Research Article

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Effect of plant growth hormones on root and shoot regeneration in Rose (*Morrasia*) under in-vitro conditions.

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In vitro propagation of rose has played a very important role in rapid multiplication of cultivars with desirable traits and production of healthy and disease free plants. In first step, Axillary buds of cv. Morrasia were cultured on Murashige and skoog medium (MS medium) supplemented with different concentrations and combinations of Naphtalene Acetic Acid (NAA) (0.1 mg/l) and Benzyl Amino Purine (BAP) (2 mg/L). For shoot regeneration study, MS medium supplemented with different concentrations and combinations of NAA and BAP. And for root regeneration study, half strength MS medium supplemented with different concentrations of Indole Acetic Acid (IAA) and NAA were applied. Highest number of shoots was produced in control of NAA and 3 mg/L BAP level but longest shoots were produced by 0 mg/L BAP BAP and NAA level. In MS medium without any growth regulators enzymes, the shoots can be grows but using of regulator enzymes the shoots can be multiplicities. Highest numbers of roots were produced by IAA in third level of NAA. A reduction in number of root was observed, when concentration of IAA was increased. Add concluding sentences here.

Key words: In vitro, NAA, IAA, BAP, Shoot and root regeneration

Roses are one of the most important floral crops in the world (Castilon *et al.* 2006). The genus Rosa has been the subject of numerous studies involving the in vitro culture of axillary's buds, shoot tips, callus, anthers, and embryos (Burger *et al*. 1990). Commercial propagation of roses is usually done by cutting, although they can also be propagated by budding and grafting, which is difficult, undesirable and tedious process (Horn, 1992). This conventional method of propagation is very slow. Moreover, disease and environmental hazards make the cultivar to degenerate gradually. So, this conventional process is not satisfactory in multiplication of Rosa Spp (Roy *et al.* 2004). Micro propagation method is especially applicable to species in which clonal propagation is needed (Gamborg and Phillips, 1995).

The most important technique in micro propagation is meristem proliferation, where in shoot meristems or nodal segments harboring an axillary bud are cultured to regenerate multiple shoots without any intervening callus phase. Multiplication of a variety through shoot tip could result in development of plants immunity to fungal and viral diseases and to maintain genetically pure, healthy and vigorous stock plants (Pati *et al.* 2005; Ozel and Arsalan, 2006).

The success of micro propagation protocol to large extent, depends on the rate and mode of shoot multiplication. Various factors that influence in vitro shoot multiplication in rose are listed bellow: species, genotypes, cultivars; media; inorganic salts and organic components; carbohydrates; growth regulators; status of the medium; physical factors such as light and temperature (Pati *et al.* 2005).

For any micro propagation protocol, successful rooting of micro shoots is a pre requisite to facilitate their establishment in soil (Pati *et al.* 2005). In vitro rooting capacity depends on the interaction of internal and external factors (Hyndman *et al.* 1982). These are listed as follows: species, cultivar; age and size of micro shoots; media; inorganic salts; carbohydrate; activated charcoal;

growth regulators; cultural vessel and physical factors (Pati *et al.* 2005).

Apical and axillary buds are used for mass multiplication in vitro. In vitro propagation of rose plant has been usually tried by using cultures of axillary buds (Ishioka Tanimoto, 1990).

The first report on shoot multiplication and rooting of rose was made by Elliot (1970). In vitro shoot proliferation and multiplication was largely based on media formulations containing cytokinins rather than auxins (Rout *et al.* 1999). Murashige and Skogs medium (MS) was found to be the most commonly used for rose propagation. Davies (1980) stated that the standard MS media induced the best rate of shoot proliferation in different rose cultivars.

In vitro shoot proliferation and multiplication are largely based on media formulation containing cytokinins as a major plant grows regulators, whereas in some cases, low concentrations of Auxins and Gibberllic acid were also used. Inclusion of BAP in the culture medium was essential for bud break and shoot multiplication of Rosa (Hassegawa, 1980; wulster and Sacalis, 1980).

Bressan *et al.* (1982) reported that benzylaminopurine (BAP) at low concentrations stimulated the development of axillary buds in Cv. Gold Glow but didn't stimulated in Cv. improved Blaze. Rout *et al.* (1990) stated that the presence of cytokinin in the culture medium helped in year round multiplication of shoots in hybrid roses (Rout *et al.* 1999).

Horn (1992) reported that genotypes had a clear effect on in vitro propagation in different cultivars of rose. The influence of genotype on shoot proliferation could easily be interpreted by linking it with the recent progress in functional genomics of plants. Current studies indicate that there are genes responsible for increased number of bud initial and shoot proliferation (Pati *et al.* 2005)

In most of the earlier reports, varying concentrations of different auxins were used for root induction (Pati *et al.* 2005). The roots were easily induced from excised mature micro shoots on MS medium supplemented with low concentration of auxins (IAA, IBA or NAA) in the range of 0.1_0.5 mg/ l (Hassegawa, 1980).

The present work was undertaken to study the effect of BAP, NAA and IAA in half strength and complete MS medium on shoot and root proliferation in Rose Cv. *Morrasia*.

MATERIAL AND METHODS

The shoots of cv. Morrasia were collected from the national ornamental plant research center (Mahhallat) and investigated in Science and Technology Research Institute (NSTRI) Agriculture, Medicine and Industry Research School, Karaj. The shoots were cut and all leaves were removed. These were placed in flax of detergent water and washed for ten minutes in desiccators. Then shoots were rinsed with tap water and dipped into 70% alcohol for one minute and rinsed with distilled water.

The shoots were cut into 1 to 1.5 cm sections containing one or two buds and explanted on half strength MS proliferation medium containing BAP (2 mg/l) and NAA (0.1 mg/L). The pH of the medium was adjusted to 5.6 or 5.7 with HCl and NaOH before autoclaving. The medium was dispensed into culture jar (25-30 ml per jar) and autoclaved at 122 ^{o}C at 1.5 atm for 20 minutes.

Shoots were harvested from 12-week tissue culture plants growing on MS proliferation medium in jars under culture room conditions. Shoots were cut into 1 cm stem segment with one node and put onto the experimental mediums for shoot and root regeneration in a laminar flow hood.

The experimental medium for shoot regeneration consist of full strength MS macro and micro mineral elements, vitamin, 3% sucrose and 6% agar, supplemented with 0, 1, 2 and 3 mg/L BAP and 0, 0.5 mg/L NAA. Culture medium for rooting investigation consisted of half strength MS salts, vitamin, sucrose and 6% agar, supplemented with 0, 0.25 and 0.5 mg/L NAA and 0, 3 and 6 mg/L IAA.

The jars placed in a culture room maintained at 23±2^{o} C under white fluorescent light with 16 hours photoperiod. Individual plants in shoot regeneration experiment evaluated for the no. of shoots / explants, no. of roots / explants, wet explants weight, length of longest root and shoot, dry explants weight and in rooting experiment evaluated for wet root weight and dry root weight.

Statistical analysis: Factorial analysis in complete randomized design was used for shooting and rooting studies. Five explants were cultured per jar per observation and

three observations in each treatment with three replications. Data were taken after four weeks of culture and analyzed using SAS version 6.0. Significant differences were assessed using Duncans multiple range test ($p<0.05$).

RESULTS AND DISCUSSION

In the shooting experiment, effects of various concentrations and combination of auxin (NAA) and cytokinin (BAP) on bud formation were examined using basal MS medium. Results indicated that significant variation ($P<0.05$) in number of shoots per explants (table 1). The highest number of shoot per explants on MS medium observed with 3 mg/L BAP treatment without NAA and adventitious shoots could not be induced by the application of 0.5 mg/L NAA (table 2). Number of shoot per explants reduced with reduction in BAP concentration. The tallest shoots were observed at the lowest level of BAP (0 mg/L) however, the primary shoots did not regenerated. Number of root and longest roots per explants were highest in 0 mg/L BAP and 0.5 mg/L NAA. The average shoot number per explants increased as the BAP concentration increased but decreased as the NAA concentration increased. In control of BAP number of root per explants and length of longest root was very high but by using of BAP any root didn't observed. On the other hand in control of BAP level and complete MS medium by increasing of NAA (0.5 mg/L) number of root and length of longest root were increased.

While in rooting study with half strength MS medium by increasing of NAA, number of root and length of longest root did not changed (table 2). Many research observed variable effects of cytokinins and auxins on shoot regeneration in different varieties of rose. Alekhno and Vystoskii, (1986) obtained efficient shoot regeneration of rose on MS medium containing 0.5-1 mg/L BAP. Similarly, Singh and Syamal, (1999) used BAP, NAA and Gibberlic acid to obtain more than 5 shoots per explants. Vijaya *et al.* (1991) reported that BAP was the most effective growth regulator in stimulating shoot proliferation. Further they investigated effect of auxins in combination with BAP and found that NAA was more effective than IAA or IBA in the production of multiple shoots. Pati *et al.* (2001) optimized the BAP concentration at 5µM for shoot proliferation in R. *damascene* and R. *bourboniana*.

In the rooting experiment, the effects of auxins (IAA and NAA) on root formation were examined using the half strength MS medium (table 3,4). The results indicated that the highest number of roots was obtained 0 mg/L IAA along with 0, 0.25 and 0.5 mg/L NAA (5.49, 5.11 and 5.25 respectively) and by increasing IAA, number of root was decreased (table 4). The longest root was observed in 0 mg/L NAA and IAA. Root length was decreased as the concentration of IAA increased.

Badzian *et al.* (1991) reported the use of MS medium with major elements reduced to one quarter to one third strength for root induction. Khosh_khui and Sink (1982) reported that half-strength MS medium supplemented with NAA (0.54 µM) was adequate for inducing root regeneration in cv. Bridal Veil of hybrid rose. Sauer *et al.* (1985) reported that 10 different rose cultivars rooted within (10-14) days on third strength of MS medium supplemented with IAA at 5.7 µM. subsequently. Douglas *et al.* (1989) achieved very high percentages of root regeneration in cv. Queen Elizabeth using long shoots and by dilution of MS medium to one fourth strength without a growth regulator. In most of the earlier reports, varying concentrations of different auxins have been used for root induction (Kirichenko *et al.* 1991; Chu *et al.* 1993; Arnold *et al.* 1995). *Khosh-khui and Sink, (1982*) indicated that a combination of IAA (0-0.1 mg/L) was quite effective in induction of roots. *Arnold et al.* (1995) reported that microshoots of R. kordessi cvs. John Franklin and champlain rooted well in MS medium with low or no auxin. Optimum root regeneration in cv. Champlain was achieved at a high concentration of IAA with low concentrations of salts or intermediate concentrations of IBA and NAA with low to medium concentrations of salts.

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Table 1: Effects of plant growth regulators (BAP, NAA) on shoot regeneration from shot tip explants of Cv. Morrasia of rose under in vitro condition

	No. Shoot / explants	No. root / explants	Wet explants weight	Length of longest shoot	Length of longest root	Wet root weight	Dry root weight
BAP	1.080619 **	11.07918 **	0.955347 *	1.1287 **	11.6987 **	0.1465 ns	0.03276 ns
NAA	0.355097 *	0.21096 ns	0.104536 ns	0.0192 ns	0.25305 ns	0.0362 ns	0.01303 ns
BAP*NAA	0.08083 ns	0.0435 ns	0.22557 ns	0.33344 ns	0.24576 ns	0.0699 ns	0.0237 ns
CV%	20.52	32.54	26.42	22.1983	33.107	29.1	40
BAP							
BAP 0 mg/L	0.977 c	5.5353 a	1.6869 b	3.9212 a	6.1382 a	0.259 a	0.149 a
BAP 1 mg/L	2.16 b	0.1 b	3.094 ab	2.847 ab	0.384 b	0.14 ab	0.479 a
BAP 2 mg/L	2.637ab	0.28 b	2.592 ab	2.4875 b	0.5677 b	0.164 b	0.495 a
BAP 3 mg/L	2.864 a	0 b	3.4476 a	1.9743 b	0 b	0.15 ab	0.036 a
NAA							
NAA 0 mg/L	2.309 a	1.5419	2.6315	2.8484	10.6919	0.2 a	0.233a
NAA 0.5 mg/L	1.876 b	1.7834	2.6917	2.8802	2.3313	0.093a	0.342 a

*, ** significant at 0.5% and 0.01%, NS: non significant by ANOVA, Mean separation by Duncan multiple rage test. Number within columns followed by different letters are significantly different.

Table 2: Effect of BAP and NAA in full MS medium on no. shoot / explants, length of longest shoot, Wet explants weight, No. root / explants, Length of longest root and wet root weight.

		NAA 0 mlg/l	NAA 0.5 mlg/l
BAP 0 mg/L	**No. Shoot / explants**	0.977±0.0667	0.975±0.1669
	Length of longest shoot	4.54±0.532	4.31±0.817
	Wet explants weight	1.564±0.248	2.116±0.35
	No. root / explants	5.033±1.235	6.95±1.631
	Length of longest root	5.339±1.3915	7.283±1.441
	Wet root weight	0.2459±0.079	0.298±0.113
BAP 1 mg/L	**No. Shoot / explants**	2.6±0.385	1.85±0.45
	Length of longest shoot	2.985±0.459	3.113±0.474
	Wet explants weight	2.938±0.727	3.4746±0.633
	No. root / explants	0	0
	Length of longest root	0	0
	Wet root weight	0	0
BAP 2 mg/L	**No. Shoot / explants**	3.35±0.63	1.97±0.734
	Length of longest shoot	2.503±0.0432	3.028±0.893
	Wet explants weight	3.479±0.871	3.558±1.544
	No. root / explants	0	0
	Length of longest root	0	0
	Wet root weight	0	0
BAP 3 mg/L	**No. Shoot / explants**	3.613±0.642	2.687±0.51
	Length of longest shoot	2.229±0.465	2.99±0.709
	Wet explants weight	3.221±0.653	3.6172±0.736
	No. root / explants	0	0
	Length of longest root	0	0
	Wet root weight	0	0

Table 3: Effects of plant growth regulators (IAA, NAA) on root regeneration from shot tip explants of Cv. Morrasia of rose under in vitro condition

	No. root / explants	Wet explants weight	Dry shoot weight	Length of longest root	Wet root weight	Dry root weight
IAA	4.2626 *	0.3487 *	0.1201 ns	1.1016 **	2.2404 ns	0.1777 ns
NAA	0.5421 ns	0.0043 ns	0.0529 ns	0.5536 *	2.4991 ns	0.3283 ns
IAA*NAA	0.8132 ns	0.1135 ns	0.061 ns	0.233 ns	2.9239 ns	0.0721 ns
CV%	29.29	16.9598	28.7151	20.744	17.496	48.33
IAA						
IAA 0 mg/L	5.3322 a	0.8599 b	0.3134 a	2.3492 a	0.1511 a	0.2837 a
IAA 3 mg/L	2.3563 b	1.2808 a	0.2568 a	2.1448 a	0.037 a	0.0204a
IAA 6 mg/L	1.5417 b	1.3817 a	0.2044 a	1.1983 b	0.5353 a	0.0489 a
NAA						
NAA 0 mg/L	3.493 a	1.0981 a	0.2199 a	2.1877 a	0.0768 a	0.0894 a
NAA 0.25 mg/L	2.984 a	1.1972 a	0.302 a	1.4108 b	0.5399 a	0.531 a
NAA 0.5 mg/L	2.774 a	1.2237 a	0.2515 a	2.1287 a	0.0895 a	0.0423 a

*, ** significant at 0.5% and 0.01%, NS: non significant by ANOVA, Mean separation by Duncan multiple rage test. Number within columns followed by different letters are significantly different.

Table4: Effect of IAA and NAA in half strength MS medium on No. shoot / explants, length of longest shoot, Wet explants weight, No. root / explants, Length of longest root and Wet root weight.

		IAA 0 mg/L	IAA 3 mg/L	IAA 6 mg/L
NAA 0 mg/L	No. root / explants	5.49±1.236	3.075±1.48	1.556±0.853
	Length of longest root	3.119±0.965	2.019±1.078	1.273±0.635
	Wet root weight	0.175±0.146	0.0306±0.0225	0.0212±0.0168
	Dry root weight	0.01536±0.0056	0.0048±0.0027	0.0037±0.0029
	Wet explants weight	0.853±0.184	1.24±0.321	1.374±0.279
	Dry shoot weight	0.178±0.0322	0.259±0.059	0.248±0.039
NAA 0.25 mg/L	No. root / explants	5.11±1.411	1.62±0.891	1.25±1.321
	Length of longest root	1.79±0.768	1.623±0.638	0.71±0.452
	Wet root weight	0.0856±0.0458	0.0292±0.0201	0.0166±0.0087
	Dry root weight	0.0199±0.0297	0.0035±0.0021	0.0021±0.0013
	Wet explants weight	0.938±0.129	1.458±0.177	1.619±0.311
	Dry shoot weight	0.199±0.021	0.281±0.035	0.257±0.045
NAA 0.5 mg/L	No. root / explants	5.25±2.35	1.975±1.113	1.044±0.598
	Length of longest root	2.168±0.919	2.7075±1.053	1.483±0.824
	Wet root weight	0.0798±0.061	0.0541±0.0289	0.0219±0.0137
	Dry root weight	0.0093±0.007	0.0072±0.0052	0.0031±0.0016
	Wet explants weight	0.959±0.366	1.226±0.373	1.374±0.412
	Dry shoot weight	0.2±0.057	0.238±0.068	0.243±0.075

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