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Full Length Research Paper

An efficient protocol for *in vitro* propagation of *Rosa gruss an teplitz* and *Rosa centifolia*

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Rose is a beautiful flower having commercial and ornamental value. In order to establish protocol shoot tips explants of *Rosa gruss an teplitz* and *Rosa centifolia* were proliferated *in vitro* using MS medium supplemented with different levels of benzylaminopurine (0, 0.5,1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}). Maximum numbers of shoots (3.906), shoot length (3.106 cm), fresh weight (178.47 mg) and dry weight (43.06 mg) was recorded at 1.0 mg l^{-1} BAP. For induction of root, uniform micro-shoots were excised and transferred to the rooting medium (1/2 MS macro, micro elements and vitamins) supplemented with 20 g l^{-1} sucrose and different concentrations (0.00, 0.25, 0.50, 1.0, 1.5 and 2.0 mg l^{-1}) of indole-3-butyric acid (IBA). IBA increased culture rooting percentage (89.375), number of roots (8.7188) and root length (3.5781 cm) more efficiently at 0.50 mg l^{-1}.

Key words: *In vitro* propagation, BAP, indole-3-butyric acid (IBA), *Rosa gruss an teplitz*, *Rosa centifolia.*

INTRODUCTION

Rose is a beautiful flower of immense horticultural importance (Hameed et al., 2006) due to its commercial and ornamental value. There are two main prospects of production of roses, that is, food products and cut flowers. The most important products are rose water, rose conserve and rose oil. They have very high economic value in the international market. One important rose specie, *Rosa centifolia* blooms in small clusters and have strong fragrance (Beales et al., 1998). Commercially, it is used for the production of essential rose oil (Farricielli, 2008). One of the most important rose species, *Rosa gruss-an-teplitz* has double red color flowers and blooms in small clusters having a strong fragrance (Beales et al., 1998). Predominantly, it is used for fresh and dried petals, making of bouquets and wreaths (Saeed, 2005).

Commercial propagation of roses is usually done by cuttings, although, they can also be propagated by budding and grafting which is a difficult and tedious process (Horn et al., 1992). Similarly, dependence on season and slow multiplication rate are some major limiting factors in conventional propagation (Pati et al., 2006). Moreover, some rose species are difficult to root. Tissue culture on the other hand is becoming increasingly popular as an alternative means for vegetative propagation of roses (Khosravi et al., 2007). Moreover, micro propagated plants are well suited for cut flower production as they are more compact, branch better and sometimes yield more flowers (Pati et al., 2006). Significant features of *in vitro* propagation are its enormous multiplicative capacity in short span of time, production of healthy and disease free plants and ability to generate propagules round the year (Razavizadeh and Ehsanpour, 2008). Therefore, this study was aimed to develop an efficient *in vitro* protocol for culture establishment and multiplication of *R. gruss an teplitz* and *R. centifolia.*

MATERIALS AND METHODS

Plants of rose species (*R. gruss-an-teplitz* and *R. centifolia*) were collected from the germplasm repository of the University of Agriculture Faisalabad and kept in pots in the glass house of the Department of Horticulture, PMAS-Arid Agriculture University, Rawalpindi.

Rose explants were propagated *in vitro* using shoot tips with partly folded leaflets. These explants were washed in running tap water for 15 min and for another 5 min in 5% aqueous solution of Teepol (a liquid detergent) followed by washing with running tap

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Table 1. Effect of different concentrations of BAP on number of shoots in *R. gruss an teplitz* and *R. centifolia.*

Treatment (BAP mgl^{-1})	Number of Shoot per explant		Mean
	Rosa gruss an teplitz	***Rosa centifolia***	
T_O (0.0)	1.090^{g}	1.063^{g}	1.076^{d}
T_1 (0.5)	2.075^{ef}	2.000^{ef}	2.037^{c}
T_2 (1.0)	4.625^{a}	3.188^{c}	3.906^{a}
T_3 (1.5)	3.787^{b}	3.063^{c}	3.425^{b}
T_4 (2.0)	2.850^{cd}	1.875^{f}	2.362^{c}
T_5 (2.5)	2.425^{de}	1.762^{f}	2.093c
Mean	2.809^{a}	2.158b	
LSD $_{0.05}$			
Variety	0.1898		
Treatment	0.3288		
Interaction (VXT)	0.4649		

Any two means not sharing a letter differ significantly at $P < 0.05$.

water. They were disinfected with 70% ethanol for 30 s. followed by surface sterilization with NaOCl solution (10%) for 10 min and then, washed thoroughly in sterile distilled water (Datta et al., 2005).

Explants were inoculated in culture jars containing shoot proliferation medium (MS macro, micro elements and vitamins) supplemented with indole-3-butyric acid (IBA) (0.01 mg l^{-1}), GA_3 (0.4 mg l^{-1}) and different levels of benzylaminopurine (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l). The medium was fortified with 30 g l^{-1} sucrose, gelled with 7 g l^{-1} agar and the pH was adjusted to 5.8. The medium was autoclaved at 121°C for 20 min. Explants were placed in growth room at 24°C (Dubois et al., 2000) under cool white light for 16 h photoperiod (Philips TL 40 W Fluorescent tubes). Data was recorded on number of shoots, shoot length (cm) per explant, fresh weight (mg) and dry weight (mg).

For induction of root, uniform micro-shoots were excised and transferred to the rooting medium (1/2 MS macro, micro elements and vitamins) supplemented with 20 g l^{-1} sucrose and different concentrations (0.00, 0.25, 0.50, 1.0, 1.5 and 2.0 mg l^{-1}) of indole-3-butyric acid (IBA). The experiments were designed according to CRD with two factor factorial. Observations on cultures rooted (%), number of roots per shoot and root length (cm) were collected after six weeks.

RESULTS AND DISCUSSION

Number of shoots

Different levels of BAP interacted significantly with both species of *R. gruss an teplitz* and *R. centifolia* in terms of number of shoots (Table 1). Both *R. gruss an teplitz* and *R. centifolia* yielded maximum number of shoots which were 4.625 and 3.188, respectively with 1 mgl^{-1} BAP (T_2) as shown in Figure 1. Increase in the concentrations of BAP showed an ascending order up to a level of 1 mgl^{-1} (T_2), then a declining trend with increase in concentrations. *In vitro* shoot proliferation and multiplication are largely based on medium formulations containing cytokinins as major plant growth regenerators (Kim et al., 2003). Its presence in the culture medium helped in the year round multiplication of shoots in hybrid roses (Rout et al., 1999). BAP is the most effective growth regulator in stimulating shoot proliferation (Vijaya et al., 1991). Axillary bud outgrowth, which is considered a process of apical dominance release, can be enhanced in response to exogenous cytokinins (Kapchina et al., 2000). Purine-type cytokinins effectively release the apical dominance in *in vitro* propagated ornamentals (Bollmark et al., 1995; Kapchina et al., 2000). Purine cytokinin N6-benzyladenine (BA) also stimulated the axillary bud break and increased the number of open buds on the *in vitro* cultured *Rosa hybrid* L. (Kapchina et al., 2000).

Among the treatments, BAP produced the highest number of shoots (3.906) at 1 mgl^{-1} (T_2) followed by 3.425 at 1.5 mgl^{-1} (T_3). Increase in BAP concentrations reduced the number of shoots up to 2.093 at 2.5 mgl^{-1} (T_5). The fact that higher doses failed to manifest their effect could be attributed to an obnoxious effect at higher concentrations, whereas the ineffectiveness of the lower dose indicated inadequate doze of hormone as a consequence indicating poor performance (Waseem et al., 2009). With increase in the concentrations of BAP, average number of shoots per culture formed decreased (Hameed et al., 2006). Inclusion of BA in the culture medium was essential for bud break and shoots multiplication of *R. hybrida* cultivars (Hasegawa, 1980; Wulster and Sacalis, 1980).

R. gruss an teplitz and *R. centifolia* showed significant results having 2.809 and 2.158 number of shoots, respectively. Shoot multiplication rate varied in different species and was specific to culture medium (Senapati and Rout, 2008). Bressan et al. (1982) also reported that, benzylaminopurine (BA) at low concentrations stimulates the development of axillary buds in cv. gold low more than cv. improved blaze.

Figure 1. Maximum number of shoots in *R. gruss an teplitz* (4.625) *(*A) and *R. centifolia* (3.188) (B) at 1 mgl^{-1} BAP, respectively.

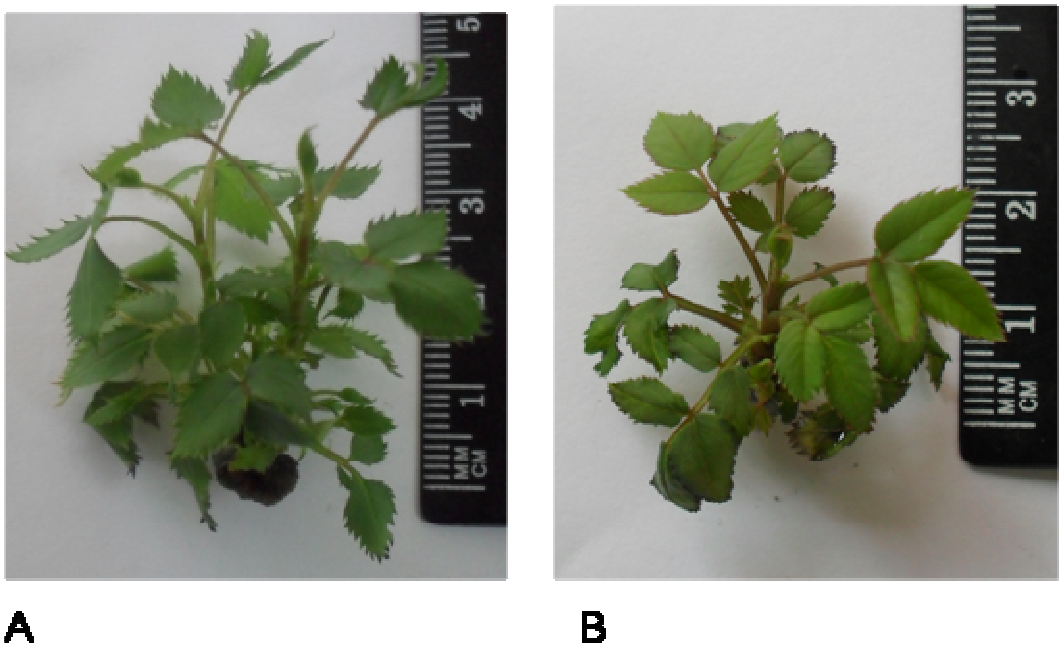

Figure 2. Shoot length of *R. gruss an teplitz* (A) (3.48 cm) and *R. centifolia;* (B) (2.72 cm) at 1 mgl^{-1}.

Shoot length

Different concentrations of BAP interacted significantly with both species regarding shoot length. Maximum shoot length (4.355 cm) was obtained by *R. gruss an teplitz* with BAP at 0.5 mgl^{-1}(T_1) followed by 3.487 cm at 1 mgl^{-1}(T_2). However, *R. centifolia* secured maximum length (2.725 cm) at 1 mgl^{-1} BAP (T_2) followed by 2.100 cm at 1.5 mgl^{-1} (T3) as depicted in Figure 2. Both species depicted a declining trend after attaining maximum shoot

Table 2. Effect of different concentrations of BAP on shoot length in *R. gruss an teplitz* and *R. centifolia.*

Treatments (BAP mgl^{-1})	Shoot Length (cm)		Mean
	Rosa gruss an teplitz	***Rosa centifolia***	
T_O (0.0)	0.8875^{g}	1.294^{f}	1.091^{e}
T_1 (0.5)	4.355^{a}	1.380^{f}	2.868^{a}
T_2 (1.0)	3.487^{b}	2.725^{c}	3.106^{a}
T_3 (1.5)	2.392^{cd}	2.100^{de}	2.246^{b}
T_4 (2.0)	1.548^{f}	1.981^{e}	1.764^{c}
T_5 (2.5)	1.390^{f}	1.506^{e}	1.448^{d}
Mean	2.343^{a}	1.831^{b}	
LSD $_{0.05}$			
Variety	0.1465		
Treatment	0.2537		
Interaction (VXT)	0.3588		

Any two means not sharing a letter differ significantly at $P < 0.05$.

length at 0.5 mgl^{-1} (T_1) and 1 mgl^{-1} BAP (T2), respectively (Table 2) Purine type cytokinin BA more positively affects the growth, development and the appearance of micro plants. Elongation decreases with increase in number of shoots at high level of BA (Carelli and Echeverrigary, 2002). This can be caused either by better uptake and more efficient utilization of the purine cytokinin or by the lower level of endogenous cytokinins (Veneta et al., 2005). BAP being a strong cytokinin depresses shoot length by an increase in number of axillary buds (Hameed et al., 2006) as all the nutrients are utilized for the formation of lateral shoots (Yakimova et al., 2000). Shoot length may also be decreased as purine-type cytokinins have been found to effectively release the apical dominance in *in vitro* propagated ornamentals (Kapchina et al., 2000). Axillary bud outgrowth, which is considered a process of apical dominance release, can be enhanced in response to exogenous cytokinins (Cline, 1994).

Among the treatments, BAP produced maximum shoot length (3.106 cm) at 1.0 mgl^{-1}(T_2) followed by 0.5 mgl^{-1} (T_1) with shoot length of 2.868 cm. All the other treatments also showed shoot length better than the medium without BAP (T_0). However, a gradual increase in the concentration of cytokinin above optimum levels showed a declining trend regarding shoot length. Ahmad et al. (2003) explained that, the higher concentrations of BAP increases the ethylene levels in the plants which block the basipetal transport of endogenous auxin in the shoots resulting in minimum shoot length. Increased levels of BAP in the medium suppress shoot growth due to increased number of shoots (Waseem et al., 2009).

R. gruss an teplitz showed better length of proliferated shoot (2.343 cm) than *R. centifolia* (1.831 cm) as genotypic differences could have a perceptible influence on growth performance of rose cultures (Nasser et al., 2005). According to Horn et al., (1992), rooting response in rose was cultivar dependent and in certain species up to 100% success could be achieved. A lower rooting ability was also recorded in old world spp. (*Rosa canina* and *Rosa damascena*) when compared with *Rosa hybrida* (Pati et al., 2006). Kirichenko et al. (1991) reported that, rooting of micro shoots of the essential oil bearing roses was difficult when compared with the ornamental varieties. Current studies indicate that, there are genes responsible for increased number of bud initials and shoot proliferation. Moreover, the possible involvement of the gene in modulating hormone levels has also been reported (Tantikanjana et al., 2001).

Fresh and dry weight

Maximum fresh weight (172.44 and 184.5 mg) was observed at 1.0 mgl^{-1} (T_2) in *R. gruss an teplitz* and *R. centifolia,* respectively (Table 3). It was inferred from the results that BAP at 1.0 mg l^{-1} (T_2) is the optimum concentration for the better performance of this particular hormone. However, fresh weight declined when concentrations was further increased. Both species depicted significant difference by attaining 82.96 and 100.4 mg fresh weight, respectively. Dry weight accumulation also showed synergism to fresh weight. Better accumulation of dry weight (46.18 and 39.94 mg) was observed at 1 mg l^{-1} (T_2) in *R. centifolia* and *R. gruss an teplitz,* respectively (Table 4). Like fresh weight, dry weight also decreased with further increase in BAP concentrations. Significant dry weight accumulation (19.99 and 23.84 mg) was recorded in *R. gruss an teplitz* and *R. centifolia* respectively.

According to Veneta et al. (2005) in medium supplemented with BA concentrations, the accumulation of dry

Table 3. Effect of different concentrations of BAP on fresh weight in *R. gruss an teplitz* and *R. centifolia.*

Treatments (BAP mgl^{-1})	Fresh Weight (mg)		Mean
	Rosa gruss an teplitz	***Rosa centifolia***	
T_O (0.0)	33.88^{g}	37.06^{g}	35.47^{f}
T_1 (0.5)	40.80^{g}	50.96^{f}	45.88^{e}
T_2 (1.0)	172.44^{b}	184.5^{a}	178.47^{a}
T_3 (1.5)	94.13^{c}	175.06^{b}	134.59^{b}
T_4 (2.0)	79.63^{de}	82.63^{d}	81.13^{c}
T_5 (2.5)	76.92^{de}	72.19^{e}	74.56^{d}
Mean	82.96^{b}	100.4^{a}	
LSD $_{0.05}$			
Variety	3.3486		
Treatment	5.8000		
Interaction (VXT)	8.2024		

Any two means not sharing a letter differ significantly at $P < 0.05$.

Table 4. Effect of different concentrations of BAP on dry weight in *R. gruss an teplitz* and *R. centifolia.*

Treatments (BAP mgl^{-1})	Dry Weight (mg)		Mean
	Rosa gruss an teplitz	***Rosa centifolia***	
T_O (0.0)	11.74^{g}	15.00^{ef}	13.37^{e}
T_1 (0.5)	$13.65f^{g}$	15.47^{ef}	14.56^{de}
T_2 (1.0)	39.94^{b}	46.18^{a}	43.06^{a}
T_3 (1.5)	21.46^{d}	32.75^{c}	27.10^{b}
T_4 (2.0)	17.27^{e}	17.12^{e}	17.20^{c}
T_5 (2.5)	15.87^{ef}	16.50^{ef}	16.18^{cd}
Mean	19.99^{b}	23.84^{a}	
LSD $_{0.05}$			
Variety	1.2724		
Treatment	2.2038		
Interaction (VXT)	3.1166		

Any two means not sharing a letter differ significantly at $P < 0.05$.

mass is pronounce better than on plants grown without cytokinins. Moreover, plants with increased cytokinin content have more branches (Schmülling, 2004), increase number of leaves, shoots and shoot length by stimulating cell division and elongation (Peres et al., 2001). BAP increases the physiological strength of sink regions in the plant which makes the plant more efficient to convert the sugars into dry matter (Gollagunta et al., 2004). It regulates important physiological parameters that determine biomass formation and distribution via central genes of primary metabolite pathways, including invertases, hexose transporters, key genes of phosphate, nitrogen metabolism and signalling (for example, nitrate reductase). Transported cytokinins may have a role in coordinating shoot development by carrying information about nutrient availability (Schmülling, 2004).

Rooting percentage

Maximum rooting percentage (95%) was observed in the interaction of IBA with *R. gruss an teplitz* at 0.50 mgl^{-1} (T_2) followed by 91.25% at 0.75 mgl^{-1} (T_3) as shown in Table 5. Similarly, *R. centifolia* also produced maximum rooting percentage of 83.75% followed by 77% at 0.50 mgl^{-1}(T_2) and 0.75 mgl^{-1} (T_3), respectively. Rooting percentage increased in both species with an increase in the level of IBA. However, a declining trend was observed after a certain level. Therefore, it can be concluded that 0.50 mgl^{-1} IBA was the best to initiate roots in both

Table 5. Effect of different concentrations of IBA on rooting percentage in *R. gruss an teplitz* and *R. centifolia.*

Treatment (IBA mgl^{-1})	Rooting percentage		Mean
	Rosa gruss an teplitz	*Rosa centifolia*	
T0 (0.00)	47.500^{f}	53.750^{ef}	50.625^{c}
T1 (0.25)	68.750^{cde}	62.500^{def}	65.625^{b}
T2 (0.50)	95.000^{a}	83.750^{abc}	89.375^{a}
T3 (0.75)	91.250^{ab}	77.000^{bcd}	84.125^{a}
T4 (1.00)	75.000^{bcd}	67.500^{cde}	71.250^{b}
Mean	75.500^{a}	68.900^{a}	
LSD $_{0.05}$			
Variety	7.7703		
Treatment	12.286		
Interaction (VXT)	17.375		

Any two means not sharing a letter differ significantly at $P < 0.05$.

species. IBA is by far the most commonly used auxin to obtain root initiation. It is readily converted to IAA but probably has an effect on its own (George et al., 2008). Maximum rooting percentage is probably due to the reason that optimum concentration of IBA might be responsible to increase the cambial growth at the base of micro cuttings that result in differentiation of root primordia (Haq et al., 2009). The lower concentrations of IBA were found more effective (Bhatt and Tomar., 2010) as many researchers also reported that, roots were induced from excised mature microshoots on MS medium supplemented with low concentration of auxins in the range of 0.1 to 0.5 mg l^{-1} (Senapati and Rout, 2008). Achievement of minimum rooting in medium without IBA rather than complete inhibition of rooting indicated that, endogenous auxin along with some root inducing factors might occur naturally within the micro cuttings that may help for root primordia initiation (Haq et al., 2009) Furthermore, root inducing factors are believed to be essential for rooting, which combine with auxin to form a complex that directs RNA to activate enzymes that cause root initiation (Hartmann et al., 2007).

IBA at 0.50 mgl^{-1}(T_2) and 0.75 mg l^{-1} (T_3) depicted the highest rooting percentage that were 89.375 and 84.125%, respectively. However, increase in IBA above optimum level showed an inhibiting effect on rooting percentage. IBA appears to be a better auxin but its higher concentrations can reduce the level of root regeneration percentage as it appear to have deleterious effect (Iqbal et al., 2003). Moreover, IBA is stable and may remain present for a long time and can inhibit the outgrowth of root primordia resulting in massive ethylene accumulation in the tissue culture container (De Klerk, 2002).

R. gruss an teplitz and *R. centifolia* depicted non significant result having 75.500 and 68.900 rooting percentage, respectively. The exogenous application of IBA is reported to be better suited for inducing rooting in cuttings of various species (Pati et al., 2004).

Number of roots

The most superior interaction of IBA was observed with *R. gruss an teplitz* which produced maximum number of roots as 12.256 at 1 mgl^{-1} (T_4), while *R. centifolia* gained 5.500 (Figure 3) at 0.50 mgl^{-1}(T_2) as exhibited in Table 6. Early stages of lateral root formation are regulated by polar auxin transport. Therefore, development of lateral roots is stimulated by auxin which is known for its ability to promote adventitious root formation due to stimulation of cell division (George et al. 2008). Higher concentration of IBA induces higher level of degradative metabolites in tissues which might lead to the blockage of root formation process (Baker and Wetzstein, 2004).

The analysis of variance indicated non significant difference among the treatments in relation to the number of roots. However, maximum number of roots (8.7188) was depicted at 0.50 mgl^{-1} (T_2). Number of roots increased with increasing level of IBA up to a concentration of 0.50 mgl^{-1} (T_2) then a descending order was noticed because higher concentrations of IBA imposed reduction in root number and vigor. Iqbal et al. (2003) explained that, application of IBA brought changes in the protein synthesis and RNA production which increase number of roots by stimulating cell division processes (Husen and Pal, 2007). Many researchers have also reported that, roots were induced from excised mature microshoots on MS medium supplemented with low concentration of auxins in the range of 0.1 to 0.5 mg l^{-1} with the reduction of the sucrose level (Senapati and Rout, 2008).

R. gruss an teplitz was much superior regarding number of shoots with mean number of shoots of 10.289 when compared with 3.541 for *R. centifolia*. Auxin-

A B

Figure 3. Number of roots in *R. gruss an teplitz* (A) (11.938) and *R. centifolia* (B) (5.500) at 0.50 mgl^{-1} IBA, respectively.

Table 6. Effect of different concentrations of IBA on number of roots in *R. gruss an teplitz* and *R. centifolia.*

Treatment (IBA mgl^{-1})	Number of root		Mean
	Rosa gruss an teplitz	***Rosa centifolia***	
T_O (0.00)	5.125^{b}	1.375^{d}	3.2500^{c}
T_1 (0.25)	11.500^{a}	4.188^{bc}	7.8438^{ab}
T_2 (0.50)	11.938^{a}	5.500^{b}	8.7188^{a}
T_3 (0.75)	10.625^{a}	3.990^{bc}	7.3075^{b}
T_4 (1.00)	12.256^{a}	2.652^{cd}	7.4544^{ab}
Mean	10.289^{a}	3.541^{b}	
LSD $_{0.05}$			
Variety	0.8211		
Treatment	1.2983		
Interaction (VXT)	1.8361		

Any two means not sharing a letter differ significantly at $P < 0.05$.

containing medium affect *in vitro* rooting of various species differently (Al-Bahrany, 2002).

Root length

IBA with 0.50 mgl^{-1}(T_2) concentration showed better interaction with *R. gruss an teplitz* and *R. centifolia* by producing 3.600 and 3.556 cm root length, respectively (Figure 4). Increase in concentration of IBA promoted root length up to certain level and beyond that, inhibiting effect on root length was recorded (Table 7). Auxin is produced in shoots and migrated to the lower portion of stem cuttings to effect the development of another tissue. It promotes cell elongation and has variety of other growth-regulating effects (Camellia et al., 2009). IBA has also greater ability to promote rooting and induce less callus formation (Panjaitan et al., 2007). The most

Figure 4. Maximum root length at 0.50 mgl^{-1} IBA in *R. gruss an teplitz* (A) (3.60 cm) and *R. centifolia* (B) (3.55 cm), respectively.

Table 7. Effect of different concentrations of IBA on root length in *R. gruss an teplitz* and *R. centifolia.*

Treatments (IBA mgl^{-1})	Root length (cm)		Mean
	Rosa gruss an teplitz	***Rosa centifolia***	
T0 (0.00)	0.762^{g}	1.278^{f}	1.020^{d}
T1 (0.25)	1.857^{e}	2.975^{c}	2.416^{c}
T_2 (0.50)	3.600^{a}	3.556^{ab}	3.578^{a}
T_3 (0.75)	3.218^{bc}	2.530^{d}	2.874^{b}
T_4 (1.00)	2.918^{c}	2.100^{e}	2.509^{c}
Mean	2.472a	2.487^{a}	
LSD $_{0.05}$			
Variety	0.1568		
Treatment	0.2480		
Interaction (VXT)	0.3507		

Any two means not sharing a letter differ significantly at P < 0.05.

favorable concentration of IBA induces cell enlargement by extruding protons actively into the cell wall region and resulting in a decrease in pH activating wall loosening enzyme that promotes the breakage of key cell wall bonds and increase cell wall extensibility; hence, causing an increase in cell size and elongation (Taiz and Zeiger, 2006). Han et al. (2009) revealed that, auxins induces the sprouting of shoot buds which then stimulates the growth substances present in the roots for their growth and elongation.

A gradual increase in root length was observed with an increasing level of IBA concentration up to 0.50 mgl^{-1} (T_2) giving maximum root length of 3.578 cm. Previous studies have also indicated that, application of IBA significantly enhanced the root number and root length of *Rosa damascene* (Pati et al., 2004); whereas at higher

concentration, it acts as a root growth inhibitor (Haq et al., 2009).

Non significant differences were observed in *R. gruss an teplitz* and *R. centifolia* showing mean root length of 2.471 and 2.487 cm, respectively. Length of root could be attributed to genetic constitution. Genetic factor is a combination of set of genes favorable for the establishment of root system. Moreover, the extent of foliage could be responsible for enhanced development of root as leaves are the site of food manufacture which is translocated to the roots for development, regardless of the genetic potential (Hussain and Khan, 2004).

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