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Full Length Research Paper

In vitro regeneration of *Gerbera jamesonii* cv. Sunglow

Khaliqa Shabbir[1], Touqeer Ahmad[1], Ishfaq Ahmad Hafiz[1]*, Azhar Hussain[1], Nadeem Akhtar Abbasi[1] and Jamil Ahmad[2]

[1]Department of Horticulture, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.
[2]Faculty of Agriculture, University of Rawalakot, Azad Jammu and Kashmir.

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This study demonstrates a protocol for *in vitro* regeneration of *Gerbera jamesonii* cv. 'Sunglow' developed by culturing leaf segments on Murashige and Skoog (MS) medium supplemented with 1.0, 1.5, 2.0, 2.5 and 3.0 mg L^{-1} indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), 2,4-dichlorophenoxy acid (2,4-D) and 1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1} benzyl adenine (BAP) and kinetin (Kin). The treatment, 1.5 mg l^{-1} 2,4-D gave 100% callus induction with a friable, nodular and creamish white callus. BAP gave fair callus growth with compact and brownish callus. However, Kin failed to produce callus. Shoot regeneration was assayed with 1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1} BAP and Kin. BAP at 3.0 mg l^{-1} showed 76.67% shoot regeneration, 4 shoots per explant and shoot length of 9 cm, whereas same concentration of Kin gave only 16.67% regenerated shoots, 1.97 shoots per explant and length of 5.167 cm. *In vitro* root induction was determined by using 0.5, 1.0, 1.5 and 2.0 mg L^{-1} indole-3-acetic acid (IAA) and NAA. IAA at 1.5 mg L^{-1} exhibited a rooting percentage of 97.67%, whereas similar concentration of NAA gave relatively less rooting percentage of 60.67%. IAA gave thick roots with maximum root number (7.567/per explant) and length (7.33 cm), on the contrary, NAA gave relatively thin roots having less number of 3.567 roots with a length of 4.667 cm.

Key words: *Gerbera jamesonii*, *in vitro* regeneration, leaf segments, culture.

INTRODUCTION

Gerbera jamesonii is one of the widely grown ornamental plants, used as a cut flower as well as potted plant with an increasing demand in domestic and international market. Its propagation can be carried out by both sexual and asexual methods. Seed propagation is usually undesirable because it takes longer time to produce flowers (Nhut et al., 2007). Vegetative propagation overcomes this problem and produce plants of better performance (Topoonyanont and Debergh, 2001). Among vegetative propagation, leaf cutting, stem cutting and divisions of clumps are the most common techniques (Kumar and Kanwar, 2007). Disadvantage in vegetative propagation through divisions is that it is very slow and only five plantlets per year are produced from a single one year old plant (Kumar et al., 2004). *In vitro* technique is a world-wide applied tool for propagation in the horticultural industry and can be helpful to solve such problem.

In gerbera, the chances of production of solid mutants from adventitious shoot regeneration techniques are much higher than those of axillary shoot techniques. Therefore, adventitious shoots are useful in mutation breeding as a tool for the production of solid mutants (Jerzy and Lubomski, 1991). The most important application of regeneration is its use in conjunction with genetic transformation. A reliable protocol for regeneration acts as a fundamental for genetic transformation, offering an opportunity for the introduction of wide range of beneficial traits in gerbera. In a previous work, adventitious shoots in gerbera were regenerated from callus by using different explants (Kumar et al., 2004). However, vegetative propagation through adventitious buds by using midrib as explant source was not successful (Nhut et al., 2007). Furthermore, callus

*Corresponding author. E-mail: decenthafiz60@yahoo.com.

Abbreviations: MS, Murashige and Skoog; **IBA,** indole-3-butyric acid; **NAA,** naphthalene acetic acid; **2,4-D,** 2,4-dichlorophenoxy acid; **BAP,** benzyl adenine; **Kin,** kinetin.

Table 1. Effect of different levels of NaOCl on necrosis, infection and survival percentages of cultured leaf explants of *G. jamesonii* cv. "Sunglow" after 4 weeks.

NaOCl solution (% v/v)	Necrosis (%)*	Infection (%)*			Survival (%)*
		Bacterial	Fungal	Total	
5	0	31	69	100	0
10	04	23	47	70	26
15	37	18	26	44	19

*, 100 explants/treatment.

cultures give inconsistency in organ regeneration. The regeneration from floral buds and capitulum cultures has also been reported in gerbera by many workers (Modh et al., 2002), however, the regeneration percentage from these methods is very low. In the present investigation, an attempt was made to develop an efficient method to improve the *in vitro* regeneration rate of *G. jamesonii* cv. "Sunglow".

MATERIALS AND METHODS

Immature young leaves of *Gerbera jamesonii* cv. Sunglow with petiole size 10 × 2 cm (first leaf from apical portion) were excised and washed thoroughly under running tap water for about half an hour to remove soil and other foreign contaminants. Leaves were divided into 3 to 4 equal strips each of 2 × 2 cm size and surface sterilized with 5, 10 and 15% (v/v) sodium hypochlorite (NaOCl) solution (Fluka RdH active chlorine 6 to 14%) for 6 min with continuous agitation followed by 3 rinses with sterilized distilled water to remove the NaOCl residues. Sterilized leaf sections with petiole were resized to 5 x 5 mm pieces (Kumar et al., 2004) and cultured aseptically in test tubes containing callus induction medium (CIM) comprising MS salts, macro and micro elements, and vitamins (Murashige and Skoog, 1962) supplemented with 1.0, 1.5, 2.0, 2.5 and 3.0 mg L^{-1} IBA, NAA, 2,4-D, and BAP and Kin at 1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1}. The medium was also complemented with 30 g L^{-1} sucrose and solidified with 7 g L^{-1} agar. The pH was adjusted to 5.8 before sterilization at 121°C for 8 min. The cultures were incubated in dark at 25 ± 1°C for 30 days and then transferred to light under 16/8 h photoperiod (2,000 lux) with white fluorescent tubes (Philips TL 40W/54). The experiment was arranged in completely randomized design with three replications per treatment and ten explants per replication. Visual observations of necrosis, bacterial and fungal contamination were recorded after two weeks. Data was recorded for callus induction percentage, callus growth and type of callus (friable and compact).

For shoot regeneration studies, one month old calli of size 4 x 6 mm were transferred to culture jars on shoot regeneration media (SRM) based on MS medium (MS macro and micro elements, and vitamins) supplemented with 1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1} BA and Kin together with 30 g l^{-1} sucrose and 7 g L^{-1} agar. The pH of media was adjusted to 5.8 before autoclaving. Cultures were incubated at 25 ± 1°C under 16/8 h photoperiod (2,000 lux) with white fluorescent tubes (Philips TL 40W/54). Data was recorded for shoot regeneration percentage, number of shoots per regenerated callus and shoot length (cm) after four weeks.

In vitro regenerated shoots were uniformly prepared to a size of 1 to 1.5 cm by separating them from a multiple shoot complex originating from the callus after shoot initiation and were transferred to rooting media (RM) consisting of MS macro and micro elements, and vitamins plus 30 g L^{-1} sucrose and 7 g L^{-1} agar with 0.0, 0.5, 1.0, 1.5 and 2.0 mg L^{-1}. The pH was adjusted to 5.8 before sterilization. The cultures were incubated at 25 ± 1°C under 16/8 h photoperiod (2,000 lux) with white fluorescent tubes (Philips TL 40W/54). After four weeks, the data on rooting percentage, average number of roots per shoot and root length of explants (cm) were recorded. Statistical analysis of the data was carried out by using analysis of variance (ANOVA) technique and differences among treatment means were compared by using least significance difference (LSD) test at 5% probability level (Steel et al., 1997).

RESULTS AND DISCUSSION

Disinfestation of leaf explants of *G. jamesonii* cv. 'Sunglow'

Visually screened data pertaining to effect of various concentrations of NaOCl on disinfestation and survival percentages of leaf explants of *G. jamesonii* cv. 'Sunglow' (Table 1) shows that 10% NaOCl had significantly increased the survival percentage (26%) which decreased to 19% when the concentration of NaOCl was further increased to 15%. NaOCl at the concentration of 15% reduced infection (44%) but increased necrosis (37%). It indicates that together with disinfestation, immature leaf sections might have been damaged and caused the death of the explants. No visible symptoms of necrosis were observed at 5% NaOCl but all the explants exhibited 100% infestation. The selection of a safe sterilant is of prime importance for the control of infection and establishment of explant tissue. Furthermore, appropriate concentration of NaOCl is required for the removal of fungal and bacterial contamination (Ahmad et al., 2003). In the present investigation, an effective control of contamination with maximum survival percentage (26%) and minimum necrosis (4%) was observed from leaf explants of *G. jamesonii* cv. 'Sunglow' by using 10% NaOCl solution.

Callus induction percentage

Significant difference was observed between treatments regarding callus induction percentage among all treatments, 2,4-D was the most effective auxin at 1.5 mg L^{-1} (T_{12}) which gave 100% callus induction (Figure 1a) followed by NAA (T_8 2.0 mg L^{-1}) with 83.33% callus

induction (Figure 1b). The callusing percentage recorded in IBA (T_2 1.5 mg L^{-1}) was 58.33%. When different concentrations of BAP were introduced to the callus induction medium, the results showed poor percentages in all concentrations, except T_{18} (3.0 mg L^{-1}) where 25% callus induction was noted.

Many researchers observed auxins (IBA, NAA and 2,4-D) as the best growth regulator for callus induction (Suzuki et al., 2002) which is in agreement with the results obtained in the present investigation where auxins in comparison with cytokinins gave better callus induction percentage (Koroch et al., 2003). Auxins stimulate RNA metabolism and induce the transcription of messenger RNA which code the proteins that are required for the chaotic cell proliferation and ultimately the callus formation. Nahid et al. (2007) reported that 2,4-D, NAA and IBA induce cell division and enlargement at optimum concentrations which is associated with increase in the activities of autolytic and synthetic enzymes by effecting cell wall plasticity and by synthesizing new cell wall materials. A decrease in callus induction percentage of IBA as compared to 2,4-D and NAA was observed which is probably due to its instability by making it less effective than other auxins like 2,4-D and NAA (Obukosia et al., 2005).

BAP had positive effect on callus induction, whereas, Kin is generally less effective. Modh et al. (2002) reported that BAP stimulate RNA and protein synthesis which activate enzyme activity for cell division and cell wall loosening. Previously, poor callus induction on MS medium supplemented with different concentrations of Kin from leaf explants of *G. jamesonii* was also reported by Kumar and Kanwar (2007).

Among different concentrations of auxins (IBA, NAA and 2,4-D) and cytokinins (BAP and Kin), it was found that by increasing the concentration of growth regulators, there is a great fall in the callusing percentages which agrees with the report of Can et al. (2008).

Callus growth and types of callus

Data for the growth and type of callus on MS medium supplemented with different concentrations of growth regulators was analyzed (Table 2). The first visible response was the initiation of callus along the wounded edges of leaf explants within 7 to 15 days on all concentrations of IBA, NAA, 2,4-D and BAP which gradually covered the partial or entire leaf surface within 30 days of inoculation. It is because the cut ends of leaf explants provided a way for the nutrients and growth regulators to be absorbed efficiently from the medium and start functioning according to the nature of the growth regulator (Reynoired et al., 1993). This response was not observed in the explants cultured on different concentrations of Kin. A friable, nodular, creamish white callus with excellent callus growth (Figure 1c) was recorded on 2,4-D at T_{12} (1.5 mg L^{-1}). NAA at T_8 (2.0 mg L^{-1}) and T_9 (2.5 mg L^{-1}) also gave excellent callus growth with creamish white, friable and nodular type of callus. Similar results were obtained from IBA (1.5 mg L^{-1}) at T_2. BAP at T_{18} (3.0 mg L^{-1}) gave compact type of callus with brown colour and fair growth (Figure 1d) whereas all concentrations of Kin gave extremely poor callus growth.

According to Thorpe (1980), inclusion of an auxin in the media is necessary for callus induction and growth by influencing the development of progressively more random planes of division. Taiz and Zieger (2002) showed that auxins (IBA, NAA and 2,4-D) directly stimulated the early phases of cell elongation by causing responsive cells to actively transport hydrogen ions out of the cell and lower the pH around cells. This acidification of the cell wall region activates wall-loosening proteins known as expansions to allow slippage of cellulose microfibrils in the cell wall and breaking of bonds in wall polysaccharides, allowing the walls to stretch more easily and to increase the callus growth more rapidly. Can et al. (2008) reported that auxins make nuclear DNA to become more methylated than usual state which revert the cell to dedifferentiated state and initiate cell division and enlargement. In the present work, compact callus was observed on all concentrations of BAP which according to Suzuki et al. (2002) was characterized with dense cytoplasm and conspicuous nuclei with comparatively thicker cell walls which were tightly packed together giving rise to an undifferentiated mass.

It was observed that callus colour turned brown and dark brown when the concentrations of IBA, NAA, 2,4-D and BAP were increased from an optimum level of 3.0 mg L^{-1}. Shirin et al. (2007) observed that meristematic cell division is blocked at supra optimal concentrations of auxins (IBA, NAA and 2,4-D) and cytokinins (BAP and Kin) which causes the inhibition of protein synthesis, leading to the browning and death of callus. Numerous small white threads like roots were observed originating directly from leaf explants on all concentrations of IBA. It is because IBA activated those genes in the leaf explant which are involved in direct rooting and in certain cases IBA indirectly affected polyamine synthesis which control rooting. According to the study of Minocha et al. (1993), synthesis of cellular polyamine levels respond to the exogenous IBA concentrations which cause direct rooting from leaf explants.

Shoot regeneration percentage

Among different treatments, shoot regeneration percentage varied significantly (Table 3). BAP at the concentration of T_3 (3.0 mg L^{-1}) gave highest shoot regeneration percentage of 76.67%. Comparatively less shoot regeneration frequency was recorded with Kin application which is in line with the findings of Karim et al. (2003).

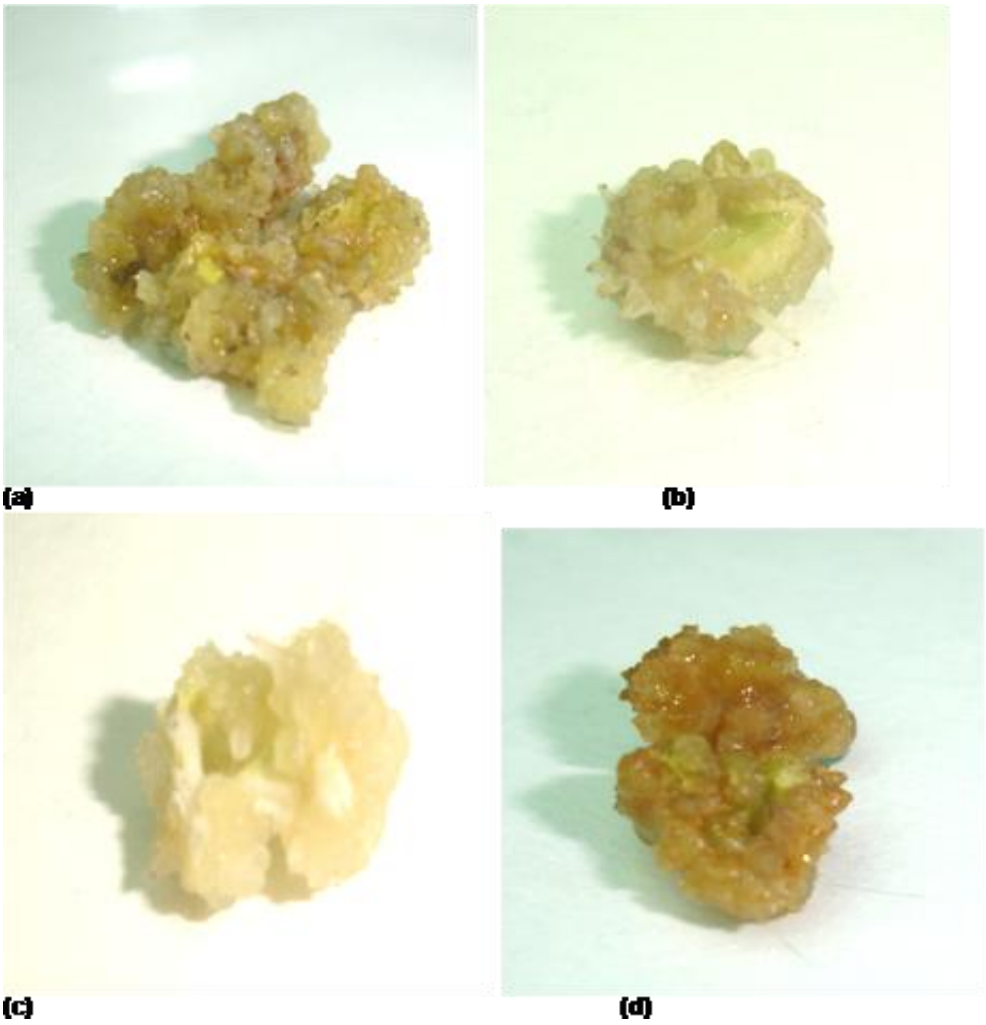

Figure 1. (a) Callus induction percentage in 2,4-D at T_{12} (1.5 mg L^{-1}), with complete callus covering; (b) NAA at T_8 (2.0 mg L^{-1}) showing 83% of leaf explant covered with callus; (c) A friable, nodular, creamish white callus observed with excellent growth on 2,4-D at T_{12} (1.5 mg L^{-1}); (d) Fair growth with compact and brownish type callus observed on BAP at T_{18} (3.0 mg L^{-1}).

Kin proved less effective for shoot regeneration and gave comparatively lower regeneration percentages then BAP. Moreover Can et al. (2008) observed the inefficiency of Kin over BAP for obtaining better regeneration percentages. A decreasing trend in regeneration percentages with increasing concentrations of BAP and Kin after an optimum level (3.0 mg L^{-1}) was found among different treatments. Shirin et al. (2007) suggested that among cultures where cytokinins are in limited concentrations, the division of cell nuclei becomes arrested at one stage of the cell cycle which affects the regeneration percentage.

Number of regenerated shoots

Results show that the number of regenerated shoots per callus ranged between 0.23 and 4.0, depending on the type and concentration of growth regulators (BAP and Kin). BAP at T_3 (3.0 mg L^{-1}) produced significantly higher number of shoots by producing an average of 4.0 shoots per callus (Figure 2a and Table 3), whereas Kin at T_8 (3.0 mg L^{-1}) gave 1.967 shoots. Initially, shoot buds with pink and green colour appeared within 3 to 4 weeks of subculturing almost in all concentrations of BAP and Kin. They took 6 to 8 weeks to develop into a healthy shoot. In Kin substituted media, 1 to 5 shoot buds appeared occasionally but almost all of them failed to develop into a shoot with the exception of only 1 or 2. BAP being the chemical analogue of cytokinin not only affect different phases of regeneration but also indicate cytokinin specificity for obtaining higher number of regenerants and shoots which is in corroboration with the findings of Aswath and Wazneen (2004). Haberer and Kieber (2002)

Table 2. Effect of different growth regulators on callus induction, growth and type.

Treatment (mg L^{-1})	Callus induction (%)	Callus growth*	Callus type
IBA			
T_0 0.0	0.0000^{k}	-	0
T_1 1.0	50.00^{efg}	++	Friable
T_2 1.5	58.33^{defg}	+++	Friable
T_3 2.0	41.67^{ghi}	++	Friable
T_4 2.5	25.00^{hij}	++	Friable
T_5 3.0	16.67^{j}k	++	Friable
NAA			
T_6 1.0	45.00^{fgh}	++	Friable and nodular
T_7 1.5	53.33^{defg}	++	Friable and nodular
T_8 2.0	83.33^{abc}	+++	Friable and nodular
T_9 2.5	70.00^{bcde}	+++	Friable and nodular
T_{10} 3.0	66.67^{cdef}	++	Friable and nodular
2,4-d			
T_{11} 1.0	91.67^{ab}	+++	Friable and nodular
T_{12} 1.5	100.0^{a}	+++	Friable and nodular
T_{13} 2.0	75.00^{bcd}	+++	Friable and nodular
T_{14} 2.5	20.00^{ijk}	++	Friable and nodular
T_{15} 3.0	8.333^{jk}	++	Friable and nodular
BA			
T_{16} 1.0	6.000^{jk}	+	Compact and nodular
T_{17} 2.0	11.67^{jk}	+	Compact and nodular
T_{18} 3.0	25.00^{hij}	+	Compact and nodular
T_{19} 4.0	8.333^{jk}	+	Compact and nodular
T_{20} 5.0	0.0000^{k}	-	0
kin			
T_{21} 1.0	0.0000^{k}	-	0
T_{22} 2.0	0.0000^{k}	-	0
T_{23} 3.0	0.0000^{k}	-	0
T_{24} 4.0	0.0000^{k}	-	0
T_{25} 5.0	0.0000^{k}	-	0
$LSD_{5\%}$ 22.82			

Different letters within a column indicate significant difference at $p < 0.05$. +++: Excellent callus growth, ++: very good callus growth, +: fair callus growth and -: poor/no callus growth.

observed that addition of BAP in the regeneration media generally provide a stimulus for the regeneration and allow the cell cycle regulation required for the induction of cell division and specialization during plant development which not only stimulated shoot meristemoid formation but also increased shoot number. Rashotte et al. (2003) showed that BAP enhanced shoot number by increasing cell division as BAP might be required to regulate the synthesis of those proteins which are involved in the formation of meiotic spindle apparatus.

Kin gave comparatively less shoot number than BAP which is in accordance with the findings of Geetha et al. (1998). Furthermore, Haberer and Kieber (2002) reported that Kin appeared to be ineffective in shoot development process because of insufficient cytokinin activity which blocks early developmental stage of adventitious shoot formation and reduces shoot number. It appeared that shoot number had a tendency to decrease as BAP and Kin concentrations reduced very much or increase beyond 3.0 mg L^{-1}. Therefore, it can be suggested that the optimal BAP and Kin concentrations in the medium should be high enough to satisfy the basic requirement

Table 3. Effect of different concentrations of BAP and Kin on shoot regeneration percentage, shoot number and shoot length (cm).

Treatment (mg L^{-1})	Shoot regeneration (%)	Shoot number	Shoot length (cm)
BAP			
T_0 0.0	0.00^{i}	0.00^{h}	0.00^{i}
T_1 1.0	53.33^{c}	2.700^{c}	6.800^{bc}
T_2 2.0	64.67^{b}	3.533^{b}	7.767^{b}
T_3 3.0	76.67^{a}	4.000^{a}	9.000^{a}
T_4 4.0	45.33^{d}	2.577^{c}	6.067^{cd}
T_5 5.0	36.67^{e}	2.230^{cd}	5.133^{de}
Kin			
T_6 1.0	4.000^{h}	0.633^{fg}	3.167^{f}
T_7 2.0	7.333^{gh}	1.233^{e}	4.233^{e}
T_8 3.0	16.67^{f}	1.967^{d}	5.167^{de}
T_9 4.0	12.33^{fg}	1.067^{ef}	1.967^{g}
T_{10} 5.0	5.333^{h}	0.233^{g}	0.833^{h}
$LSD_{5\%}$	6.022	0.4474	0.9858

Different letters within a column indicate significant difference at $p < 0.05$.

for cell division and differentiation and low enough not to impose any negative effect on number of shoots per callus. Rashotte et al. (2003) reported that suboptimal (<3 mg L^{-1}) concentrations of BAP and Kin reduce cell division during a cell cycle which causes a marked decrease in shoot number. Whereas supraoptimal levels (>3 mg L^{-1}) of BAP and Kin have inhibitory effect on number of shoot per explant. This inhibitory effect has been related with the negative effect of BAP and Kin on protein synthesis (Casimiro et al., 2001).

Length (cm) of regenerated shoots

Significant difference for shoot length was noticed for different concentrations of BAP and Kin (Table 3). BAP at T_3 (3.0 mg L^{-1}) gave maximum shoot length of 9.0 cm (Figure 2b) followed by Kin at T_8 (3.0 mg L^{-1}) producing 5.167 cm long shoots. Furthermore, highest Kin concentration at T_{10} (5.0 mg L^{-1}) gave lowest shoot length of 0.833 cm. Chen et al. (1985) reported that BAP increased the amount of mRNAs which promote cell expansion, cell division and chlorophyll synthesis by activating synthesis of protein that bind chlorophyll for shoot elongation. D' Angeli et al. (2001) observed more shoot length when optimal concentration of BAP was applied, it produced more diffused and well distributed endogenous isopentenyladenine (iP) and isopently adenosine (iPR) in the tissues of the vegetative shoot apex which is considered to be the precursor of all the naturally occurring cytokinins.

Furthermore, Haberer and Kieber (2002) observed that BAP increased the cytokinin biosynthesis by coding those enzymes which are involved in the conversion of dimethylallyl diphosphate (DMAPP) and adenisine-monophosphate (AMP) to isopentenyleadenosine-5-monophosphate (iPMP) through the action of *ipt* genes, thus causing a marked increase in shoot length.

The results obtained in the present investigation might be in line with those of D' Angeli et al. (2001) who reported that maximum shoot development was found in BAP than Kin in Agnihothri. Bennet et al. (1994) observed that reduced shoot length can be connected to less efficiency of exogenous Kin to trigger endogenous cytokinin level of the cell for normal growth. Werner et al. (2001) mentioned that the inhibition of shoot elongation is induced by excess cytokinin due to the production of ethylene in cells which increased the production of protein synthesis inhibitors and inferred the inhibitory effect on shoot length.

Rooting percentage (%)

Significant difference was observed among treatments; rooting percentages (Table 4) varied from 0 to 97.67% with the varying concentrations of auxins. IAA at T_7 (1.5 mg L^{-1}) gave the highest rooting percentage of 97.67% followed by IAA at T_8 (2 mg L^{-1}) giving 85% rooting. No root induction was observed in auxin free media. According to the study of Metaxas et al. (2004) metabolic changes in the rooting zones of cuttings due to auxins are capable of promoting adventitious root formation which indicates that auxin is an essential growth regulator required for the process of root formation of gerbera. Husen and Pal (2001) reported that IAA application strongly enhances root induction through the endogenous IAA acropetal movement from the shoot towards the central cylinder (phloem or precursor procambial cells). Metaxas et al. (2004) found that IAA plays an important

Figure 2. (a) Maximum shoot number of 4 with BAP at T_3 (3.0 mg L^{-1}) and (b) Shoot length of 9.0 cm with BAP at T_3 (3.0 mg L^{-1}).

Table 4. Effect of different concentrations of NAA and IAA on rooting percentage, root number and root length (cm).

Treatment (mg L^{-1})	Rooting percentage	Root number	Root length (cm)
NAA			
T_0 0.0	0.000^{i}	0.000^{i}	0.000^{i}
T_1 0.5	36.67^{h}	2.533^{h}	2.933^{h}
T_2 1.0	43.33^{g}	3.567^{g}	3.567^{g}
T_3 1.5	60.67^{e}	4.433^{e}	4.667^{e}
T_4 2.0	51.67^{f}	4.067^{f}	4.233^{f}
IAA			
T_5 0.5	68.33^{d}	5.800^{d}	5.267^{d}
T_6 1.0	76.67^{c}	6.567^{c}	5.767^{c}
T_7 1.5	97.67^{a}	7.567^{a}	7.333^{a}
T_8 2.0	85.00^{b}	7.033^{b}	6.333^{b}
$LSD_{5\%}$	5.404	0.3116	0.2365

Different letters within a column indicate significant difference at $p < 0.05$.

role in rhizogenesis through the process of redifferentiation of the cells in which predetermined cells switch from their morphogenetic path to act as mother cells for root primordial initiation. Furthermore, IAA promoted rooting of the cuttings because it increases sugar availability at the site of primordium development (Saxena et al., 2000). Haissig (1986) observed that IAA plays an important role in mobilization of carbohydrates in

Figure 3. (a) Healthy and thick roots with a number of 7.599 at IAA (T_7 1.5 mg L^{-1}) and (b) Maximum root length of 7.333 cm at IAA (T_7 1.5 mg L^{-1}).

the upper stem and increases its transportation towards the rooting zone where it gives energy to initiate the root primordium.

Among both auxins, NAA resulted in less rooting percentage as compared to IAA which is in line with the findings of Ali et al. (2009) who proposed that NAA is less effective in inducing rooting. According to the study of Dunlap et al. (1986) NAA is a more stable auxin than IAA and do not oxidize rapidly once absorbed within the cell. Casimiro et al. (2001) also reported that the rate of degradation of NAA and its subjection to the enzymatic activity is also less because of its stability; therefore, it is less active in the process of root initiation. The results indicate that rooting percentage reduce after attaining a peak value at optimum concentrations (1.5 mg L^{-1}) of IAA and NAA. Similar results were obtained by Ali et al. (2009) who reported that higher than optimum concentration of auxin inhibit the root primordia formation.

Root number

Exogenous application of NAA and IAA had a positive effect on root number. Root number varied significantly among different treatments (Table 4). IAA at T_7 (1.5 mg L^{-1}) gave maximum root number of 7.567 (Figure 3a) and NAA at T_3 (1.5 mg L^{-1}) gave 4.433 roots, which was significantly low as compared to all the concentrations of IAA applied. Furthermore, no root appeared in basal MS media without NAA and IAA supplementation. Celenza et al. (1995) found that IAA and NAA promoted root initiation and growth by inducing the cells to the pericycle and parenchyma to dedifferentiate and start initial cell division process. Laskowski et al. (1995) also mentioned that auxin accumulation within the root tissues may cause an increase in the number of adventitious root formation. Mockaitis and Howell (2000) reported an increase in myelin basic protein (MBP)-kinase activity in response to auxin treatment which provides a stimulus for mitogen activated protein kinase (MAPK) activation and initiated mitotic process which induces dedifferentiation of xylematic or parenchyma cells that acquired meristematic activity resulting in cell division and increased number of adventitious roots.

IAA proved to be more efficient in producing maximum number of good quality, healthy and thick roots than NAA. Pagnussat et al. (2004) reported that IAA increases the number of roots through the development of meristematic tissues and regulation of cell differentiation. Bruce and West (1989) reported that once root primordial

have been developed in the cuttings, then IAA causes a considerable metabolic activity within the cells which results in the formation of new root tissues that later grow into a root.

Less number of low quality and thin roots were observed with NAA as compared to IAA which is almost similar to the results of Mariska et al. (1989) who reported that NAA produced less root number, whereas IAA gave more number of roots.

Root length (cm)

The results show that root length was synergistic to the previous two trends of rooting percentage and rooting number. Among various treatments, a significant difference in root length was recorded (Table 4). IAA at T_7 (1.5 mg L^{-1}) gave the longest roots of 7.333 cm (Figure 3b), whereas NAA at T_3 (1.5 mg L^{-1}) gave root length of 4.67 cm, which was significantly low as compared to all the concentrations of IAA applied. Posada et al. (1999) observed that auxin not only stimulated rhizogenesis but also increased the early phases of cell elongation by causing responsive cells to actively transport hydrogen ions out of the activating wall loosening proteins known as expansins which allow the slippage of cellulose microfibrils in the cell wall and ultimately increased the root length by making the cell wall less rigid. Furthermore, Pagnussat et al. (2004) reported that auxin is necessary for gibberellin (GA) mediated control of root growth, causing a marked increase in root length.

In accordance with the previous parameters (rooting percentage and root number), less root length was observed with NAA as compared to IAA. Puchooa (2004) observed small, thin and delicate roots with NAA. Karim et al. (2003) reported that among auxins, reduced effects of NAA might be linked with the fact that within the root tissues, NAA is a more persistent auxin which restrain the successful development of root meristemoids and may obstruct the synthesis of enzymes concerned with cell elongation.

With different concentrations of IAA and NAA, it was observed that root length decreased immediately after an optimum level (1.5 mg L^{-1}). Puchooa (2004) reported that increased IAA and NAA concentrations inhibit cytoplasmic streaming in plant cells which cause the acidification of the cytoplasm by disturbing the orientation of actin filament in the cell wall and retard the process of cell wall loosening, and ultimately arrest the root growth. Moreover, Salisbury and Ross (2005) observed that higher concentrations of IAA and NAA reduce root growth due to increased level of endogenous ethylene biosynthesis.

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