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Research Article

In vitro Regenration of Pomegranate (*Punica granatum* L.) from Nodal Explant

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Abstract

Reliable and reproducible protocols to get healthy and well formed plants from nodal explants of the pomegranate (*Punica granatum* L) Nodal segments were cultured on M.S. media at full strength Murashige and Skoog media was prepared as a basal medium supplemented with organic acids and vitamins. Sucrose was added at 30.0 g/L. The pH of the prepared media was adjusted between 5.6 to 5.8 and agar-agar was added as 8.0 g/L for media solidification. For establishment stage, BAP 0.2 to 2.0 mg/L, NAA 0.1 to 1.0 mg/L, BAP 0.1 to 0.5 mg/L and NAA 0.1 to 0.5 mg/L were tested. Also, for rooting stage, two different auxins; IBA and NAA were tested at 0.5, 0.25 and 0.50 mg/L on MS medium at full strength. MS medium containing 0.50 mg/L NAA (97%) and 0.50 mg/L IBA (95%). Both NAA and IBA therefore showed same rooting response.

Key words: Micro propagation Rooting, *Punica granatum*.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is generally known in a distinct family (Punicaceae), which comprises only one genus (*Punica*) and two species; *P. granatum* and *P. protopunica* .[1] Pomegranate is an economically important species of the tropical and subtropical regions of the world due to its delicious edible fruits, and pharmaceutical and ornamental usage .[2] It is considered native to Iran, Afghanistan and Southern Pakistan's Baluchistan region to the Himalayas in Northern India. Pomegranate has been naturalized over the whole Mediterranean region and the Caucasus since ancient times. It has been widely cultivated throughout India, drier parts of Southeast Asia, Malaysia, the East Indies and tropical Africa.The cultivation of pomegranate in india 100,000 ha yielding 0.45 million ton of fruit per year. The pomegranate fruit juice is a good source of sugars, vitamin C, vitamin B, pantothenic acid, potassium, antioxidant polyphenols and a fair source of iron. Wild pomegranate is too acidic and of little value except as souring agent (Anardana). Pomegranate parts have been used traditionally for their medicinally for their medicinal properties. The double-flowered pomegranates (which do not bear fruits) are grown in parks and ornamental gardens for their beautiful red flowers [3]. Wild pomegranate is too acidic and of little value except as souring agent (Anardana). *In vitro* propagation of pomegranate *Punica granatum* has been reported by several workers using different explants shoot tip and nodal explants [4.5.6.7] and cotyle-donary nodal explants [8.9.10.] However, till date there are hardly any reports described in details of different media and serial sub culturing process using cotyledonary nodal explants in pomegranate. Therefore the main objective of the present study was to develop an *In-vitro* Regeneration of Pomegranate (*Punica granatum* L) from nodal ex-plant.

MATERIAL AND METHODS

Plant Collection

This work was done in Research institute, Biodiversity Conservation & Rural Biotechnology center Jabalpur (M.P.) during the period from January , 2014 to May, 2014 . *Punica granatum* L. were collected from the high yielding, 1 Year old tree

growing in the State Forest Research Institute Jabalpur.

Washing of the ex-plant

Isolated nodal segments were cleaned under running tap water for about 15 to 20 min. each under laminar air flow hood and followed by three times rinsing in sterile distilled water. Nodal segments were further soaked in fungicide (Bavistin) solution (.5 mg/L) for 10 min. and then again washed with sterile distilled water. Finally .1 g/L mercuric chloride solution for 5 min. was used to treat these explants followed by three times washes with sterile distilled water for complete sterilization of nodal explants. Completely sterilized explants were inoculated on establishment Patil *et al.* 18131 media. After establishing transferred explants on proliferation media for growth, completely proliferated explants were then transferred to rooting media.

Culture media

MS medium were tested for micro propagation of pomegranate cultivar Media was prepared as a basal medium supplemented with organic acids and vitamins. Sucrose was added at 30.0 g/L. The pH of the prepared media was adjusted between 5.6 to 5.8 and agar-agar (Hi-Media) was added as 8.0 g/L for media solidification. For establishment stage, BAP 0.2 to 2.0 mg/L, NAA 0.1 to 1.0 mg/L, BAP 0.1 to 0.5 mg/L and NAA 0.1 to 0.5 mg/L were tested. Also, for rooting stage, two different auxins; IBA and NAA were tested at 0.5, 0.25 and 0.50 mg/L on MS medium at full strength.

RESULT & DISCUSSION

1. Effect of BAP on regeneration of shoots

MS media were used for the growth of nodal explants for different concentration of BAP (0.5 to 2 mg/L). The highest average growth response (98%) in MS medium containing BAP 1.5 mg/L, whereas 2 to 4 shoots per explants having highest shoot length (0.5 to 1.5 cm) was recorded.

2. Effect of NAA on regeneration of shoots

MS media were used for the growth of nodal explants for different concentration of NAA. 0.2 to 1.5 mg/L the highest growth response (98%) was recorded on MS medium containing 0.9 mg/L NAA, (0.5to 1.3 cm) containing was recorded two to three shoots per explants having highest shoot length

3. Effect of NAA and IBA in MS medium on rooting

The containing of 0.50 mg/L NAA and 0.50 mg/L IBA in MS medium for showed the highest rooting responseThe data in Table 1 shows that the highest average rooting response was recorded on MS medium containing 0.50 mg/L NAA (97%) and 0.50 mg/L IBA (95%). Both NAA and IBA therefore showed same rooting response .However, thick root formation was observed in medium containing 0.50 mg/L IBA. Root length of 0.5 to 2.8 cm was recorded on medium containing IBA, whereas 0.5 to 2.5 cm root length was recorded on medium containing NAA.

SUMMARY AND CONCLUSION

The *Punica granatum* were obtained from State Forest Research Institute Jabalpur. The investigation incorporated the work carried out during January 2014 to may 2014.the shoot initiation was found to be better on BAP (0.5 to 2mg/L) highest average growth response. And the regeneration of the shoot was better on 0.9mg/L NAA. Where two to three shoots per ex-plant. And the table has shows the NAA and IBA highest response for the root development.

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Table 1
Effect of NAA and IBA in MS medium on Rooting

Medium	Con.(mg/L	Number of root	% of explants showing response	Root length
MS + IBA	0.5	1 ± 1	15 ± 1	0.2 ± 0.3
MS + IBA	0.25	2 ± 1	50 ± 1	1.5 ± 0.2
MS + IBA	0.50	4 ± 1	70 ± 1	2.8 ± 0.5
MS + NAA	0.5	1 ± 1	10 ± 1	1.2 ± 0.2
MS + NAA	0.25	2 ± 1	45 ± 1	2.5 ± 0.2
MS + NAA	0.50	4 ± 1	70 ± 1	2.5 ± 0.5

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