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Influence of Sucrose Concentration on *in vitro* Growth of Five Rose (*Rosa hybrida* L.) Cultivars

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Abstract

Influence of sucrose concentration of the medium, genotype and interaction of the two on *in vitro* growth responses of the cultures of five hybrid-tea rose (*Rosa hybrida* L.) cultivars were evident from the initiation of shoot proliferation from axillary buds on MS medium containing 4.0 mg/l BA, 0.2 mg/l Kn 1.0 mg/l GA_3 and 30 g/l sucrose. Emerging shoots were subcultured to media containing 3.0 mg/l BA, 0.2 mg/l Kn and three different concentrations of sucrose (30, 40 and 50 g/l). Number of nascent shoots produced and elongation of the main shoot in a four-week-period was recorded for three consecutive subcultures in each concen-tration of sucrose. Rooting was induced on a medium containing 1.0 mg/l of IBA and respective sucrose levels. Sucrose concentration of 40 g/l produced the best response followed by 30 and 50 g/l. Genotypic influence was also apparent on growth responses with significant differences between values for shoots produced and shoot elongation in different cultivars. A significant interaction was observed between sucrose concentration and genotype. A negative correlation was found to exist between shoot proliferation and elongation. In general, Pristine White expressed the best growth response followed by Tropicana Orange, Peace Yellow, Paradise Pink and Oklahoma Red in that order.

Introduction

Traditionally, hybrid-tea roses (*Rosa hybrida* L.) have been considered to be one of the most prized flowers of the world because of their high ornamental value (Bose and Yadav 1989). Their importance has grown over the years with the emergence of a global cut flower market (CBI 1990). Lately, Micropropagation procedures have facilitated mass production of good quality plantlets giving a boost to rose floriculture industry (Dohare et al. 1991; Short and Roberts 1991).

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In Saudi Arabia roses are considered a precious commodity. However, production of roses in this country is constrained by stress conditions prevailing in most parts for maximum time of the year. *In vitro* procedures and 'under-the-cover' cultivation can circumvent these constraints and may considerably enhance rose production in this region. It should be worthwhile to adopt and optimize tissue culture protocols for micropropagation of preferred varieties of rose.

Besides hormonal regime, other media components are also known to influence *in vitro* growth response of rose cultures (Short and Roberts 1991; Skirvin et al. 1990; Lloyd et al. 1988). Concentration of sucrose in the medium has been found to affect photosynthetic potential (Langford and Wainwright 1988) and rooting response (Hyndman et al. 1982). Genotypic effects on the *in vitro* responses of rose cultures have also been noted (Short and Roberts 1991; Skirvin et al. 1990).

In the present study, an attempt was made to assess the influence of sucrose concentration in the medium on *in vitro* shoot growth and rooting response in five cultivars of hybrid-tea rose.

Materials and methods

Five cultivars of hybrid-tea rose (*Rosa hybrida* L.), namely 'Oklahoma Red', Paradise Pink, Tropicana Orange, Peace Yellow and Pristine White were used for this study. Nodal cuttings were used as explants, which were sterilized with a 0.1 % (w/v) solution of mercuric chloride, followed by thorough washings with sterilized distilled water.

For induction of shoot proliferation, explants were planted on MS supplemented with 4.0 mg/l BA, 0.2 mg/l Kn, 1.0 mg/l GA_3 and 30 g/l sucrose. The medium was gelled with 8.0 g/l agar and pH was adjusted to 5.8 before autoclaving. Cultures were incubated at 25 ± 1°C and 16 h photoperiod.

Two-week-old bunches of proliferating shoots were transferred to media containing 3.0 mg/l BA and 0.2 mg/l Kn with three different concentrations of sucrose; i.e., 30, 40 and 50 g/l. After four weeks, individual shoots, more than one cm in height were separated from the bunch and transferred to fresh medium with the same hormonal combination and a specific concentration of sucrose. Subculture of single shoots on the same media was repeated at an interval of four weeks. Data on production of nascent shoots per culture and elongation of the main shoot in each concentration of sucrose were recorded for three consecutive subcultures. These three sets of data were treated as replicates.

For root induction, shoot cultures were transferred to medium containing 1.0 mg/l IBA and the same concentration of sucrose as that of the shoot growth

medium. Rooting response in different concentrations of sucrose was evaluated in terms of days to initiation of rooting and number of roots formed after two weeks. Three replicates of rooting cultures were maintained. Data for shoot growth as well as rooting were recorded on at least 25 cultures per treatment in each replicate. Independent and combined influences of sucrose concentration and genotype on *in vitro* responses were evaluated by factorial analysis according to Yates method.

Results and Discussion

Multiple shoot formation started in the axially region of the nodal cuttings. Peace Yellow was first to respond on eighth day of incubation followed by Pristine White (tenth day), Oklahoma Red and Paradise Pink (12th day) and Orange rose (13th day). Time taken for initiation was found to be shorter than reported in some other studies (Ara et al. 1997; Chaudhary 1991), where BA in the similar range was used. The divergence may be because of different genotypes used or due to the presence of GA_3 in the initiation medium used in the present study.

Sucrose concentration of the medium showed a distinct influence on the rate of shoot proliferation in cultures of all cultivars tested. Proliferation rate depicted by mean number of nascent shoots produced per culture in four weeks was highest (6.5) in 40 g/l sucrose, intermediate (5.3) in 30 g/l and lowest (4.2) in 50 g/l (Table 1, Fig. 1). Shoot elongation, measured as per cent gain in height in four weeks was also influenced by sucrose concentration of the medium. Maximum shoot elongation was recorded in the medium containing 40 g/l sucrose (mean: 71.1%) followed by 59.7% in 30 g/l and 50.3% in 50 g/l (Table 1, Fig. 2). Hyndman et al. (1982) and Lanford and Wainwright (1988) reported that sucrose concentration of the medium incrementally influences the photosynthetic ability of *in vitro* growing shoots up to a certain level; but higher concentrations suppress the activity. This could be the reason for inferior growth response in 50 g/l sucrose as compared to 40 g/l in the present study.

Genotypic influence on shoot multiplication and elongation could also be observed clearly. The highest number of shoots was recorded in cultures of Pristine White in all the three concentrations of sucrose (mean: 7.3), while in the Oklahoma Red (mean: 3.9) the production of shoots was lowest (Table 1, Fig. 3). Highest elongation was recorded in the cultures of Oklahoma Red (mean: 70.9%), while Pristine White (mean : 41.8%) showed the lowest increase in height (Fig. 4). Kim et al. (2003), Short and Roberts (1991) and Skirvin et al. (1990) reported that the genotypic differences have a perceptible influence on growth performance of rose cultures. Values for shoot proliferation and elongation observed in the present study are comparable to those reported in the above and some other studies (Chaudhary 1991; Bressen et al. 1982).

Within a given concentration of sucrose, significant differences were noted between rates of shoot production of different cultivars. Similarly, within the cultures of a single cultivar shoot proliferation was variable at three concentrations of sucrose used (Table 1). This trend suggests that there was a significant interaction between sucrose concentration and genotype of the culture. Apparently, as a result of this interaction, highest proliferation rate (8.8%) was recorded in 40 g/l sucrose in cultures of Pristine White and the lowest in 50 g/l sucrose in Oklahoma Red. Interaction between the sucrose concentration and the genotype was also noticed for shoot elongation (Table 1). Highest elongation (85.9%) was observed in 40 g/l sucrose in culture of Oklahoma Red and lowest (41.8%) in 50 g/l in Pristine White. Interactions between genotype and components of the medium, including sucrose and their concentrations are known to operate in rose cultures (Ara et al. 1997; Short and Roberts 1991; Skirvin et al. 1990).

Table 1. ***In vitro*** **shoot growth response of rose cultures in different concentrations of sucrose.**

Cultivar	Shoot production (No. per culture)* (g/l)				Shoot elongation (%)* (g/l)			
	30	40	50	Mean	30	40	50	Mean
Oklahoma Red	3.8 J	4.8 G	3.2 K	3.9 T	71.8 c	85.9 a	54.9 ef	70.9 p
Paradise Pink	4.1 I	5.2 F	3.8 J	4.4 S	65.9 d	76.5 b	55.8 e	66.1 q
Tropicana Orange	5.8 E	7.5 C	4.5 H	5.9 Q	51.1 fg	64.6 d	50.7 fg	55.5 s
Peace Yellow	5.2 F	6.3 D	4.1 I	5.2 R	64.7 d	70.7 c	48.4 gh	61.3 r
Pristine White	7.4 B	8.8 A	5.6 E	7.3 P	45.0 hi	57.9 e	41.8 i	48.2 t
Mean	5.3 Y	6.5 X	4.2 Z	-	59.7 y	71.1 x	50.3 z	-

*Values followed by same capital/small letters are not significantly different at 5% level of probability.

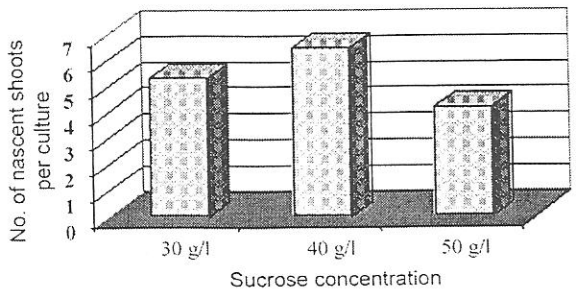

Fig. 1. Influence of sucrose concentration on shoot proliferation.

Trend of shoot proliferation was conversely proportional to shoot elongation (Table 1). While Pristine White producing the highest number of shoots (mean: 7.3) showed the lowest elongation (mean : 48.2 %), Oklahoma Red with the

lowest number of shoot production (mean: 3.9), registered highest elongation (mean: 70.9 %). Means pooled over the cultivars and sucrose concentrations showed a highly significant negative correlation between shoot proliferation and shoot elongation (r = – 0.970). This could be due to partitioning of nutrition for the two types of growth processes, which may be differentially influenced by the genotype of the cultures.

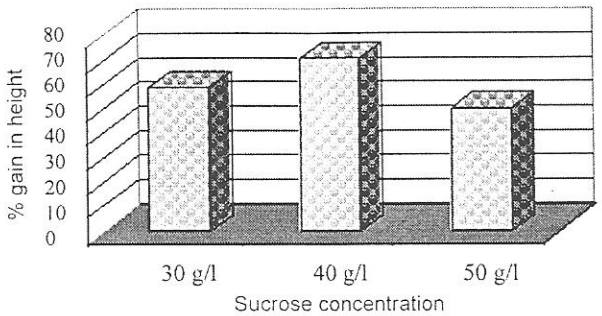

Fig. 2. Influence of sucrose concentration on shoot elongation.

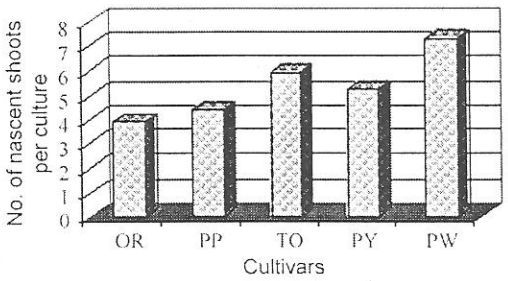

Fig. 3. Influence of genotype on shoot proliferation*. OR = Oklahoma Red, PP = Pink Paradise, TO = Tropicana Orange, PY = Peace Yellow, PW = .Pristine White.

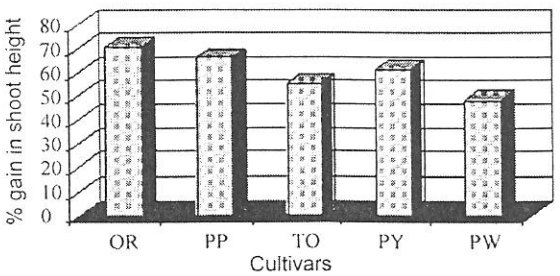

Fig. 4. Influence of genotype on shoot elongation. OR = Oklahoma Red, PP = Pink Paradise, TO = Tropicana Orange, PY = Peace Yellow, PW = .Pristine White

Rooting was rapid and prolific on the medium containing 1.0 mg/l IBA in contrast to media with IAA used by Kim et al. (2003) for induction of roots in rose cultures. Ara et al. (1997) also found IBA to be better than IAA and NAA alone or in combination for rooting in hybrid-tea rose cultures.

Average time taken for initiation of rooting was significantly shorter in 40 g/l sucrose (7.8 days) as compared to 8.5 days in 30 g/l sucrose and 8.8 days in 50 g/l, showing statistically no significant difference (Table 2, Fig. 5). Among the cultivars, Pristine White was first to produce roots (7.0 days). 'Oklahoma Red' (9.6 days) and Peace Yellow (9.4 days) were the slowest in root initiation and did not show much difference with each other (Fig. 6).

Table 2. ***In vitro*** **rooting response of rose cultures in different concentrations of sucrose.**

Cultivar	Days to initiation of rooting* (g/l)				No. of roots after two weeks* (g/l)			
	30	40	50	Mean	30	40	50	Mean
Oklahoma Red	10.0 A	9.1 B	9.8 A	9.6 P	5.4 a	3.9 hi	2.9 k	4.1 r
Paradise Pink	8.2 CDE	7.7 EF	8.6 BC	8.2 R	4.4 ef	4.7 cd	4.0 gh	4.4 q
Tropicana Orange	7.5 FG	7.4 FG	7.9 DEF	7.6 S	4.5 de	5.0 b	4.0 gh	4.5 q
Peace Yellow	9.8 A	8.5 BCD	10.0 A	9.4 P	3.7 i	3.9 hi	3.1 jk	3.6 s
Pristine White	7.0 GH	6.5 H	7.5 FG	7.0 T	4.2 fg	5.6 a	4.2 fg	4.7 p
Mean	8.5 X	7.8 Y	8.8 X	-	4.4 x	4.6 x	3.6 y	-

*Values followed by same capital/small letters are not significantly different at 5% level of probability.

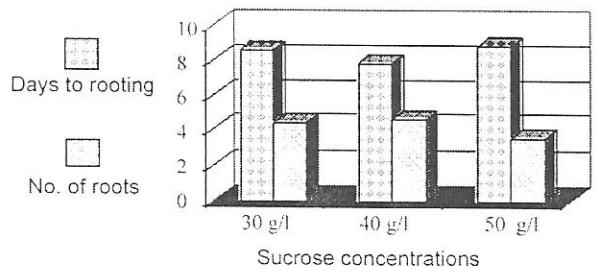

Fig. 5. Influence of sucrose concentration on rooting.

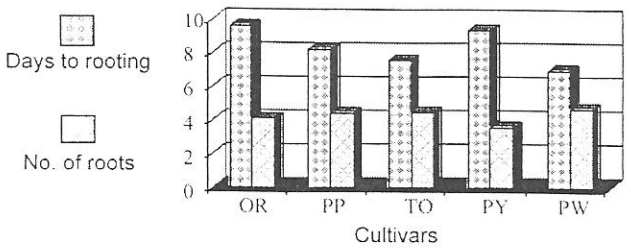

Fig. 6. Influence of genotype on rooting.

Number of roots produced in 30 g/l sucrose (4.4 roots) and in 40 g/l (4.6 roots) was not significantly different. However, significantly a smaller number of roots (3.6) were produced in 50 g/l sucrose (Fig. 5). Cultivars 'Pristine White'

(4.7 roots), Tropicana Orange (4.5 roots) and Paradise Pink (4.4 roots) did not differ much in their root production capacity. Oklahoma Red (4.1 roots) was intermediate, and Peace Yellow produced the least number of roots (Fig. 6). Interaction between concentration of sucrose and genotype was not consistent for days to initiation of rooting or root production. This observation is in agreement with the findings of Kim et al. (2003) who worked with six cultivars of hybrid-tea rose.

Sucrose concentration of 40 g/l in the medium seems to be optimal for *in vitro* multiplication of the cultivars tested in the present study. However, in view of the interactions between sucrose concentration and the genotype of the culture, optimal media regimes may be worked out for each cultivar in a micropropagation program.

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