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High Frequency Regeneration in Punica Granatum L

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Abstract: ***Punica granatum L. (Pomegranate) is an economically important commercial fruit crop. The plant is extensively grown in India. Conventionally pomegranate is propagated vegetatively by the rooting of hard wood cuttings and air layering, which is hectic and time consuming. Apart from that seed culture induces heterogeneity. In recent years the bacterial blight disease caused by Xanthomonas compestris pv. punicae and wilt caused by Ceratocysis fimbriata causing enormous losses to the crop and both bacterial blight and wilt are transmitted mainly through planting material. Plant tissue culture has been extensively utilized for the improvement of many commercial plants now days. Hence it was decided to undertake in vitro multiplication studies in pomegranate.***

Keywords: Pomegranate, Health benefits, Heterogeneity

1. Introduction

Punica granatum L. is generally known in a distinct family Punicaceae. Pomegranate is one of the oldest known fruit trees of the tropics and sub-tropics, cultivated for its delicious edible fruits. It is native to Iran and possibly also to some surrounding areas. It is exploited for nutritional value of its fruit, medicinal properties of different parts of the tree and for ornamental purpose (**Kirtikar and Basu, 2001**). The fruit is a rich source of minerals, vitamins, antioxidant polyphenols and tannins. Protocols for regeneration of pomegranate via shoot organogenesis, somatic embryogenesis and enhanced axillary bud proliferation have been reported (Naik et al., 2000).

Propagation through conventional methods does not ensure disease free healthy plantlets. Hence, an alternate strategy of sustainable nature is required to check the disease and save the crop. Use of disease free planting materials regenerated through tissue culture is most appropriate measure to manage the disease effectively. Looking towards this possibility efforts have been made to propagate this plant by tissue culture technique.

2. Materials and Methods

Source of Explant: Plant was grown in nursery in botanical garden; College of Agriculture Biotechnology, Georai Tanda, Aurangabad and explants viz. shoot tips, axillary buds, were collected from the elite plant.

Sterilization of explants: These explants were surface sterilized with $HgCl_2$ solution (0.1% w/v) for four - five min followed by three washes with sterile distilled water.

Culture Media: Nodal segments of twigs were cut (0.5 cm) and cultured on 8% (w/v) agar solidified MS supplemented with various growth regulators (NAA, IAA, IBA, BAP and Kin) at different concentrations and combinations. PVP was added into the medium to stop the phenolic leaching. Sub culturing was done at an interval of 14 - 20 days.

Culture conditions: The pH of the medium was adjusted to 5.8 before autoclaving. All cultures were incubated at 25 ± 2°C under 16/8 hr photoperiod. After 12 weeks, plantlets with roots were successfully planted in pot soil through gradual acclimation.

3. Results and Discussion

Results were recorded after 15 – 20 days and it was observed that there was phase for initiation of shoots. Within fifteen days of culture, shoot primordia were induced at the cut end of nodal explants. Explants like shoot tip and axillary buds, grown on MS supplemented with 1, 2, 3 and 5 mg/1 BAP and Kin either alone or in combination with 0.1 - 1.0 mg/1 NAA, IAA and IBA (Table 1). Maximum (80%) explants shown emergence of shoot premordium on MS medium fortified with 5.0 mg/1 BAP with 0.5 mg/1 NAA after two successive subcultures.

However, KIN was found to be more effective than BAP for induction of multiple shoots (Table 1). Highest number of multiple shoots formed was 14 using 5 mg/lit KIN and alone (Plate a, b). This was followed by 10 shoots using 4 mg/lit KIN alone. According to Faisal and anis 2005, callus forms frequently at the basal cut ends of nodal explants on cytokinin enriched medium in species exhibiting strong apical dominance.

There were significant differences in regeneration frequencies on multiplication of shoots. As stated by Martin (2002) the high morphogenic efficiency of node segments derived callus may be due to the presence of some internal components from the pre-existing axillary buds that are essential for induction of caulogenesis. Shoot buds were also developed from callus culture elongated. This continued in two subsequent subcultures made up of identical constituents at an interval of 15 days.

Mean values within columns followed by the same letter are not significantly different at 5% level. In case of Pomegranate, fewer reports on successful regeneration tissue culture studies have reported for successful regeneration protocols. Another protocol has been developed for high-frequency shoot regeneration and plant establishment of *Tylophora* from petiole-derived callus which resembles to the present work. (Faisal and Anis, 2005).

Table 1: Effect of different concentrations of growth regulators on regeneration

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Growth regulators(mg/l)					% explants producing callus	No of shoots produced
BAP	Kin	NAA	IAA	IBA		
Growth regulator Free Medium					-	-
1					20 ± 3.84	2±1.52ab
2					51 ±1.22	3± 1.52ab
5					40 ± 1.21	4 ± 2.08b
	1				18 ± 2.22	3± 3.21ab
	2				31 ± 4.44	10 ± 3.03b
	5				20±1.21	14 ± 3.46b
5		0.1			40 ± 3.84	2±1.0ab
5		0.5			82 ± 2.22	2±1.15ab
5		1.0			55 ± 0.92	Nil
5			0.1		20 ± 3.84	Nil
5			0.5		44 ± 4.44	Nil
5			1.0		27±1.13	Nil
5				0.1	31± 5.87	2 ± 3.51ab
5				0.5	40+ 7.69	*2* ±2,0ab
5				1.0	20±1.21	3 ± 3.05a

a-Callus b-Shoots

a. Multiple shoots (Early Stage) b. Multiple shoots (Late)

4. Conclusion

The fruits of pomegranate are known to possess nutritional, pharmaceutical and therapeutic properties. The potential health benefits of fruit made high demands for pomegranate in both domestic and international market. Plant rose through the seeds shows tremendous genetic variation which is not suitable for commercial cultivation. Vegetative propagation is difficult in Pomegranate due to low seed viability and germination rate (Thomas and Philip, 2005). Therefore present studies will be helpful for mass propagation of this plant.

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