**0.3**	**0.4**	**0.3**	

Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (P<.05)
- = No callus formation + = Weak Callus

Table II. Effect of different concentration of NAA on the regeneration of chrysanthemum from leaf disc explants

Treatments (NAA mg L^{-1})	Shoot initiation %	Av. shoots explant^{-1}	Av. length of shoots (cm)	Av. leaves shoot^{-1}	Av. nodes shoot^{-1}	Callus formation
0.0	26.7 BC	1.4 D	1.5 C	2.5 D	2.2 D	-
0.5	70.0 A	2.0 A	2.6 A	5.3 A	3.1 A	+
1.0	30.0 B	1.8 B	2.2 B	4.8 B	2.8 B	+
1.5	20.0 C	1.6 C	2.0 B	4.0 C	2.5 C	++
LSD (P<.05)	**12.1**	**0.1**	**0.3**	**0.4**	**0.2**	

Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (P<.05)
- = No callus formation + = Weak Callus
++ = Good Callus

Table III. Effect of different concentration of IBA on the regeneration of chrysanthemum from leaf disc explants

Treatments (IBA mg L^{-1})	Shoot initiation %	Av. shoots explant^{-1}	Length of shoots (cm)	Av. leaves shoot^{-1}	Av. nodes shoot^{-1}	Callus formation
0.0	26.7 B	1.4 C	1.5 C	2.5 C	2.2 C	-
0.3	56.7 A	1.9 A	2.4 A	4.7 A	3.0 A	+
0.7	16.7 C	1.6 B	2.1 B	3.8 B	2.7 B	++
1.0	-	-	-	-	-	+++
LSD (P<.05)	**9.4**	**0.1**	**0.1**	**0.2**	**0.2**	

Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (P<.05)
- = No callus formation + = Weak Callus
++ = Good Callus +++ = Very Good Callus

unable to produce callus. Similar results were reported by Slimmon *et al.* (1991), who observed that higher concentration of IAA in the medium induced callusing, but the callus was neither embryogenic nor organogenic.

Effect of different concentration of NAA on the regeneration of chrysanthemum plantlets from leaf disc explants. Highly significant data regarding the effect of different concentrations of NAA on the regeneration of chrysanthemum plantlets using leaf disc as explant is presented in Table II. The result revealed the supremacy of the lowest concentration of NAA i.e., (0.5 mg L^{-1}) as compared to the other concentrations of NAA used. Significantly maximum shoot initiation (70.0%), shoots per explant (2.0), longest shoots (2.6 cm), leaves (5.3) and nodes (3.1) per shoot were recorded in (0.5 mg L^{-1} NAA), followed (1.0 mg L^{-1} NAA) with 30% shoot initiation, 1.8 shoots per explant, 2.2 cm long shoots, 4.8 leaves and 2.8 nodes per shoots (Table II). The least response was reported by (0.0 mg L^{-1} NAA), as this media was devoid of any growth regulator to support the regeneration of chrysanthemum plantlets. Looking at the results, one would get the impression that higher concentration of NAA has suppressed the growth. The fact that the lowest concentration i.e., (0.5 mg L^{-1} NAA) gave the best results reflects the optimal concentration effective for the plant regeneration (Fig. 3). Similar findings were reported by Waseem *et al.* (2007), who also found that the lowest concentration of NAA (0.5 mg L^{-1}), when used alone, showed its superiority over all the other concentration of NAA by producing the maximum number of shoots per explant, leaves and nodes per shoot. Ali *et al.* (2005) also reported that an increase of NAA in MS medium resulted in decreasing the multiplication rate.

The results showed that better callus formation with fairly green color and good growth was observed in the highest concentration of NAA i.e., (1.5 mg L^{-1}), as shown in Fig. 4. Weak, small pale yellow compact callus was observed in (1.0 mg L^{-1} NAA). Whereas, (0.5 mg L^{-1} NAA) showed poor callus formation with a very weak, small brown callus, which showed mortality later on 0.0 mg L^{-1} NAA was unable to induce callus. Our results get support from the previous done by reports (Kaul *et al.,* 1990), who observed that the rate of callusing of leaf segment was faster with an increase in the concentration of NAA in the medium.

Effect of different concentrations of IBA on the regeneration of chrysanthemum plantlets from leaf disc explants. Although, the overall response of IBA was not encouraging towards the chrysanthemum plantlets regeneration, but the results showed the superiority of the lowest (0.3 mg L^{-1}) concentration of IBA, over all the other concentrations of IBA used.

Fig. 1. Initial shoot proliferation in MS leaf media supplemented with 0.1 mg L^{-1} IAA

Fig. 2. Disintegration of Callus raised from Disc explant, when cultured in MS media supplemented with 0.3 mg L^{-1} IAA

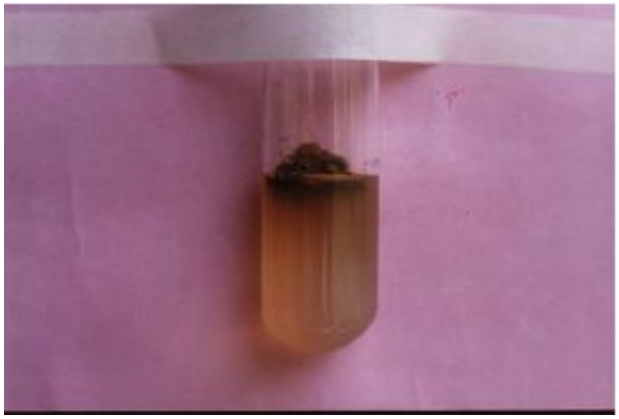

Fig. 3. Initial shoot proliferation in MS with 0.5 mg L^{-1} NAA

Maximum shoot initiation (56.7%) was observed in (0.3 mg L^{-1}) followed by the (0.0 mg L^{-1}) with 26.7% shoot initiation. The lowest shoot initiation (16.7%) was observed in (0.7 mg L^{-1} IBA). Whereas, the highest concentration (1.0 mg L^{-1} IBA) showed no organogenesis. The results further

Fig. 4. Callus initiation at MS media supplemented supplemented with 1.5 mg L^{-1} NAA

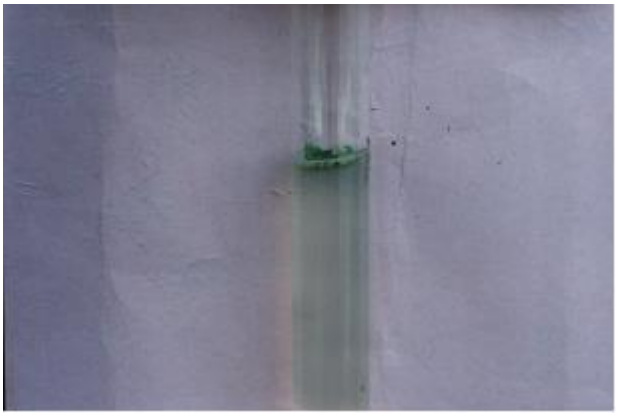

Fig. 5. Callus initiation at MS media supplemented with 1.0 mg L^{-1}IBA

showed that (0.3 mg L^{-1} IBA) had produced significantly higher number of shoots (1.9), longest shoots (2.4 cm), leaves (4.7) and nodes (3.0) per shoot followed by (0.7 mg L^{-1}) with 1.6 shoots per explant, 2.1 cm long shoots, 3.8 leaves and 2.7 nodes per shoot (Table III). Whereas, (0.0 mg L^{-1} IBA) produced the least response for direct organogenesis. Whereas, the highest concentration of IBA (1.0 mg L^{-1}) was unable to show any effect for the direct organogenesis in chrysanthemum. Waseem *et al.* (2007) also stated that the lowest concentration of IBA (0.3 mg L^{-1}) showed its superiority in the regeneration of chrysanthemum plantlets as compared to its other concentrations, when used alone.

In case of callus formation, the data depicted that the highest concentration of IBA (1.0 mg L^{-1}) showed its superiority over the other concentrations as it produced healthy green callus with good growth (Fig. 5 & 6). Small pale yellow compact callus and very weak small brown callus were obtained by (0.7 mg L^{-1}) and (0.3 mg L^{-1}), respectively. However, (0.0 mg L^{-1} IBA) was unable to initiate any callus.

The results showed that lower concentrations of IBA favored the direct organogenesis by producing good shoot

Fig. 6. Initiation of plantlets on the callus derived from MS media supplemented with 1.0 mg L^{-1} IBA

development, while the higher concentration favored the callus formation and after sub-culture of these calluses, vigorous shoot formation and plantlet development.

CONCLUSION

For direct plantlet regeneration, the lowest concentrations of all the auxins used (IAA, NAA & IBA) showed better results as compared to their higher concentrations and amongst the auxins, IAA showed better results, followed by NAA and IBA, respectively. For callus formation, higher concentrations of all the auxins used (IBA, NAA & IAA) showed better results as compared to their lower concentrations and IBA responded well as compared to other auxins.

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