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BRIEF COMMUNICATION

Anti-auxin enhance *Rosa hybrida* L. micropropagation

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Abstract

Shoots of rose cultivars Super Star and Sonia were multiplied for ten subcultures at 4-week intervals on solidified Murashige and Skoog's medium supplemented with 22.19 μM benzylaminopurine + 1.07 μM napthalene acetic acid + 0.05 μM gibberellic acid. Addition of anti-auxins 2,3,5-triiodobenzoic acid (TIBA; 2.0 μM) and 2,4,6-trichlorophenoxy-acetic acid (2,4,6-T; 0.39 μM) into proliferation medium increased number of shoots per explant and length of shoots in both cultivars. Treatment with TIBA increased also number of leaves per shoot and leaf chlorophyll content.

Additional key words: axillary shoot proliferation, chlorophyll, hybrid tea rose, TIBA, 2,4,5-T.

Several micropropagation protocols have been proposed in *Rosa hybrida* L., however, only cost efficient protocol capable of producing a large number of good quality micro-shoots in several subculture can be successful (Debergh and Maene 1981). There is a marked gradual decline is shoot proliferation rate from axillary buds after repeated subcultures (Rajeevan and Pandey 1986, Valles and Boxus 1987). Arnold *et al.* (1992) suggested this phenomenon to be cultivar dependent in *Rosa* spp. Voyiatzi *et al.* (1995) proposed it as the result of apical dominance. Anti-auxins has been reported to promote or modify morphogenetic processes *in vitro* by negating the excess of exogenous or endogenous auxins in the cultures (Cassells 1979, Jelsaka *et al.* 1984). The aim of the present paper was to study the effect of some anti-auxins in restoring the depleting shoot proliferation rate in hybrid tea rose after several subcultures.

Axillary buds from field-grown rose (*Rosa hybrida* L.) plants of cvs. Sonia and Super Star were established and repeatedly proliferated by following routine procedures on Murashige and Skoog (1962) medium with 22.19 μM BAP, 1.07 μM NAA and 0.05 μM GA_3 along with 30 g dm^{-3} sucrose and 8.0 g dm^{-3} *Bactoagar*. The pH of the medium was adjusted to 5.7 before autoclaving for 15 min (1.05 kg cm^{-2}). The plants (cultures) were cultivated under irradiance of 25 μmol m^{-2} s^{-1} (cool white lamps), 16-h photoperiod, and temperature 24 ± 1 °C. The individual microshoots (< 1.5 cm) proliferated in tenth subculture carried out at four week interval were used for experiments.

The two anti-auxins, 2,3,5-triiodobenzoic acid (TIBA; 2.0 and 4.0 μM) and 2,4,6-trichlorophenoxyacetic acid (2,4,6-T; 0.39 and 1.06 μM) were employed singly in the solidified shoot proliferation medium after filter sterilization. The treatment time was 14 d followed by subculture on the proliferation medium containing the other growth regulators except NAA. The experiment was layout in randomized block design with over 60 microshoots per treatment. The leaf chlorophyll *a+b* content was estimated by the method suggested by Bruinsma (1963).

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Abbreviations: BAP - 6 -benzylaminopurine; GA_3 - gibberellic acid; NAA - α-napthalene acetic acid; TIBA - 2,3,5-triiodobenzoic acid; 2,4,6-T - 2,4,6-trichlorophenoxy acetic acid.

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The addition of anti-auxins had an invigorating effect on microshoot proliferation as 2.0 μM TIBA gave 12.4 and 9.2 shoots per explant for Super Star and Sonia, respectively (Table 1), whereas the controls gave rise only about four shoots. There was a marked decline in shoot proliferation during subsequent subcultures in control as compared to anti-auxin treatments.

The quality of microshoots was also found to be improved with the anti-auxin treatment (Tables 1 and 2, Fig. 1). The TIBA treatments had a significant effect on the average length of shoots and number of leaves per explant. Shoot base thickness and leaf chlorophyll *a+b* content was higher in anti-auxin treated shoots. Similar results were observed in *Ixora* micropropagation after TIBA application (Lakshmanan *et al.* 1997).

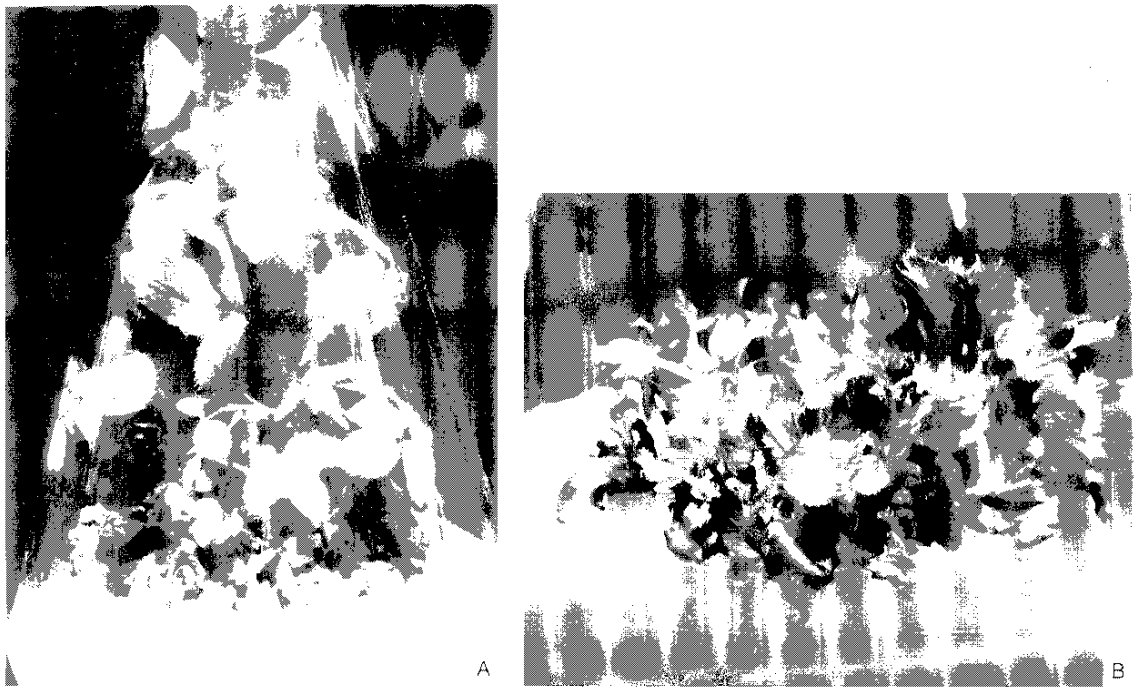

Fig. 1. *In vitro* proliferated shootlets of rose cv. Super Star: *A* - elongated shootlets after TIBA (2.0 μM) treatment, *B* - stunted shootlets on proliferation medium after tenth subculture.

Table 1. Effect of anti-auxin treatment an axillary shoot proliferaiton of hybrid tea roses after tenth subcultures (at 30 d). Mean values within each column followed by different alphabetical letters differ significantly ($P = 0.05$) according to Duncan's multiple range test ($n = 60$).

Treatment	No. of shoots per explant		Length of shoots [cm]		Shoot base thickness [mm]	
	Sonia	Super Star	Sonia	Super Star	Sonia	Super Star
Control	4.3 ± 0.5b	4.6 ± 0.3c	3.4 ± 0.1b	2.7 ± 0.2c	2.20 ± 0.01c	2.09 ± 0.03b
TIBA 2.0 μM	9.2 ± 1.1a	12.4 ± 2.2a	4.8 ± 0.3a	3.9 ± 0.3a	2.21 ± 0.03c	2.91 ± 0.10a
TIBA 4.0 μM	8.1 ± 0.8a	9.0 ± 0.8ab	4.6 ± 0.5a	4.2 ± 0.1a	2.24 ± 0.07c	1.96 ± 0.08b
2,4,6-T 0.39 μM	5.8 ± 0.3b	6.8 ± 0.7bc	3.8 ± 0.1b	3.3 ± 0.4bc	2.41 ± 0.03b	2.43 ± 0.05a
2,4,6-T 1.06 μM	5.5 ± 0.5b	5.6 ± 0.4c	4.3 ± 0.3a	3.5 ± 0.2b	2.81 ± 0.04a	2.13 ± 0.02b

Effects of 2,4,6-T on measured parameters were less expressive, but the treatment reduced callus formation at the cut edges of micro-shoots during rooting and shoot proliferation. It is anticipated that lack of callusing in 2,4,6-T treated shoots might improve transport of nutrients and cytokinins to the expanding leaves. A study will be undertaken in future to elucidate its exact role.

The results indicate that the anti-auxin treatment prior to subculture on normal shoot proliferation medium triggered the cells in the epidermal layer to become meristematic or it activated dormant lying axillary buds,

which gave rise to many microshoots as suggested by Cassells (1979), Nyman and Arditti (1984) and Belaizi *et al.* (1991). It is proposed that presence of terminal bud on microshoots during subculture has a suppressing effect on nodal buds and the anti-auxin may be acting as chemical detopping compound which activate the dormant buds by negating the apical dominance (Voyiatzi *et al.* 1995). Further, Goldworthy and Rathore (1985) and Rubery (1987) proposed that these anti-auxins act as basipetal auxin inhibitor, thereby correcting the cytokinin:auxin ratio required for optimum axillary shoot proliferation.

Table 2. Effect of anti-auxin treatment on leaf number and chlorophyll content in two cultivars of hybrid tea roses after tenth subcultures (30 d). Mean values within each column followed by different alphabetical letters differ significantly ($P = 0.05$) according to Duncan's multiple range test ($n = 60$).

Treatment	Number of leaves per shoot		Leaf chlorophyll ($a+b$) [(mg g^{-1}(f.m.)]	
	Sonia	Super Star	Sonia	Super Star
Control	4.8 ± 0.7b	3.7 ± 0.2b	1.34 ± 0.2b	0.84 ± 0.1c
TIBA 2.0 μM	5.9 ± 0.2a	4.9 ± 0.3a	2.08 ± 0.0a	0.89 ± 0.2b
TIBA 4.0 μM	6.2 ± 0.9a	5.2 ± 0.1a	1.81 ± 0.1b	0.94 ± 0.1a
2,4,6-T 0.39 μM	4.3 ± 0.3c	3.9 ± 0.3b	1.19 ± 0.2b	0.98 ± 0.2a
2,4,6-T 1.06 μM	5.1 ± 0.4b	4.6 ± 0.5a	1.31 ± 0.1c	1.01 ± 0.1a

Recently, Yoskimatsu and Shimomura (1994) advocated that anti-auxin might have a crucial role in negating the effect of high level of endogenous auxin (indole acetic acid and its derivatives) which get accumulated in the tissues under prolonged *in vitro* culture, often resulting in callus formation at the base of explants. The variability in multiplication rate of the two cultivars as experienced in the present study may be due to the genotype effect or it may also be due to culture factors (Valles and Boxus 1987, Arnold *et al.* 1992).

Results suggest that inclusion of an anti-auxin (TIBA) treatment after every 8 - 10 subcultures, can significantly improve the depleting proliferation rate, during stage II in commercial micropropagation of rose.

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