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Micro-Propagation and Bio-priming in Pomegranate - Imperative for Quality Planting Material

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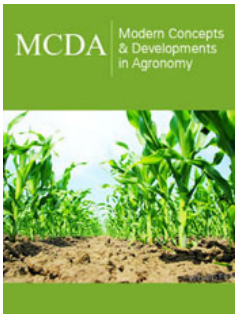

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Abstract

Pomegranate is an excellent and highly remunerative fruit crop for arid and semiarid regions the world experiencing frequent climatic vagaries, irrigation water scarcity and marred with edaphic challenges. In recent past pomegranate cultivation has grown leaps and bound across the globe particularly in India owing to its amazing nutraceutical properties, versatile adaptability, huge market demand and impressive return on investment. To match with the pace of pomegranate expansion the large-scale propagation of quality planting material is of numero uno importance. The bacterial blight, wilt and other sapling or potting mixture transmitted diseases are major threats for the sustainable pomegranate production and demand for QPM production to avoid spread of these diseases. The micropropagation offers the scope of producing disease-free elite saplings in bulk to support sustainable pomegranate production.

Keywords: Micro-propagation; *In vitro*; Pomegranate; Bio-priming

Abbreviations: AC: Activated Charcoal; PVP: Polyvinyl Pyrollidone; WPM: Lloyd and McCown (1980) Woody Plant Medium, MS: Murashige and Skoog (1962) Medium, QPM: Quality Planting Material

Introduction

Pomegranate is one of the ancient fruit crops domesticated by the mankind and is considered as a highly remunerative fruit crop under semiarid tropics. The versatile adaptability and hardy nature of the crop support fruit yield by overcoming climatic vagaries and edaphic challenges of the semi-arid regions [1]. The unmatched nutraceutical and therapeutic properties of this super fruit has attracted consumers across the globe creating a huge demand for its fruits, value added products and dietary supplements. Globally pomegranate is cultivated in about 0.5 million ha area with an annual production of approximately 6.0 million tonnes. In India, pomegranate is cultivated in about 0.234 million hectares land with an annual production of 2.85 million MT. There is significant growth in acreage and production of pomegranate globally in the recent past and India has almost registered an impressive 3 folds increase in acreage and about four folds increase in production of pomegranate during last two decades. Furthermore, the export is estimated to be 0.67 lakh MT. The average annual increase in pomegranate acreage during last 10 years in India is about 19,300 hectares, and the average annual quantity of healthy saplings required to plant this area is about 15.0 million [2]. Though, conventional methods of propagation are commercially used for propagation of pomegranate saplings, but these methods do not ensure elite and healthy/ disease free/ blight free saplings production. The bacterial blight, wilt and other sapling or potting mixture transmitted diseases are major threats for the sustainable pomegranate production and use of *in-vitro* conditions to

extend pomegranate clonal propagation has allowed for rapid mass multiplication of clonal propagation of superior plant material which are free from pathogens causing blight and wilt diseases in pomegranate. The ultimate objective is to extend pomegranate agriculture area all over the world, which demands large scale and disease-free clonal propagation of superior genotypes using micropropagation.

Micro-Propagation

Micro-propagation is a plant tissue culture technique used to produce plantlets in which aseptic tiny portions of tissues and organs are cultured in vessels with predetermined culture media and under controlled environmental conditions to develop plantlets.

There are five distinct stages in micro-propagation:

1. Stage 0: It involves the selection and maintenance of elite and healthy mother plant of a commercial variety and explant selection and pretreatments
2. Stage I: *In vitro* culture establishment
3. Stage II: *In vitro* shoot proliferation
4. Stage III: *In vitro* rooting
5. Stage IV: hardening or acclimatization

Source, Type and Pretreatments of Explant

The source of explants, explant type and pretreatments given to the explants are considered as crucial variables for *in vitro* culture establishment and success of micro-propagation protocol of pomegranate. In terms of regeneracy, not all explants are supposed to be equal. Commercial micropropagation includes meristems, shoot tips, and nodal buds [3-9]. However, *in vitro* regeneration using other explants like leaf segments and cotyledons [10-13], anthers [14] or through embryogenesis from various seedling explants, petals, and immature zygotic embryos [10] were also reported. The stage 0 also entails identifying healthy mother plants and treating them in such a method that they produce more responsive explants suited for establishment in contamination-free cultures. For the culture establishment appropriate explants are selected, sterilised, and transferred to nutrient media. Soaking of nodal segments in fungicides @0.1-0.2% (Dithane M- 45 and/or Carbendazim) solution for about 30-45 min followed by treatment with antibiotics (streptomycin solution @0.05-0.1%) for 10-20 min. and surface sterilization for 5-10 min. take care of most of the infections [6,7]. Most widely used surface sterilants in pomegranate are $HgCl_2$ and NaOCl. The explants are washed thoroughly under running tap water before the surface sterilization process [15].

In vitro Culture Establishment

In vitro propagation of pomegranate is mostly carried out on modified Murashige Skoog (MS) and Woody Plant Medium (WPM) [2,9]. The ratio of cytokinin to auxin determines shoot proliferation in pomegranate micropropagation. Among cytokinins, 6-Benzylaminopurine (6-BAP) and Kinetin and cytokinin supplements like Adenine Sulphate are mostly used for culture establishment and shoot proliferation and among auxins α-naptheleneacetic acid is commonly used [14,16,2]. The proliferation rate (10-15 shoots/explant) of the Bhagwa nodal segment at the establishment stage was highest on MS media with 1.8mg/l BAP, 0.9mg/l NAA, 1mg/l silver nitrate ($AgNO_3$), and 30mg/l adenine sulphate [6]. Two types of basal medium – MS and WPM had been used for culture establishment of nodal segments and shoot tips of pomegranate cvs. "Malas Saveh" and "Yousef Khani" [17].

Media Browning

Establishment of woody plants like pomegranate is severely affected by browning of medium due to leaching of secondary metabolites like phenols. Culture of juvenile explants, or new growth flushes during the active growth period, culture in darkness, transfer to inoculums in fresh medium at short intervals (rapid sub-culturing), culture in liquid medium with antioxidants in the culture medium, or soaking explants in water or antioxidant-containing solutions prior to inoculation, supplementing the medium with adsorbing agents like Activated Charcoal, PVP (polyvinyl pyrollidone), etc are some of the different approaches for controlling the browning of growth medium. For controlling the media browning, shoot tips and nodal segments of pomegranate cv. 'Ganesh' *in vitro* are obtained by rapid subculturing [12]. Rapid subculturing on first and third day after inoculation was suggested for *in vitro* propagation of pomegranate cv. 'Mridula' with the use of shoot tips and nodal segments of mature trees as explants [3]. The phenol exudation could be reduced if the cut edges of nodal segments are sealed with sterile wax [8]. *In vitro* rooting was best obtained on half strength MS medium containing 500mg/l of activated charcoal [18].

Shoot Proliferation

The extent and rate of multiplication is influenced by the duration and concentration of auxins and cytokinins in the multiplication medium. In a variety of plants, the cytokinin 6-benzylaminopurine (BAP) is the most often utilized growth regulator for shoot regeneration. Using cotyledonary nodes produced from axenic seedlings, a full procedure for *in vitro* regeneration of pomegranate (*Punica granatum* L.) was studied [14]. On a medium containing 9.0μM BA, the largest number of shoots (9.8 shoots/explant) was generated [14]. The treatment MS + BAP 2.0mg/l had the highest number of shoots per explant (1.73), whereas the medium containing MS + BAP 2.5mg/l had the highest shoot length and number of leaves [15]. MS medium supplemented with 2.0mg/l 6-benzylaminopurine (BAP) and 1.5mg/l naphthalene acetic acid (NAA) resulted in the maximum frequency of shoot regeneration (67.89%) along with the average shoot length of 3.62cm and the number of shoots generated (5.38) per explant [19]. Under both liquid and solidified Murashige and Skoog (1962) media, the optimum hormonal treatments for direct adventitious shoot regeneration in cotyledonary explants [20].

The micro-shoots of cvs. "Malas Yazdi" and "Shirine Shahvar" were multiplied *in vitro,* and the highest shoot height was reported on MS medium containing 2mg/l BA [17].

Photoperiod and Light Intensity Effects

Various level of light intensity with a constant temperature 26±2° C and 16/8h light/dark period in the incubation room were studied and at 3000 lux intensity, the largest number of shoots per explant and the longest length of shoots were reported [8].

In vitro Rooting

During stage III, the proliferating shoots are placed to a rooting medium. It is the final stage *in vitro* before the plantlets are transferred to *ex vitro* environments. The goal of this phase is to get fully grown plantlets to start growing. Auxins are primarily concerned with *in vitro* root induction. Among all auxin varieties, the auxin IBA was shown to be the most effective. IAA, on the other hand, although being natural, was the least effective since it was damaged by light. The salt content in rooting media is often low. Half-strength WPM medium supplemented with 0.54µ M NAA was the most effective for rooting of microshoots in cvs. "Malas Yazdi" and "Shirine Shahvar" [21]. Half strength MS medium supplemented with 0.1mg/l IBA was the most successful in inducing rooting of microshoots. *In vitro* produced shoots in half strength MS media with 1.0mg/l IBA promoted strong roots (80%) in 8-10 days [22]. Good *in vitro* rooting was observed on half strength MS medium supplemented with 0.5g/l of IBA [12]. According to Singh and Khawale, the MS medium containing 2.0mg/l IBA, 200mg/l AC, and 40g/1 sucrose increased root quality [23]. In comparison to control, a considerable increase in average root number per shoot (7.4) was observed after a brief dip of *in vitro* produced shoots in 1000mg/lit IBA [24]. Half strength MS medium with 0.1mg/l NAA and 0.02% activated charcoal was found to be the most efficient for rooting shoots, resulting in an 80.12 % root regeneration frequency [19].

Hardening/ Acclimatization

Hardening is the process of acclimating *in vitro* grown plantlets to regular outdoor conditions by gradually exposing them to diffused light in humid chambers. Hardening *in vitro* grown plantlets is critical for their long-term survival and establishment. *Ex vitro* survival of cv. "Ganesh" plantlets was reported to be 68 % when transplanted to vermicompost [12]. The plantlets in cv. "G-137" had the highest survival rate (89%) and took the shortest time to field transfer (35 days) [8]. The method of using a glass jar with a polypropylene cap packed with wet peat: Soilrite® (1:1) was shown to be the most effective, resulting in the highest plantlet survival rate of 86.5 % of cv. G-137 *in vitro* raised plants [23]. The rooted micro-cuttings registered 80% survival when shifted to a mixture vermiculite (60%), perlite (30%), and cocopeat (10%) v/v [25]. Acclimatization of regenerated plantlets in plastic cups containing autoclaved peat moss resulted into 85 % survival rate [19]. Pots containing sterilized cocopeat-perlite mixture and covered with polythene bags was successfully used for hardening for *in vitro* rooted plantlets, plantlets were kept at 25± 1ºC in artificial light (50µmol m-2s-1) for 3-4 weeks for proper hardening (10). Commercially, primary hardening of *in vitro* raised rooted pomegranate plantlets in India is carried out in nursery trays or net pots and hardened under low tunnel polyhouses with very high relative humidity. Bio-priming is utilization of plant beneficial microbes and/or their formulations and inoculating them in rhizosphere and/or phyllosphere of *in vitro* raised plants or saplings at *in vitro* or *ex vitro* or nursery stage to improve field survival and performance inoculated saplings [26,9]. The bioprimed/biohardened plantlets exhibit higher survival, more root and shoot biomass production, enhanced photosynthesis, and better nutrient uptake [27-33,9].

Conclusion

To date, significant progress has been made in the creation of pomegranate *in vitro* plant regeneration systems that are fast, repeatable, and dependable. To meet out the demands of rapid expansion in pomegranate cultivation a commensuration production of Quality Planting Material (QPM) is compulsory for sustainable production..

References

1. Mahishi DM, Muralikrishna A, Shivashankar G, Kulkarni RS (1991) Shoot tip culture method for rapid clonal propagation of pomegranate (*Punica granatum* L.). In Horticulture-New Technologies and Applications, Springer, Dordrecht, pp. 215-217.
2. Kumar R, Jokhar ML, Verma R, Jat HR (2017) Pomegranate micropropagation - a review. International Journal of Pure and Applied Bioscience 5(5): 1138-1149.
3. Chaugule RR, More TA, Kamble AB, Karale AR (2007) Studies on micropropagation in pomegranate (*Punica granatum* L.). In: recent trends in horticultural biotechnology (Vol I) and II-ICAE National Symposium on Biotechnological Interventions for Improvement of Horticultural Crops: Issues and Strategies, Vellanikkara, Kerala, India, 10-12, pp. 195-199.
4. Murkute AA, Patil S, Singh SK (2004) *In vitro* regeneration in pomegranate cv. Ganesh from mature trees. Indian Journal of Horticulture 61(3): 206-208.
5. Naik SK, Pattnaik S, Chand PK (1999) *In vitro* propagation of pomegranate (*Punica granatum* L. cv. Ganesh) through axillary shoot proliferation from nodal segments of mature tree. Scientia Horticulturae 79(3-4): 175-183.
6. Patil VM, Dhande GA, Thigale DM, Rajput JC (2011) Micro-propagation of pomegranate (*Punica granatum* L.) 'Bhagava' cultivar from nodal explant. African Journal of Biotechnology 10(79): 18130-18136.
7. Singh J, Gupta S, Khoshe P (2014) *In vitro* regeneration of pomegranate (*Punica granatum* L.) from nodal explant. International Journal of Advances in Pharmacy, Biology and Chemistry 3(3): 734-736.
8. Singh NV, Singh SK, Patel VB (2007) *In-vitro* axillary shoot proliferation and clonal propagation of 'G 137'pomegranate (*Punica granatum*). Indian Journal of Agricultural Science 77(8): 505-508.
9. Singh NV, Karimi HR, Babu KD, Sharma J (2021) Propagation techniques and nursery management. In: Zamani Z, Sarkhosh A, et al. (Eds.), The Pomegranate: Botany, Production and Uses. CABI- Wallingford OX10 8DE, United Kingdom, pp.196-224.
10. Kanwar K, Joseph J, Deepika R (2010) Comparison of *in vitro* regeneration pathways in *Punica granatum* L. Plant Cell Tissue Organ Culture 100: 199-207.

11. Guranna P, Hosmani I, Sathyanarayana R, Hegde R, Hipparagi K (2017) Micropropagation in pomegranate (*Punica granatum* L.) cv. Bhagwa through indirect organogenesis and assessment of genetic fidelity by RAPD marker. Biotechnology Journal International 20(3): 1-8.

12. Murkute AA, Patil S (2003) Exudation and browning in tissue culture of pomegranate. Agricultural Science Digest 23(1): 29-31.

13. Raj D, Kanwar K (2010) *In vitro* regeneration of *Punica granatum* L. plants from different juvenile explants. Journal of Fruit and Ornamental Plant Research 18(1): 5-22.

14. Naik SK, Pattnaik S, Chand PK (2000) High frequency axillary shoot proliferation and plant regeneration from cotyledonary nodes of pomegranate (*Punica granatum* L.). Scientia Horticulturae 85(4): 261-270.

15. Kalalbandi BM, Waskar DP, Khandare VS, Gorad DS (2014) Micropropagation studies on pomegranate var. Bhagwa. Indian Journal of Horticulture 71(4): 564-566.

16. da Silva JAT, Rana TS, Narzary D, Verma N, Meshram DT, et al. (2013) Pomegranate biology and biotechnology: A review. Scientia Horticulturae 160: 85-107.

17. Mulaei S, Jafari A, Shirmardi M, Kamali K (2020) Micropropagation of arid zone fruit tree, pomegranate, cvs. 'Malase Yazdi' and 'Shirine Shahvar. International Journal of Fruit Science 20(4): 825-836.

18. Deepika R, Kanwar K (2010) *In vitro* regeneration of *Punica granatum* L. plants from different juvenile explants. Journal of Fruit Ornamental Plant Research 18(1): 5-22.

19. Parmar N, Kanwar K, Thakur AK (2015) High efficiency plant regeneration from cotyledon explants of pomegranate (*Punica granatum* L.) cv. Kandhari Kabuli. Vegetos 28(2): 160-165.

20. Soukhak F, Khalighi A, Ghaemmaghami SA (2012) Study of direct adventitious shoot regeneration in pomegranate (*Punica granatum* cv. Malas Saveh) through cotyledonary explants. International Journal of Agricultural Science and Research 2(3): 19-26.

21. Kaji BV, Ershadi A, Tohidfar M (2013) *In vitro* propagation of pomegranate (*Punica granatum* 1.) Cv.'Males Yazdi'. Albanian Journal of Agricultural Sciences 12(1): 1-5.

22. Naik SK, Chand PK (2011) Tissue culture-mediated biotechnological intervention in pomegranate: a review. Plant Cell Reports 30(5): 707-721.

23. Singh SK, Khawale RN (2006) Plantlet regeneration from the nodal segments of pomegranate (*Punica granatum* L.) cv. Jyoti. Plant Biotechnology and its Applications in Tissue Culture 105-113.

24. Golozan AB, Shekafandeh A (2010) Effects of plant growth regulators on pomegranate (*Punica granatum* L. cv. Rabbab) shoot proliferation and rooting. Advances in Horticultural Science 24(3): 207-211.

25. Bonyanpour A, Khui MK (2013) Callus induction and plant regeneration in *Punica granatum* L. 'Nana' from leaf explants. Journal of Central European Agriculture 14(3): 75-83.

26. Kajla S, Poonia A, Kharb P, Duhan JS (2013) Role of Biotechnology for Commercial Production of Fruit Crops. In: Salar RK, Gahlawat SK, et al. (Eds.), Biotechnology: Prospects and Applications. Springer, New Delhi, India. pp. 27-37.

27. Puthur JT, Prasad KVSK, Sharmila P, Pardhasaradhi P (1998) Vesicular arbuscular mycorrhizal fungi improves establishment of micropropagated Leucaena leucocephala plantlets. Plant Cell Tissue Organ Culture 53: 41-47.

28. Rupnawar BS, Navale AM (2000) Effect of VA-mycorrhizal inoculation on growth of pomegranate layers. Journal of Maharashtra Agricultural University 25(1): 44-46.

29. Singh NV, Singh SK, Singh AK, Meshram DT, Suroshe SS, et al. (2012) Arbuscular Mycorrhizal Fungi (AMF) induced hardening of micropropagated pomegranate (*Punica granatum* L.) plantlets. Scientia Horticulturae 136: 122-127.

30. Bharose AA, Khandagale DJ Damse DN (2014) Regeneration media standardization of pomegranate (*Punica granatum* L.) cv. Bhagava from Shoot Tip, Cotyledonary Node, Hypocotyls and Leaves. Vegetos 27(2): 338-342.

31. Chauhan RD, Kanwar K (2012) Biotechnological advances in pomegranate (*Punica granatum* L.). *In Vitro* Cellular & Developmental Biology-Plant 48(6): 579-594.

32. Gadapa M, Singh G (2022) Recent trends in pomegranate propagation: A review. The Pharma Innovation SP-11(5): 405-414.

33. Singh P, Patel RM (2014) Factors influencing *in vitro* growth and shoot multiplication of pomegranate. The Bioscan 9(3): 1031-1035.