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Full Length Research Paper

Efficient *in vitro* regeneration of chrysanthemum (*Chrysanthemum morifolium* L.) plantlets from nodal segments

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Efficient plant regeneration system has been developed from the nodal segments of chrysanthemum (*Chrysanthemum morifolium* L). Nodal segments, after being sterilized with 1.0% mercuric chloride for three minutes, were inoculated in Murashige and Skoog (MS) media with varied concentrations of indole acetic acid (IAA), benzylaminopurine (BAP) and their combinations. Different parameters including shoot initiation percentage, average number of shoots per explant, length of shoots (cm), number of leaves per shoot and number of nodes per shoot were studied during the course of study. Intermediate level (0.3 mg/l) of IAA exceeded all the other concentrations of IAA by producing 80.0 % shoot initiation, an average of 4.0 shoots per explants, 5.1 cm long shoots, 11.3 leaves and 5.6 nodes per shoot, when used alone. Similarly, intermediate level of BAP (1.0 mg/l) showed its supremacy over all the other concentrations as it produced 100% shoot initiation, 4.9 shoots per explant, 5.8 cm long shoots, 13.4 leaves and 6.3 nodes per shoot, when used alone. When the combination of different concentrations of IAA and BAP were used, significant results regarding the regeneration of chrysanthemum plantlets were also achieved. MS media supplemented with lower concentrations of IAA (0.1 and 0.2 mg/l) along with intermediate levels of BAP (1.0 and 2.0 mg/l) had a favorable effect on the regeneration of chrysanthemum plantlets using nodal segments of chrysanthemum, as compared to other concentrations and combinations. Satisfactory rooting response was obtained in half strength MS media supplemented with 0.2 mg/l indole butyric acid (IBA), followed by 0.2 mg/l naphthalene acetic acid (NAA) and IAA, respectively.

Key words: Chrysanthemum, *Dendranthema morifolium*, growth regulators, *in vitro* culture, nodal segments, auxins, rooting.

INTRODUCTION

Chrysanthemum commonly known as Gul-e-Daudi or Autumn Queen belongs to the family Compositeae (Asteraceae) (Arora, 1990). It is highly valued as a cut flower worldwide with its diverse floral types and colors (Teixeira da Silva, 2003). It is globally an important cut flower and pot plant species usually cultivated by vegetative cuttings (Jaime and Teixeiria, 2004). It is generally propagated using suckers and terminal cuttings (Rout and Das, 1997). This approach, however, is inadequate to attain fast multiplication rate, as these conventional propagating methods are very slow, time consuming and tiring. Secondly, cuttings obtained repeatedly from mother plants may be subjected to any virus infection and degeneration, thereby increasing production costs (Hahn et al., 1998). These problems have been solved by apply-

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Abbreviations: IAA, Indole acetic acid; **BAP,** benzylaminopurine; **IBA,** indole butyric acid; **NAA,** naphthalene acetic acid; **MS,** Murashige and Skoog medium.

ing micro propagation methods, which are routinely applied to the clonal propagation of a variety of horticultural plants including Chrysanthemum (Ben-Jaacov and Langhans, 1972). Micro propagation and other *in vitro* techniques have been used for plans which present particular problems in conventional horticulture (Fay, 1992). Chebet et al. (2003) reported the use of biotechnological approaches to improve horticultural crop production. The regeneration of plants from tissue culture is an important and essential component of biotechnological research. High frequency regeneration of plants from the *in vitro* cultured tissue is a pre-requisite for successful application of tissue culture techniques for crop improvement (Akter, 2001). It is possible now to obtain a large number of plants from one explant *in vitro* (Bajaj, 1992). A decade ago, the protocols for rapid true to type, disease-free propagation has been developed in chrysanthemum through bud/shoot proliferation (Grewal et al., 1996). In tissue culture, the use of plant growth regulators play a pivotal role in influencing different plant processes comprising mostly of growth, differentiation and development for example, culture establishment, shoot initiation, callogenesis, embryogenesis, rooting, etc (Hobbie, 1998). Pierik (1987) stated that cytokinins are often used to stimulate growth and development, Kin and benzylaminopurine (BAP) being in common use. Although the presence of a cytokinin is almost always advantageous, and is often all that is required, optimum rates of shoot initiation generally occur with the combinations of auxins and cytokinin (George, 1993).

The presence of auxin in defined combinations with cytokinins in the culture medium is also necessary to obtain adventitious shoot formation (Caboni and Tonelli, 1999). Presence of BAP in the culture medium was necessary for the shoot regeneration, although concentrations higher than 4.44 uM reduced the shoot regeneration frequency (Hodson et al., 2008). Waseem et al., (2006) inoculated chrysanthemum nodal segments in Murashige and Skoog (MS) media supplemented with different concentrations of indole acetic acid (IAA), naphthalene acetic acid (NAA) and indole butyric acid (IBA) and reported that 0.3 mg/l IAA, 0.5 mg/l NAA and 0.3 mg/l IBA showed their superiority over all their other respective concentrations, when used alone. Therefore, the attempts were made to determine the effect of different growth regulators on the shoot proliferation and rooting of chrysanthemum plantlets using nodal segments explant.

MATERIALS AND METHODS

The experiments regarding the effect of different concentrations of growth regulators and their combinations on the shoot regeneration and rooting of chrysanthemum (*Chrysanthemum morifolium* L) plantlets using nodal segments as explants were conducted at the Plant Tissue Culture Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, during 2007 in completely randomized design (CRD) with three replications and each replication has seven test tubes with nodal segments. For the regeneration of chrysanthemum plantlets, MS media were supplemented with varied concentrations of IAA (control, 0.1, 0.3 and 0.5 mg/l), BAP (control, 0.5, 1.0 and 2.0 mg/l) and their combinations. For rooting of chrysanthemum micro-shoots, half strength MS media supplemented with various concentrations of IBA (0.1, 0.2 and 0.5 mg/l), NAA (0.1, 0.2 and 0.5 mg/l) and IAA (0.1, 0.2 and 0.5 mg/l) were used. The following procedure was adopted.

Explants

The explants material was collected from 6 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. 1 cm long nodal segments (Bhattacharya et al., 1990) (containing a single node) were prepared, by using forceps and scalpel.

Sterilization

The excised explants were dipped in 70% ethanol for 60 sec. After pretreatment with ethanol, the explants were rinsed with double distilled water twice, so as to lower the toxic effect of ethanol. Nodal segments were then surface sterilized with 1.0% mercuric chloride ($HgCl_2$) for 3 min (Illahi 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 effects of $HgCl_2$.

Shoot Multiplication

Explants were cultured on solidified MS media with agar (8 mg/l) 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 and cytokinins. One explant in each tube (15 × 2.5 cm) containing 10 ml media was placed, vertically. Each treatment was replicated three times and each replication has seven explants. The tubes were covered with autoclaved poly praline sheets after culturing, which were held in place with rubber band. The cultured tubes were incubated for 16 h daily light of fluorescent, Philip white tubes with intensified 1000 LUX, at 25 ± 1°C temperature.

For shoot multiplication, the data was recorded for different parameters including shoot initiation percentage (number of explants initiating shoots were counted and percentage was calculated), average number of shoots per explant (number of shoots initiated, of five explants per replication, were counted and average was calculated), average shoot length (cm) (shoot length of five explants per replication, were counted and average was calculated), average number of leaves per shoot (number of leaves of five explants per replication, were counted and average was calculated) and average number of nodes per shoot (number of nodes per shoots were noted for five explants per replication and average was calculated). Shoot initiation percentage was calculated after one week, whereas all the other parameters were taken after eight weeks interval.

Rooting

For rooting, micro-shoots raised were harvested after 6 weeks and each shoot was transferred to a test tube containing 10 ml of half strength MS medium supplemented with different levels of IBA, IAA and NAA. Data was recorded for different parameters including root initiation percentage (number of roots initiated from five micro shoot

Table 1. Effect of different concentration of IAA on the regeneration of chrysanthemum from nodal segment explants.

Treatment	Shoot initiation %	Av. shoots explant^{-1}	Av. length of shoots (cm)	Av. leaves shoot^{-1}	Av. nodes shoot^{-1}
Control	33.3 D	1.5 C	2.1 D	2.7 D	2.7 D
0.1 mg/l IAA	70.0 B	3.5 AB	4.6 B	8.8 B	5.0 B
0.3 mg/l IAA	80.0 A	4.0 A	5.1 A	11.3 A	5.6 A
0.5 mg/l IAA	56.7 C	3.1 B	4.0 C	6.8 C	4.5 C
LSD (P<.05)	7.7	0.6	0.3	0.8	0.4

Av., Average. Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (P<.05).

Figure 1. Shoot multiplication on MS media supplemented with 0.3 mg/l IAA.

per replication were counted and average was calculated), average number of roots per explant (number of roots per five micro shoots per replication were counted and average was calculated), average root length (cm) (root length of five micro shoots per replication, were counted and average was calculated).

Statistical analysis

Recorded data were analyzed statistically using analysis of variance technique (ANOVA) and means were compared by Duncan's multiple range test (Steel et al., 1997).

RESULTS AND DISCUSSION

Effect of different concentration of IAA on the regeneration of chrysanthemum plantlets from nodal segment explants

Highly significant data regarding the effect of different concentrations of IAA (Control, 0.1, 0.3 and 0.5 mg/l) on the regeneration of chrysanthemum plantlets clearly depicted that an intermediate level of IAA (0.3 mg/l) showed its superiority amongst all the other IAA levels, in all the parameters studied (Table 1). Maximum shoot initiation percentage (80), shoots per plant (4.0), length of shoot (5.1 cm), leaves (11.3) and nodes per shoot (5.6) were recorded in 0.3 mg/l IAA (Figure 1). It was followed by 0.1 mg/l IAA producing 70% shoot initiation, an average of 3.5 shoots per explant, 4.6 cm long shoots, 8.8 leaves and 5.0 nodes per shoot. Whereas, the least response was found in control in all the parameters studied. This might be due to the fact that IAA is not usually considered to be a shoot proliferation growth regulator rather than root promoting regulator, as reported by Lazar and Cachita (1983). Our results gets support from a previous work done by Rout et al. (1997) who stated that the lower the indole acetic acid concentration, the higher would be the shoot bud regeneration in chrysanthemum. These results are in agreement with our previous findings (Waseem et al., 2008) who also reported that a low concentration of IAA (0.3 mg/l) excelled all the other concentrations by producing the best results for shoot proliferation.

Effect of different concentration of BAP on the regeneration of chrysanthemum plantlets from nodal segment explants

The highly significant data regarding the prolificacy of nodal segments towards plantlets regeneration under different concentrations of benzylaminopurine (control, 0.5, 1.0 and 2.0 mg/l) is shown in Table 2. The result revealed that an intermediate level of BAP (1.0 mg/l) showed its supremacy amongst all the other treatments,

Table 2. Effect of different concentration of BAP on the regeneration of chrysanthemum from nodal segment explants.

Treatment	Shoot initiation %	Av. shoots explant^{-1}	Av. Shoot Length (cm)	Av. number of leaves shoot^{-1}	Av. nodes shoot^{-1}
Control	33.3 D	1.5 D	2.1 C	3.2 C	2.7 D
0.5 mg/l BAP	83.3 B	4.0 B	4.9 B	10.6 B	5.4 B
1.0 mg/l BAP	100.0 A	4.9 A	5.8 A	13.4 A	6.3 A
2.0 mg/l BAP	66.7 C	3.6 C	4.6 B	10.2 B	5.0 C
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).

Figure 2. Shoot multiplication on MS media supplemented with 1.0 mg/l BAP.

in all the parameters. Maximum shoot initiation (100%), shoots per explant (4.9), shoot length (5.8 cm), leaves (13.4) and nodes (6.3) per shoot were recorded in 1.0 mg/l BAP (Figure 2). It was followed by 0.5 mg/l BAP with 83.3% shoot initiation, 4.0 shoots per explant, 4.9 cm long shoots, 10.6 leaves and 5.4 nodes per shoot. The least response for all the parameters was found in Control. Mok and Mok (2001) also reported that BAP and kinetin act as a cytokinin that have long been recognized as an essential plant hormone that are involved in diverse process of plant growth and development including cell division, shoot initiation, etc.

The results showed that an intermediate concentration (1.0 mg/l) of benzylaminopurine produced the best results in almost all the parameters and no other treatment was better than this. The entire plus or minus deviation from this particular BAP concentration showed poor results. The fact that higher dozes fail to manifest their superiority could be attributed to an obnoxious effect at higher concentration, whereas, the ineffectiveness of the lower dose indicate inadequate doze of hormone as a consequence indicating poor performance. Similarly, Hodson et al. (2008) stated that the presence of BAP in the culture medium was necessary for the shoot regeneration, although concentrations higher than 4.44 µM reduced the shoot regeneration frequency. Our results are also being supported by Ali et al. (2007) and Waseem et al. (2009) who also recommended 1.0 mg/l BAP as the most optimum BAP concentration for the regeneration of plantlets and by any increase or decrease in its concentration caused a decrease in multiplication rates. Similar results were also reported by a number of other scientists (Gul, 2001; Karim et al., 2003) stating higher percentage of chrysanthemum plantlets formation in MS media supplemented with 1.0 mg/l BAP.

Effect of different concentrations of BAP and IAA on the regeneration of chrysanthemum plantlets from nodal segment explants

Cytokinins along with auxins also play a vital role in shoot regeneration in chrysanthemum (Karim et al., 2003). Among the different treatments shoot regeneration frequency varied significantly (Table 3). The regeneration frequency could be improved by manipulating the compositions of the hormones in the culture media. The reason is that juvenility played an important role in regeneration is not clear and the number of regenerated shoot buds depends on the composition of culture medium, especially on the levels of plant growth regulators (Rout and Das, 1997). Highly significant differences regarding the effect of different combinations of benzylaminopurine (BAP) and indole acetic acid (IAA) on the regeneration of chrysanthemum plantlets using nodal segments as explants

Table 3. Effect of different concentrations of BAP + IAA on the regeneration of chrysanthemum from nodal segment explants.

BAP+IAA (mg/l)	Shoot initiation (%)	Av. shoots explant^{-1}	Av. length of shoots (cm)	Av. Leaves shoot^{-1}	Av. nodes shoot^{-1}
0.5 + 0.1	76.7 EFG	6.5 EF	4.7 DE	18.3 C	5.0 EF
0.5 + 0.2	70.0 G	6.1 G	4.3 FG	17.7 E	4.8 FG
0.5 + 0.5	60.0 H	5.8 H	3.4 I	15.7 I	3.9 HI
0.5 + 1.0	50.0 IJ	5.0 JK	2.5 K	14.4 L	3.0 K
1.0 + 0.1	96.7 A	8.0 A	5.5 A	19.4 A	6.0 A
1.0 + 0.2	90.0 ABC	7.6 B	5.1 BC	19.1 B	5.5 BC
1.0 + 0.5	80.0 DEF	6.7 DE	4.5 EF	16.3 H	5.0 EF
1.0 + 1.0	56.7 HI	5.5 I	3.0 J	14.0 M	3.6 I
2.0 + 0.1	93.3 AB	7.7 B	5.3 AB	18.5 C	5.8 AB
2.0 + 0.2	90.0 ABC	7.0 C	5.0 CD	18.0 D	5.3 CD
2.0 + 0.5	83.3 CDE	6.7 DE	3.7 H	15.1 J	4.1 H
2.0 + 1.0	53.3 HIJ	5.2 J	2.9 J	13.5 N	3.3 J
5.0 + 0.1	86.7 BCD	6.9 CD	4.9 CD	17.2 F	5.2 DE
5.0 + 0.2	83.3 CDE	6.8 CD	4.8 DE	16.7 G	5.1 DE
5.0 + 0.5	73.3 FG	6.3 FG	4.0 GH	14.8 K	4.5G
5.0 + 1.0	46.7 J	4.8 K	2.3 K	13.0 O	2.8 K
LSD P<.05)	7.5	0.2	0.3	0.2	0.2

Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (P<.05).

Figure 3. Initial Shoot initiation in MS media supplemented with 1.0 mg/l BAP + 0.1 mg/l IAA.

are presented in Table 3. The result showed that out of 16 different hormonal combinations, 1.0 mg/l BAP + 0.1 mg/l IAA, 2.0 mg/l BAP + 0.1 mg/l IAA, 2.0 mg/l BAP + 0.2 mg/l IAA and 1.0 mg/l BAP + 0.2 mg/l IAA showed their superiority over all the other combinations, as they excelled in all the parameters as compared to other combinations, as shown in Table 3.

Maximum shoot initiation (96.7%), shoots per explant (8.0), shoot length (5.5 cm), leaves (19.4) and nodes per shoot (6.0) were recorded in 1.0 mg/l BAP+ 0.1 mg/l IAA, followed by 2.0 mg/l BAP + 0.1 mg/l IAA, 1.0 mg/l BAP + 0.2 mg/l IAA and 2.0 mg/l BAP + 0.2 mg/l IAA with 93.3, 90.0 and 90.0% shoot initiation, 7.7, 7.6 and 7.0 shoots per explant, 5.3, 5.1 and 5.0 cm long shoots 18.5, 19.1 and 18.0 leaves and 5.8, 5.5 and 5.3 nodes per shoot, respectively (Figures 3 and 4). The least response was observed in 5.0 mg/l BAP + 1.0 mg/l IAA and by 2.0 mg/l BAP + 1.0 mg/l IAA for almost all the parameters studied. All the other treatments showed an intermediate behavior for all the parameters.

Similar results were quoted by Waseem et al. (2009) and Karim et al. (2003) stating that best results were obtained in MS media supplemented with 1.0 mg/l BAP + 0.1 mg/l IAA, followed by 2.0 mg/l BAP + 0.1 mg/l IAA for the regeneration of Chrysanthemum plantlets. Our results were also confirmed by Sivanesan and Murugesan (2008) who stated that a combination BAP and IAA is responsible for an increment in shoot length.

It is evident from the given result tabulated in Table 3, that 1.0 mg/l BAP + 0.1 mg/l IAA showed the best results in almost all the parameters closely trailed by 2.0 mg/l

Figure 4. Shoot multiplication in MS media supplemented with 1.0 mg/l BAP+ 0.1 mg/l IAA.

BAP + 0.1 mg/l IAA, 1.0 mg/l BAP + 0.2 mg/l IAA and 2.0 mg/l BAP + 0.2 mg/l IAA, which also produced better results as compared with the other hormonal combinations. This result showed that the intermediate levels of BAP (1.0 and 2.0 mg/l) along with the lower concentrations of IAA (0.1 and 0.2 mg/l) had a favorable effect on the regeneration of chrysanthemum plantlets using nodal segments as explants. On the other hand the highest (5.0 mg/l) and the lowest (0.5 mg/l) concentrations of benzylaminopurine (BAP) combined with higher concentrations of indole acetic acid (IAA). That is, 0.5 and 1.0 mg/l did not respond well and showed their least interest towards regeneration of chrysanthemum.

As BAP belongs to cytokinin group so it would have its tendency towards the shoot development and thus its influence on the shoot development of chrysanthemum was not affected by the lower concentrations of IAA, whereas the higher concentrations of IAA would had affected its influence on the shoot development. Similar results were also reported by Singh and Arora (1995) stated that increased levels of BAP in the medium increased the number of shoots but suppressed their growth. An addition of IAA did not supplement the effect of BAP on shoot morphogenesis.

A number of research workers have also reported the encouraging results of different combinations of BAP + IAA on the regeneration of chrysanthemum plantlets that is, Lazar and Cachista (1983), Bhattacharya et al. (1990), Singh and Arora (1995), Hoque et al. (1998), Gul (2001), Karim et al. (2003) and Waseem et al. (2009).

Effect of different concentrations of indole butyric acid (IBA), naphthalene acetic acid (NAA) and indole acetic acid (IAA) on the rooting of micro-shoots of chrysanthemum raised from nodal segment explants

Effect of different concentrations of indole butyric acid (IBA), naphthalene acetic acid (NAA) and indole acetic acid (IAA) on the rooting of micro-shoots of chrysanthemum from nodal segment explant showed a statistically significant behavior for all the parameters (Table 4).

A very highly competitive and encouraging result was observed regarding root initiation percentage as affected by different concentration of auxins such as IBA, NAA and IAA. Maximum (100%) rooting was observed in ½ MS + 0.2 mg/l IBA and ½ MS + 0.2 mg/l NAA, very closely followed by ½ MS + 0.5 mg/l IBA, ½ MS + 0.5 mg/l NAA and ½ MS + 0.2 mg/l IAA with 96.7, 93.3 and 93.3% root initiation and all these treatments were statistically at par with each other. Statistically similar results were found in ½ MS + 0.1 mg/l IBA, ½ MS + 0.1 mg/l NAA and control with 83.3, 83.3 and 80.0% root initiation, respectively. However, the minimum of 70 and 76.7% rooting was observed in ½ MS + 0.5 mg/l IAA and ½ MS + 0.1 mg/l IAA respectively. Our findings are in agreement with the results obtained by Khan et al. (1994) who stated 100% root initiation in half strength MS medium containing 0.25 mg/l IBA.

The results once again showed the supremacy of ½ MS + 0.2 mg/l IBA as compared to all other treatments as it produced significantly maximum number of roots per plantlet (16.0) and longest roots (11.0 cm). It was followed by ½ MS + 0.2 mg/l NAA and ½ MS + 0.2 mg/l IAA with an average of 15.0 and 14.3 roots per plantlet and 10.6 and 10.0 cm long roots, respectively (Figures 5 and 6). The least response was reported in ½ MS + 0.1 mg/l IAA and ½ MS + 0.5 mg/l IAA with an average number of 7.8 and 8.9 roots per plantlets; 7.7 and 7.2 cm long roots, respectively. The results showed that the lowest (0.1 mg/l) and the highest (0.5 mg/l) concentrations of IAA used, had shown the least response towards all the parameters regarding rooting. Our results are confirmed by the findings of Hoque et al. (1998) who reported that in case of *in vitro* rooting; best response was obtained when micro-shoots were cultured on half strength MS medium supplemented with 0.2 mg/l IBA, in chrysanthemum. Similarly, Karim et al. (2002) also declared that the maximum length of roots were recorded in half strength MS medium supplemented with 0.2 mg/l IBA, chased by 0.2 mg/l NAA.

While comparing the effect of different auxins for root induction and development, it is evident from the data tabulated in the Table IV, that half strength MS medium fortified with different concentrations of IBA had radically did better and their supremacy was absolute for all the

Table 4. Effect of different concentrations of IBA, NAA and IAA on the rooting of micro shoots raised from nodal segment explants of chrysanthemum.

Treatment (½ Strength MS +)	Root initiation (%)	Av. roots plantlet^{-1}	Av. length of roots (cm)
(Control))	80.0 CD	9.3 H	8.0 F
0.1mg/l IBA	83.3 BC	12.4 E	9.7 CD
0.2 mg/l IBA	100.0 A	16.0 A	11.0 A
0.5 mg/l IBA	96.7 A	13.3 D	8.9 E
0.1 mg/l NAA	83.3 BC	10.9 F	9.4 D
0.2 mg/l NAA	100.0 A	15.0 B	10.6 B
0.5 mg/l NAA	93.3 AB	10.1 G	8.1 F
0.1 mg/l IAA	76.7 CD	7.8 J	7.7 G
0.2 mg/l IAA	93.3 AB	14.3 C	10.0 C
0.5 mg/l IAA	70.0 D	8.9 I	7.2 H
LSD (P<.05)	13.2	0.3	0.4

Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (P<.05)

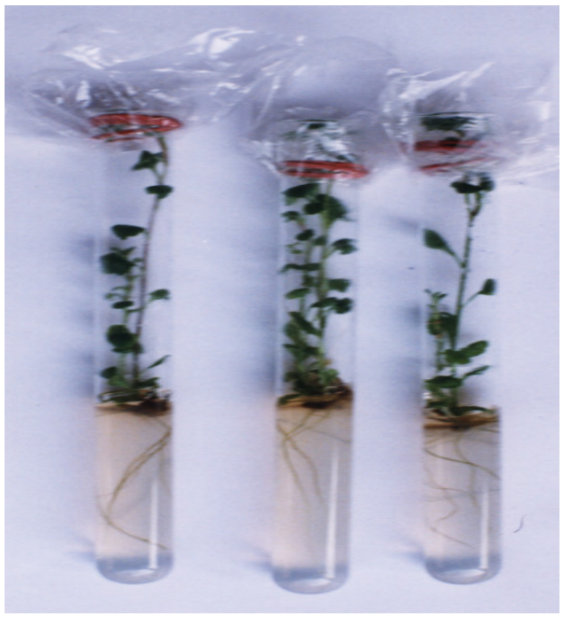

Figure 5. Rooting of micro shoots in 1/2 MS media fortified with 0.2 mg/l NAA.

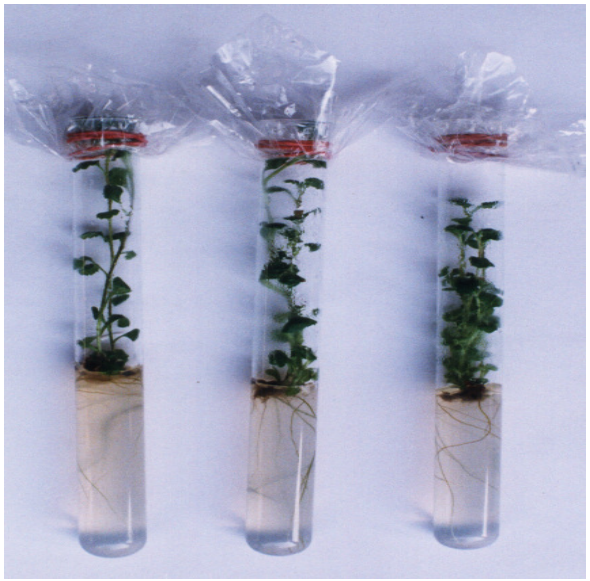

Figure 6. Rooting of micro shoots in 1/2 MS media fortified with 0.2 mg/l NAA.

other treatments, followed by different concentrations of NAA and IAA, respectively. IBA is considered as the most effective auxin in root induction (Litz and Jaiwal, 1990). Our results were further confirmed by the previous findings of Komalavalli and Rao (2000), Sarker and Shaheen (2001), Munshi et al. (2004), Awal et al. (2005), Din et al. (2005) and Rajani and Patil (2009) who suggested indole butyric acid as the best auxin for root induction and development.

Half strength MS media supplemented with the lower concentrations (0.1 and 0.2 mg/l) of all the auxins responded well. Our results are supported by Kaul et al. (1990) who reported that at higher concentrations of auxins, shoots failed to regenerate roots.

However, the best response was obtained in 0.2 mg/l for all the auxins as it had produced the best results for average number of days for root emergence, root induction percentage, high number of roots per plantlets and average length of roots. A number of research workers including Hoque and Fatema (1995), Hoque et al. (1998), Rout (2005), and Liu and Gao (2007), mentioned ½ MS medium fortified with 0.2 mg/l auxins as the most optimum media for root initiation and development.

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