Advances in Bioresearch
Adv. Biores., Vol 8 (5) September 2017: 33-36
©2017 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.5.3336

Advances in Bioresearch

ORIGINAL ARTICLE

A Suitable Explant For Culture Establishment In Pomegranate (*Punica granatum* L.)

Suhasini, S. C.*, S. N. Patil, Prabhuling, G., Venkateshalu and S. L. Jagadeesh
Department of Fruit Science, College of Horticulture, Bagalkot
University of Horticultural Sciences, Udyanagiri, Bagalkot- 587104, Karnataka (INDIA)
*E-mail: suhasinisc92@gmail.com

ABSTRACT
Pomegranate is one of the oldest known fruit trees of the tropics and sub-tropics, cultivated for its delicious edible fruits. In vitro propagation of pomegranate has been reported through axillary shoot proliferation from nodal segments and also using parts of the plant which eliminates the disease and increases the early maturity of the crop. An investigation was conducted to standardize suitable explants for protocol for aseptic culture establishment in pomegranate (Punica granatum L.) Though the conventional method of propagation of pomegranate is time consuming, it does not ensure disease free and healthy planting material Micropropagation is the only aspect of plant tissue culture that has potential to circumvent these problems in pomegranate. Bhagwa is commercial leading variety of North Karnataka due to its attractive skin color and taste. In present investigation, a second nodal segment recorded the highest aseptic culture establishment in pomegranate cv. 'Bhagwa', when explants were sterilized with mercuric chloride ($HgCl_2$) at 0.01 % for 3 minutes followed by incubation on full strength MS medium.
KEY WORDS: *Axillary shoot, In vitro, Mercuric Chloride, Bhagwa and Aseptic culture*

Received 16/04/2017 Revised 09/06/2017 Accepted 20/07/2017

How to cite this article:
Suhasini, S. C., S. N. Patil, Prabhuling, G., Venkateshalu and S. L. Jagadeesh. A Suitable Explant For Culture Establishment In Pomegranate (*Punica granatum* L.). Adv. Biores., Vol 8 [5] September 2017: 33-36.

INTRODUCTION

Pomegranate is one of the oldest known fruit trees of the tropics and sub-tropics, cultivated for its delicious edible fruits. It is generally known in a distinct family Punicaceae, which comprises only one genus (*Punica*) and two species; *P. granatum* and *P. protopunica,* chromosomal number, 2n = 16 and 18, respectively [1]. Pomegranate is exploited for nutritional value of its fruit, medicinal properties of different parts of the tree and for ornamental purpose [2-5]. The fruit is a rich source of minerals, vitamins, antioxidant polyphenols and tannins. Tannins occur in all parts of the tree, particularly in the fruit rind, stem bark, root bark and leaves. The natural dyes from flowers and dried fruit are used for dyeing wool, silk and other textiles. The fruit juice is a good source of sugars, vitamin C, vitamin B, pantothenic acid, potassium, antioxidant polyphenols and a fair source of iron. It is native to Iran and possibly also to some surrounding areas. Protocols for regeneration of pomegranate *via* shoot organogenesis, somatic embryogenesis and enhanced axillary bud proliferation have been reported [6-13].

However in commercial tissue culture, plant multiplication is carried out through axillary shoot proliferation. It is preferred mainly because of its true to type and genetically stable plantlets. Reliable and efficient protocols for plant regeneration *in vitro* through stimulation of axillary shoot proliferation from nodal stem segments and apical buds or through organogenesis or embryogenesis directly from various explants or callus have been developed for many important tropical and temperate fruit trees [14-17]. *In vitro* propagation of pomegranate has been reported through axillary shoot proliferation from nodal segments [9-11], shoot tips [11] and cotyledonary nodes [18]. Regeneration of *P. granatum* plantlets *in vitro* can occur through organogenesis from callus derived from leaf segments [19].The conventional method of propagation through seed is not preferred because of the resulting variability in

tree and fruit characters; while, propagation through hardwood or softwood cuttings does not ensure disease free and healthy plants. In addition, this method is time consuming and labour intensive [20] and a large number of cuttings do not survive transplanting. Therefore micropropagation in fruit tree would help in overcoming difficulties of vegetative propagation, producing true to type and rapid mass production of planting material [1]. The present investigation was, therefore carried out to standardize protocol for selection of suitable explants for culture establishment in cv. 'Bhagwa' of pomegranate.

MATERIAL AND METHODS

Explants

The young, healthy and green shoots with first 3-4 nodes were taken from mother plant. These shoots were washed thoroughly in tap water to remove adhered dust and other impurities. Washing continued (4-5 washes) with soap water or few drops of antiseptic solution (Tween 20). They were treated with a solution containing streptocycline (300 mg/l) + carbendazim (750mg/l) and then with cetrimide (500 mg/l) for 25-30 minutes. Further thoroughly washed 4-5 times with sterilized distilled water.

Following explants were prepared for present study (Fig 1)

Shoot tip : Explants from apical portion of shoot (1.5-2 cm) were taken from the current season growth; **First nodal segment**: A nodal segment of (1.5-2 cm) below the shoot tip was taken from current season shoot. **Second nodal segment** : A segment of node of 1.5- 2 cm length below the first node was taken from current season shoots and **Double nodal segment**: A segment of double nodes of 2-5 cm length below the shoot tip were taken from current season shoots (**Fig. 2**). These trimmed explants were surface sterilized with four different mercuric chloride ($HgCl_2$) concentrations for varied duration. T_1: 0.01 % for 3, T_2: 0.01 % for 5 minute, T_3: 0.03 % for 2 minute and T_4: 0.03 % for 3 minute.

Figure 1. Different type of explants of Pomegranate (*Punica granatum* L.) ***cv*. 'Bhagwa'**

Figure 2. Establishment of aseptic culture from second nodal segment

A suitable explant for culture establishment

Choice of suitable explant

 ©2017 Society of Education, India

Four different **e**xplants were treated with different concentration and duration of mercuric chloride and cultured on full strength MS media containing 3% sucrose, 35 mg/l adenine sulfate, 35 mg/l citric acid and 2.00 mg/l BAP. The observations at 5 weeks after incubation. The data in percentages were transformed to arcsine values for statistical analysis. The data were subjected to ANOVA. Critical difference values were tabulated at one per cent probability where "F" test was significant.

RESULTS AND DISCUSSION

A suitable explants for establishment of aseptic culture in pomegranate cv. 'Bhagwa' with respect to $HgCl_2$ treatments

Results obtained are furnished in Table 1.

Results are expressed according Two Factorial Design (*i.e*. Factor I: Explants and Factor II : $HgCl_2$ treatments)

There was no significant differences were observed among the different explants; however second nodal segment showed superior response to per cent aseptic culture establishment (23.53). $HgCl_2$ treatments were also non significant, however 0.01 % $HgCl_2$ for 3 minute was recorded superior culture establishment (27.60 %).

In the interaction effect, results were found to be significant. The maximum culture establishment was noted in second nodal segment with 0.01 % $HgCl_2$ for 3 minute (48.85 %, Fig 2).

It may be attributed that higher survivality of matured explants due to lesser exposure of them with reduced concentration or duration of $HgCl_2$ which might have led to less bleaching activity of chlorine and also attributed due to hardy nature of nodal segment made them survive better. Similar results were reported by Singh *et al.* [21] in pomegranate.

Table 1: A suitable explants for establishment of aseptic culture in pomegranate cv. 'Bhagwa' with respect to $HgCl_2$ treatments

Explants	$HgCl_2$ Treatments				
	T_1	**T_2**	**T_3**	**T_4**	**Mean**
Shoot tip	34.82	30.78	28.67	28.67	20.49
First nodal segment	42.89	35.01	30.78	28.67	22.89
Second nodal segment	**48.85**	32.90	30.78	28.67	**23.53**
Double nodal segment	39.04	39.04	32.90	26.56	22.92
Mean	**27.60**	22.95	20.52	18.76	
	Explant	**$HgCl_2$ Treatment**	**Explant × $HgCl_2$ Treatment**		
SEm	**0.74**	**0.91**	**1.81**		
CD @ 1%	**NS**	**NS**	**6.60**		

Concentration and duration of $HgCl_2$

T_1: 0.01% for 3 minute
T_2: 0.01% for 5 minute
T_3: 0.03% for 2 minute
T_4: 0.03% for 3 minute

REFERENCES

1. Samir, Z, El-Agamy, Rafat A.A., Mostafa, M.M., Shaaban, M., Marwa ,K. and E-Mahdy, T. (2009). *In vitro* propagation of Manfalouty and Nab El-gamal pomegranate Cultivars. *Res. J. Agric. Biol. Sci.,* **5**(6): 1169-1175.
2. Parmar, C. and Kaushal, M.K. (1982). *Punica granatum* In: Wild fruits. Kalyani, New Delhi, India, pp. 74–77
3. Naovi, S.A.H., Khan, M.S.Y. and Vohora, S.B. (1991). Antibacterial, antifungal and anthelmintic investigations on Indian medicinal plants. Fitoterapia 62: 221–228.
4. Jayesh K.C. and Kumar, R. (2004). Crossability in pomegranate (*Punica granatum* L.). Indian J. Hort., 61(3): 209–210.
5. Johanningsmeier, S.D and Harris, G.K. (20110. Pomegranate as a functional food and nutraceutical source. Ann. Rev. Food Sci. Technol., 2: 181–201.
6. Jaidka, K. and Mehra, P.M .(1986). Morphogenesis in *Punica granatum* (Pomegranate). Canadian J. Bot., 64: 1644-1653.
7. Omura, M., Matsuta, N., Moriguchi, T. and Kozaki, I. (1987). Adventitious shoot and plantlet formation from cultured pomegranate leaf explants. Hort. Sci., 22: 133-134.
8. Nataraja, K. and Neelambika, G.K. (1996). Somatic embryogenesis and plantlet from petal cultures of pomegranate (*Punica granatum* L.). Indian J. Experimental Bio., 34: 719-721.

9. Naik, S.K., Pattnaik, S. and Chan, P.K. (1999). *In vitro* propagation of pomegranate (*Punica granatum* L. cv. Ganesh) through axillary shoot proliferation from nodal segments of mature tree. Sci. Hort.,79: 175-183.
10. Naik, S.K., Pattnaik, S. and Chand, P.K. 2000. High frequency axillary shoot proliferation and plant regeneration from cotyledonary nodes of pomegranate (*Punica granatum* L.). Sci Hort., 85: 261–270.
11. Murkute, A. Patil, S., Patil, B.N. and Mayakumari, M.S. (2002). Micropropagation in pomegranate, callus induction and differentiation. South Indian Hort. 50:49–55.
12. Shao, T.Z., Chen, C.L. and Deng, X.X. (2003). *In vitro* induction of tetraploid in pomegranate (*Punica granatum* L. var. Nana). Plant Cell Tissue Organ Cult., 75(3): 241–246.
13. Terakami, S., Matsuta, N., Yamamot, T., Sugaya, S., Gemma, H. and Soejima, J. (2007). Agrobacterium-mediated transformation of the dwarf pomegranate (*Punica granatum* L. var. Nana). Plant Cell Rep.,24:1243–1251.
14. Hutchinson, J.F. and Zimmerman, R.A. (1987). Tissue culture of temperate fruit and nut trees. Hort. Rev., 9: 273-349.
15. Litz, R.E. and Jaiswal, V.S. (1991). Micropropagation of tropical and subtropical fruits. In: Debergh P.C., Zimmerman, R. H. (Eds.), Micropropagation. Kluwer Academic Publishers, Dordrecht, pp. 247-263
16. Grosser, J.W. (1994). *In vitro* culture of tropical fruits. In: Vasil, I. K., Thorpe, T. A. (Eds.), Plant Cell and Tissue Culture. Kluwer Academic Publishers, Dordrecht, pp. 475-496.
17. Zimmerman, R.H., Swartz, H.J. (1994). *In vitro* culture of temperate fruits, In: Vasil, I. K., Thorpe, T. A. (Eds.), Plant Cell and Tissue Culture. Kluwer Academic Publishers, Dordrecht, pp. 457-474.
18. Sharon, M. and Sinha, S. (2000). Plant regeneration for cotyledon node of *Punica granatum* L. Indian J. Plant Physiology, 5(4): 344-348.
19. Deepika and Kawar, K. (2010). *In vitro* regeneration of *Punica granatum* L. plant from different juvenile expalnts. J. Fruit Orna. Plant Res., 18(1): 22.
20. Kanwar, K., Jomy, M., Joseph, S. and Deepika, R. (2010). Comparison of *in vitro* regeneration pathways in *Punica granatum* L. Plant Cell Tiss. Organ Cult., 100: 199–207.
21. Singh, N.V., Singh, S.K. and Patel, V.B. (2011). *In vitro* culture establishment studies on pomegranate. Indian J. Hort., 68(3):307-311.

Copyright: © 2017 Society of Education. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.