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Short communication

Micropropagation studies on pomegranate var. Bhagwa

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ABSTRACT

In 'Bhagawa' pomegranate, micropropagation study was undertaken using different explants from fruiting mother plants. Shoot tip was found the best explant for culture establishment and microbial contamination (14.28%). Surface sterilization with 0.1% mercuric chloride for 10 min. showed the maximum survival (90.58%) and minimum microbial contamination (9.52%). Maximum number of shoots per explant (1.73) was recorded in treatment MS + BAP 2.0 mg/l, while maximum shoot length and number of leaves were observed on medium containing MS + BAP 2.5 mg/l. Half-strength MS medium along with NAA 8 mg/l gave the best rooting with maximum number of roots per shoot (4.00) and root length (3.72 cm). Hardening of rooted plantlets was ideal with maximum survival of plantlets (71.72%) on medium containing soil + sand (1:1; v/v).

Key words: Micropropagation, pomegranate, mature plant.

INTRODUCTION

Pomegranate (*Punica granatum* L.) belonging to the family Punicaceae is one of the favourite table fruit of tropical and subtropical countries. Although the conventional methods of vegetative propagation has reached commercial acceptability but for want of better alternatives, recently, tissue culture techniques have shown definite and indispensable advantages over the former, as ensures an extremely rapid rate of multiplication (Teixeira da Silva *et al.*, 8). Presently, Bhagwa is the most popular variety in demand. Many herbaceous species have been propagated extensively through this technique, this tree species has received very little attention, may be because of contamination of explants (Naik *et al.*, 6), slow growth of shoot (Kantharajah *et al.*, 4) and problem of excessive phenolic exudation (Singh and Patel, 5). There are stray reports on *in vitro* cultures of pomegranate, Chaugule (2) in Mridula, (Nataraja *et al.* (7) in Jyoti, Singh and Patel (5) in Ganesh; using shoot tip, nodal segment, cotyledons, etc. as explant. Thus, an effort was made to develop a dependable protocol for *in vitro* large scale clonal multiplication of pomegranate cv. Bhagawa.

MATERIALS AND METHODS

The investigation was carried out during 2011-12 at the Tissue Culture Laboratory, Vasantrao Naik Marathwada Krishi Vidhyapeeth, Parbhani. Five sub-experiments were carried out, in which CRD was applied for laboratory experiment and RBD for field experiments. In first experiment, the suitable explants was tried by using different explants, namely T_1 = Shoot tip, T_2 = Nodal segment, and T_3 = Leaf segment. Experiment two dealt with surface sterilants, *i.e.* T_1 = $NaOCl_2$ (0.5%) 5 min., T_2 = $NaOCl_2$ (0.5%) 10 min., T_3 = $NaOCl_2$ (0.5%) 15 min., T_4 = $HgCl_2$ (0.1%) 5 min., T_5 = $HgCl_2$ (0.1%) 10 min., T_6 = $HgCl_2$ (0.1%) 12 min. Murashige and Skoog (1962) medium was used as basal medium with pH adjusted between 5.7-5.8 and supplemented with 30 g/l sucrose and solidified using 8 g/l agar-agar. Experiment three was carried for standardization of suitable shoot proliferation medium, which consisted of T_1 = MS + BAP 1.0 mg/l, T_2 = MS + BAP 1.5 mg/l , T_3 = MS + BAP 2.0 mg/l, T_4 = MS + BAP 2.5 mg/l, T_5 = MS + Kn 0.4 mg/l, T_6 = MS + Kn 0.6 mg/l, T_7 = MS + Kn 0.8 mg/l, T_8 = MS + Kn 1.0 mg/l, T_9 = MS + (control). Standardization of rooting medium was carried out in experiment four where T_1 = ½ MS + NAA 2 mg/l, T_2 = ½ MS + NAA 4 mg/l, T_3 = ½ MS + NAA 6 mg/l, T_4 = ½ MS + NAA 8 mg/l, T_5 = ½ MS + IBA 2 mg/l, T_6 = ½ MS + IBA 4 mg/l, T_7 = ½ MS + IBA 6 mg/l, T_8 = ½ MS + IBA 8 mg/l, T_9 = $^1/_2$ MS + control applied to find suitable rooting media. Experiment five dealt with standardization of hardening mixture, which comprised of T_1 = Sand, T_2 = Soil + sand (1:1 v/v), T_3 = Soil + sand + vermicompost + (1:1: v/v). All aseptic manipulations were carried out under horizontal laminar air-flow cabinet. All the cultures were incubated in a culture room at temperature of 25 ± 1°C relative humidity of 65 ± 5% and were exposed to 16/ 8 h photoperiod period provided by cool white fluorescent tubes (light intensity of 2500-3000 lux).

Different explants of pomegranate cv. Bhagawa were obtained from four-year-old healthy, high

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yielding and disease-free mother plants established at Horticulture Research Scheme, VNMKV, Parbhani. Ten units having one explant in each treatment were used for recording different observations and mean values were used for statistical analysis. The data were subjected to ANOVA.

RESULTS AND DISCUSSION

Perusal of data in Table 1 indicates the nodal segment gave better result regarding number of shoot, number of leaves, length of shoot but less survival with more microbial contamination after initiation shoot tip. The shoot tip was the next best explant with regard to the number of shoots per explant, number of leaves and shoot length. Significantly, more survival and less microbial contamination after culture initiation were recorded as compared to nodal segments. Micropropagation establishment from field grown plants is a very critical stage as it is met with an array of problems such as microbial contamination, phenol exudation, media browning etc. Phenol secretion from the cut ends of explants lead to the browning of the medium and reduces development of explants. Similar results were reported by *Patil et al.* (5) in pomegranate.

It is evident from the data presented in Table 2 that the survival and microbial contamination of shoot tip differed significantly under different treatments. The highest survival and the lowest microbial contamination were recorded in treatment $HgCl_2$ 0.1% for 10 min. It was found that the explants turned necrotic when the treatment duration was enhanced beyond 10 min. for both surface sterilants ($NaOCl_2$ 0.5% and $HgCl_2$ 0.1%). The results obtained in present study were on similar lines as reported by *Patil et al.* (5) and Singh and Patel (6).

Data presented in Table 3 with regard to shoot regeneration revealed that significantly more number of leaves per shoot tip and microshoot length was recorded in treatment T_4, *i.e*. MS + BAP 2.5 mg/l. The lower number of leaves and shoot length was recorded in treatment MS + Kn 0.6 mg/l. No shoot proliferation was recorded in treatment MS medium devoid of cytokinin. About 1-2 shoots proliferated in the present study. Treatment MS + BAP 2.0 mg/l performed the best by allowing the maximum number of shoots to sprout. For the shoot regeneration,

Table 2. Effect of surface sterilants and exposure duration on culture establishment using shoot tip explant.

Treatment (min.)	Survival (%)	Contamination (%)
$NaOCl_2$ (0.5%) 05	0.00 (0.00)*	100.00 (0.00)*
$NaOCl_2$ (0.5%) 10	21.91 (33.15)*	78.07 (62.03)*
$NaOCl_2$ (0.5%) 15	0.00 (0.00)*	100.0 (90.00)*
$HgCl_2$ (0.1%) 05	57.55 (49.31)*	42.45 (40.63)*
$HgCl_2$ (0.1%) 10	90.58 (82.96)*	9.42 (17.85)*
$HgCl_2$ (0.1%) 12	0.00 (0.00)*	0.00 (0.00)*
CD at 1%	3.87	17.90

*Transformed Arc Sin value

Table 3. Effect of treatment combinations of growth regulators on shoot growth of shoot tip explant.

Treatment	No. of shoots	No. of leaves	Shoot length (cm)
MS + BAP 1.0 mg/l	1.33	5.66	1.72
MS + BAP 1.5 mg/l	1.53	7.46	2.61
MS + BAP 2.0 mg/l	1.73	7.26	2.71
MS + BAP 2.5 mg/l	1.60	7.73	2.80
MS + Kn 0.4 mg/l	1.13	6.73	1.78
MS+ Kn 0.6 mg/l	1.00	5.60	1.68
MS+ Kn 0.8 mg/l	0.93	5.86	1.68
MS + Kn 1.0 mg/l	0.93	6.33	1.92
MS (control)	0.00	0.00	0.00
CD at 1%	0.52	1.05	0.41

Table 1. Effect of type of explants on culture establishment in pomegranate.

Treatment detail	No. of shoots per explant	No. of leaves per shoot	No. of leaves per shoot	Microbial contamination (%)
Shoot tip	1.48	4.68	4.68	14.28 (22.14)*
Nodal segment	2.40	7.28	7.28	20.00 (26.56)*
Leaf segment	0.00	0.00	0.00	0.00
CD at 1%	0.47	0.41	0.41	1.57

*Transformed Arc Sin value

cytokinin is effective when used in combination with auxin (Zimmerman and Sartz, 8). Similar findings have been reported by Naik *et al*. (3) as well as by Chaugule (1) who reported regenerated roots on the same medium to form shoots complete plantlets. However, in the present study none of the shoot could root unless they were transferred onto rooting medium.

The data depicted in Table 4 show that the best treatment for *in vitro* rooting was half-strength MS medium supplemented with 8.0 mg/l IBA. Similar result was reported by Chaugule (1). Studies on primary hardening in pots having different mixtures of hardening media is depicted in Tables 5 & 4. The maximum survival of plantlets was recorded in treatment containing (soil + sand 1:1 v/v), while treatment soil + sand + vermi-compost (1:1 v/v) recorded minimum.

Table 4. Effect of treatment combinations of growth regulators used for root growth of shoot tip explants.

Treatment	No. of roots per shoot	Root length (cm)	No. of days for rooting
$^1/_2$ MS + NAA 2 mg/l	2.20	2.75	10.46
$^1/_2$ MS + NAA 4 mg/l	2.40	2.22	9.80
$^1/_2$ MS + NAA 6 mg/l	3.06	2.79	9.66
$^1/_2$ MS + NAA 8 mg/l	3.00	2.28	9.80
$^1/_2$ MS + IBA 2 mg/l	3.13	2.15	10.53
$^1/_2$ MS + IBA 4 mg/l	3.20	2.52	11.06
$^1/_2$ MS + IBA 6 mg/l	3.86	2.94	10.13
$^1/_2$ MS + IBA 8 mg/l	4.00	3.20	8.33
$^1/_2$ MS + control	0.00	0.00	0.00
CD at 1%	0.44	0.60	1.18

Table 5. Effect of different proportion of hardening media on plantlets survival of shoots tip explants.

Treatment	Survival (%)
Sand	68.72 (55.98)*
Soil + sand (1:1 v/v)	71.87 (57.92)*
Soil + sand + vermi-compost (1:1 v/v)	50.05 (45.00)*
CD at 1%	2.60

*Transformed Arc Sin value

Thus, an efficient micropropagation protocol for large scale micropropagation of pomegranate cultivar Bhagawa was developed which can be employed commercially.

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