



Performance evaluation of plants raised through different propagation methods in pomegranate (*Punica granatum*)

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ABSTRACT

Disease free and elite planting material propagated through *in vitro* propagation may prevent the spread of diseases particularly bacterial blight through infected planting material. However, there are certain misapprehensions about fruit quality of harvest from micro-propagated plants as compared to air layered or hardwood cutting raised plants. Keeping these facts under consideration, an elaborate study on comparative qualitative and quantitative evaluation of harvest from different types of planting material had been carried out during 2015-17 at ICAR-NRC on Pomegranate, Solapur. The terminal bearing non-significantly ranged from 25 to 28.30% across the different types of planting material. Fruit weight and aril to fruit ratio were found at par in harvest from the three types of planting material. Rind thickness and PLW, which generally play critical role in governing fruit shelf life, were also found at par in fruits from all the three types of planting materials. The rind to fruit ratio of fruits from hardwood cutting raised plants (0.41) was found significantly higher than air layered plants. The maximum 'L*' and 'b*' values of fresh fruits were recorded for fruits from air layered plants (60.76 and 31.65, respectively) and 'a*' value for fruits from TC raised plants (39.70). The results proved parity among fruit quality of harvests from different types of planting material in pomegranate (*Punica granatum* L.) cv. Bhagwa.

Key words: Micro-propagation, Pomegranate, PLW, Planting material

Pomegranate (*Punica granatum* L.) is an important fruit crop for livelihood security of farmers in dry land regions of the world. It's a diploid ($2n=2x=16$) perennial shrub from the family Lythraceae (Nath and Randhawa 1956, Smith 1976). The popularity of pomegranate has grown immensely during recent years due to its health benefits, high returns on investment, less water requirement, therapeutic and functional properties. As a result of all these benefits, India is witnessing a pomegranate revolution with more than 80% expansion in area and 300% increase in pomegranate production during last one decade. India is the world leader in pomegranate acreage and production with 208.73 thousand ha area and 2442.39 thousand tonnes of annual production (NHB 2017). Pomegranate cultivation is expanding at a rapid pace which requires huge availability of elite planting material and based on last 5 years pomegranate expansion rate, the annual planting material demand for pomegranate in India is more than 10 million which is expected to increase in years to come (Singh *et al.* 2017). Commercially, pomegranate is propagated through air layering, cutting and micro-propagation and among these only micro-propagation

ensures rapid production of a large quantity of uniform disease free plants (Sheela and Nair 2001). The utilization of micro-propagated plants should be made mandatory for expansion of pomegranate to non-traditional areas, so as to avoid spread of pathogens like *Xanthomonas axonopodis* pv. *punicae* to new areas through infected planting material as bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae* is a major production constraint in pomegranate (Chand and Kishun 1991, Ravikumar *et al.* 2009, Sharma *et al.* 2012).

There are certain apprehensions about fruit quality of harvest from micropropagated plants of cv. Bhagwa like poor shelf life, lesser rind thickness, higher proportions of terminal bearing, etc. as compared to fruits of same variety raised on air layered or hardwood cutting propagated plants. To investigate this cause for concern among farmers, an elaborate study had been carried out to find out qualitative and quantitative variations, if any, in plants of the same variety propagated through different propagation methods.

MATERIALS AND METHODS

The study was carried out during the period 2015-17 at H4 block planted in the year 2013 located at Hiraj Research Farm, ICAR-National Research Centre on Pomegranate, Solapur, India, at 17°43' N latitude, 75°50' E longitude and

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475 m amsl. The average relative humidity for the entire growing season of both the years ranged between 85.35 to 56.26 and the average maximum and minimum temperature were 33.66 and 19.94°C, respectively.

The planting of pomegranate was done in 0.3 m raised beds of 1.25 m width in newly developed experimental plot having sub-marginal land with slightly heavy textured soil with good drainage. Six months old saplings of cv. Bhagwa raised through air layering (Al), hardwood cutting (HWC) and micro propagation (TC) were planted at 4.5 m × 3.0 m distance. Branches for making hardwood cuttings were separated from mother plants in the month of June when plants were under rest, more than six months old upright growing branches were pruned for making cuttings, individual cuttings of about 20 cm length with 4 nodes and 8-12 mm in diameter were made ready and treated with luke warm solution (about 45°C) of Carbendazim 50% WP @0.1% and 2-bromo-2-nitro 1,3-propane diol @0.05% for 20 min followed by surface sterilization for 10 min in the solution of 2.0% NaOCl and finally treatment of basal potion of the cuttings with 2500 ppm IBA solution before planting them on cocopeat medium. Air layers were tied on mother plants in the month of August during rainy season and well rooted layers separated from mother plants after two months and planted in nursery bag having pre-sterilized mixture till planting in the field. Micropropagation protocol standardized by ICAR-NRCP using nodal segments and at optimum growth regulator concentration had been utilized for propagation of plantlets; the primary hardened plantlets were inoculated with the plant beneficial microbes (AMF and *Aspergillus niger* strain AN27) in their rhizosphere during secondary hardening stage to produce bio-hardened micro-propagated saplings for planting in the field. *Mrig bahar* crop (June-July flowering) was taken in both the years and fruits were harvested during December-January. The crop regulation was done during *mrig bahar* by withholding the water during April-May months. Standard practices of *bahar* treatment, viz. pruning, defoliation, manuring, etc. were done during last week of May to first week of June. Two prunings were done after harvest, first heavy pruning immediately after harvest in January-February followed by application of basal dose and second light pruning at the time of *bahar* regulation during first week of June followed by resuming irrigation. Basal fertilizer and manure application were also carried out at the time of first and second pruning along with the standard fertigation schedule during fruiting season. One shoot pinching was carried out 60 days after defoliation to encourage side shoots. All horticultural practices were kept uniform across the plants raised through different types of planting material. Fresh ripe fruits of pomegranate at commercial stage were harvested from all the directions of the tree canopy. The fruits were kept at 5°C until analyzed.

Plant height and canopy spread (east-west and north-south directions) were recorded during middle of fruiting season. Percent axillary and terminal bearing was recorded in six plants of each type (2 units per replication) by

individually counting them. Fruits from plants raised through different propagation methods were individually analyzed for different physico-chemical characteristics. The fruits were weighed using a high precision electronic balance. The arils and rind were separated manually from the fruits to estimate total arils and rind weight per fruit. Total aril weight was taken and subsequently, 100 arils were counted manually and weighed. Aril to fruit and rind to fruit ratio were estimated by dividing whole aril and rind weight of a fruit with total fruit weight. Fruit number and fruit yield per plant were also recorded.

The titrable acidity (TA) was determined by titration against 0.1 N NaOH solution and expressed in terms of gram citric acid per 100 ml of juice (Ranganna 2001). The total soluble solids (TSS) were determined using a digital refractometer (model SMART-1, ATAGO, Tokyo) and reported as °B at 25°C. Subsequently, Brix/acid ratio was calculated by dividing total soluble solids to titrable acidity (Tehranifar *et al.* 2010).

Physiological loss in weight (PLW) was calculated by taking initial and final fruit weight using precision electronic balance (Babu *et al.* 2015). The experiment was set up at room temperature with 27°C and 35% relative humidity and observations were recorded at harvest (0 DAS) and 5, 10, 20 and 30 days after storage (DAS).

$$PLW (\%) = IW-FW/IW \times 100$$

IW, initial/fresh fruit weight (g); FW, final fruit weight (g).

Fruit appearance was measured using Colour Difference Meter (Hunter Lab) as L*, a*, b* values of the fruit rind at 0, 5, 10, 20 and 30 DAS. The Color parameters were observed using a LabScan XE colorimeter according to Shwartz *et al.* (2009)

- 'L*' scale: Light vs. Dark where a low number (0-50) indicates dark and a high number (51-100) indicates light.
- 'a*' scale: Red vs. Green where a positive number indicates red and a negative number indicates green.
- 'b*' scale: Yellow vs. Blue where a positive number indicates yellow and a negative number indicates blue.

Bioyield point is defined as the force required for rupturing the aril of pomegranate and seed rupture point is the force at which seed breaks. Texture Analyzer (Stable Micro Systems, Model TAXT-Plus) was used to estimate bioyield and seed rupture points. The experiment was conducted following randomized block design with 5 replications and each replication having three units. The mean data of two years was subjected to analysis of variance (ANOVA) and differences among the treatment means were determined for significance at P<0.05 (Gomez and Gomez 1983). The analysis was done using Web Based Agri Stat Package (WASP 2.0) developed by ICAR-CCARI, Goa.

RESULTS AND DISCUSSION

Vegetative growth parameters including plant height (cm), canopy spread (cm) in east-west and north-south direction of different types of planting material did

Table 1 Comparative evaluation of vegetative growth parameters of different types of planting material

Treatment	Plant height (cm)	Canopy spread E-W (cm)	Canopy spread N-S (cm)
AL	231.944	252.00	239.944
HWC	240.278	257.50	244.389
TC	242.639	247.25	237.583
CD (P=0.05)	NS	NS	NS

not show any significant variations (Table 1). Though, micropropagated saplings because of their fast growing and precious nature expected to exhibit better vegetative growth as compared to other types of planting material but due to pruning practice twice a year (one heavy and other light pruning -bringing all types of planting material to almost same canopy level) and pinching during flowering stage resulted in compact canopy of all types of planting material without showing any significant differences in vegetative parameters.

The terminal bearing ranged from 25 to 28.30% across the different types of planting material without any significant difference. Similarly, axillary bearing was also found at par in all the three types of planting material (Table 2). Axillary and terminal bearing in pomegranate can be significantly influenced by pruning practices and the differences observed at farmers' field might be due to difference in pruning practices. ICAR-NRCP recommends two pruning and one cropping, first pruning should be heavy and immediately after harvesting and second pruning should be light and performed after defoliation (Sharma *et al.* 2014). Fruit weight (ranged between 313.45 to 266.75 g), average yield per plant (8.11 to 9.08 kg) and aril to fruit ratio (0.53 to 0.54) were found at par in all the three types of planting material (Table 2). Rind thickness which generally plays a critical role in governing shelf life of fruits in pomegranate also found at par in fruits from all the three types of planting materials (ranged from 3.08 to 3.36 mm). Arils boldness measured as 100 aril weight also didn't differ significantly among fruits from plants raised through different propagation methods. Though, rind to fruit ratio in fruits harvested from hard wood cutting raised plants (0.41) found significantly higher than the fruits on air layered plants (0.38) but this parameter is not going to affect the shelf life of fruits as other parameters governing shelf life were found

at par (Table 2). Brix to acid ratio also known as maturity index is an important index of fruit quality. This ratio is often better related to palatability of fruit than either sugar or acid level alone. It ranged non-significantly among the fruits raised on plants propagated through different propagation methods (33.18 to 35.07). Similarly, aril crunchiness and seed texture measured as bioyield and seed rupture point, respectively were also found at par among different types of planting material (Table 2). Similar values of physico-chemical properties of fruit and its juice were reported in pomegranate cv. Bhagwa by Babu *et al.* (2015, 2017) and Gaikwad *et al.* (2017).

Physiological loss in weight (PLW) upon storage is an important parameter which governs fruit quality and marketability. It indicates the total moisture lost during storage and ripening, which results in desiccation and shriveled appearance (Naik *et al.* 2017). PLW at different storage duration was found at par in fruits harvested from plants raised through different propagation methods, this result is also supported by at par rind thickness in fruits on plants raised through different types of planting material (Fig 1). Similar trend for PLW upon storage in Bhagwa fruits was observed by Babu *et al.* (2015).

The 'L*' value for each scale indicates the level of light or dark, the 'a*' value redness or greenness, and the 'b*' value yellowness or blueness. The maximum 'L*' value of fresh fruits was recorded for fruits on air layered plants (60.76) and the maximum 'a*' value for fruits on TC raised plants (39.70) and the maximum 'b*' value was found again for fruits on air layered plants (31.65), however, at 30 days after storage the 'L*' and 'b*' values of fruits harvested from HWC raised plants was found higher than other types of planting material (46.35 and 21.37, respectively) (Fig 2). The 'L*', 'a*', 'b*' values in most of the cases didn't

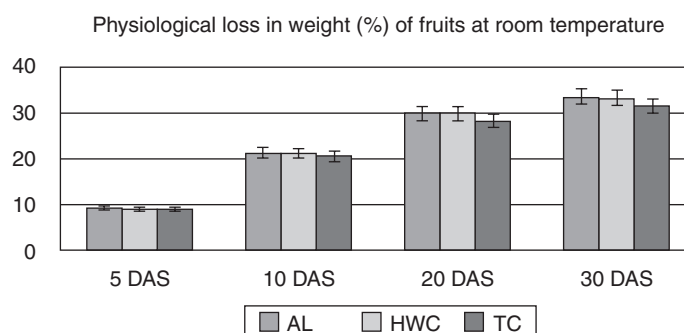


Fig 1 PLW (%) of fruits at room temperature.

Table 2 Comparative evaluation of fruits of cv. Bhagwa harvested from plants raised through different propagation methods

Treatment	Terminal bearing	Axillary bearing	Avg. fruit wt. (g)	Rind wt. (g)	Rind/Fruit ratio	Rind thickness (mm)	Yield/plant (kg)	Aril/Fruit ratio	(Brix/Acid ratio)	100 Aril weight (g)	Bioyield point (N)	Seed rupture point (N)
AL	28.30	61.69	313.45	118.85	0.38	3.08	8.11	0.53	33.18	34.10	6.38	35.72
HWC	25.00	65.00	285.25	118.35	0.41	3.36	9.12	0.53	34.84	37.30	6.06	35.94
TC	27.47	62.53	266.75	107.10	0.40	3.27	9.08	0.54	35.07	35.05	6.83	33.63
CD (P=0.05)	NS	NS	NS	NS	0.02	NS	NS	NS	NS	NS	NS	NS

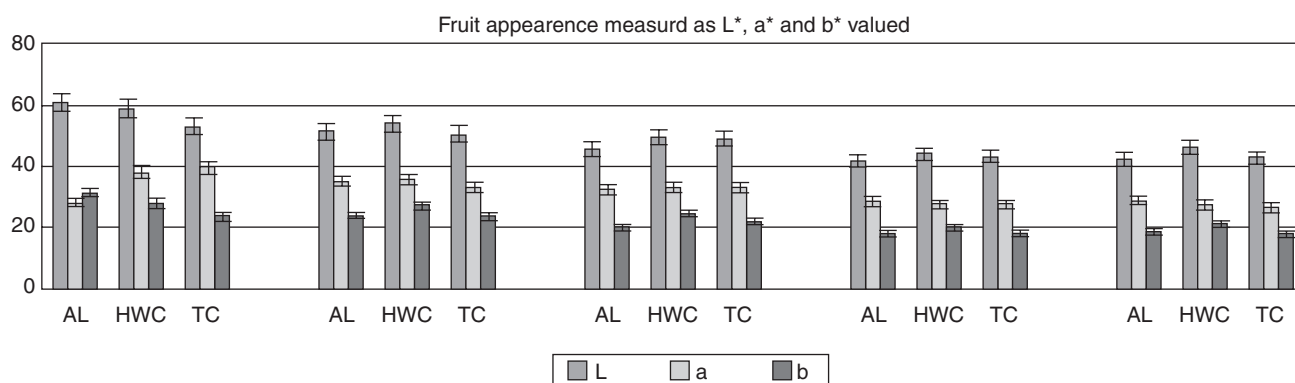


Fig 2 L*, a*, b* values of fruits at 0, 5, 10, 20 and 30 days after storage

vary significantly amongst the different types of planting material. Increased shriveling and reduction in appearance upon storage of fruits was also found by Babu *et al.* (2015).

The present article lays to rest to all the misapprehensions and concludes that quality differences among fruits raised on various types of planting material (HWC, air layering and micro propagation) of the same variety may be due to variable horticultural practices until unless there is occurrence of somaclonal variations in tissue culture raised plants. Identification of somaclonal variants and molecular marker based clonal fidelity testing offer scope for further investigation.

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REFERENCES

- Babu K D, Singh N V, Chandra R, Sharma J, Maity A and Pal R K. 2015. Improvement in keeping quality of pomegranate fruits during storage (*Punica granatum*). *Research on Crops* **16**(2): 281–7.
- Babu K D, Singh N V, Gaikwad N N, Maity A, Suryavanshi S and Pal R K. 2017. Determination of maturity indices for harvesting of pomegranate (*Punica granatum*). *Indian Journal of Agricultural Sciences* **87**(9): 1225–30.
- Chand R and Kishun R. 1993. Systemic movement of *Xanthomonas campestris* pv. *punicae* (Hingorani and Singh) dye from leaf to node in pomegranate. *International Journal Tropical Plant Disease* **11**: 85–90.
- Gaikwad N N, Pal R K, Suryavanshi S, Babu K D, Maity A and Sarkar S. 2017. Effect of extraction method and thermal processing on retention of bioactive compounds of pomegranate (*Punica granatum*) (cv. Bhagwa) juice. *Indian Journal of Agricultural Sciences* **87**(11): 1445–52.
- Gomez K A and Gomez A A. 1983. *Statistical Procedure for Agricultural Research*, 2nd edn. John Wiley and Sons Inc., New York.
- Naik R, Prasad M D and Joshi V. 2017. Shelf life and quality of minimally processed pomegranate arils cv. Bhagwa as influenced by antioxidants. *International Journal of Pure and Applied Bioscience* **5**(4): 1022–7.
- Nath N and Randhawa G S. 1959. Studies on cytology of pomegranate (*Punica granatum* L.). *Indian Journal of Horticulture* **16**: 210–5.
- NHB. 2017. Horticultural Statistics at a Glance 2017. Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Government of India, New Delhi (www.agricoop.nic.in).
- Ranganna S. 2001. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*, 2nd edition. Tata McGraw–Hill, New Delhi.
- Ravikumar M R, Shamarao J, Yenjereapp S T and Patil H B. 2009. Epidemiology and management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*. *Acta Horticulturae* **818**: 291–6.
- Sharma J, Chandra R, Babu K D, Meshram DT, Maity A, Singh N V and Gaikwad N N. 2014. *Pomegranate: Cultivation, Marketing and Utilization*. Technical Bulletin No. NRCP/2014/1. ICAR–National Research Centre on Pomegranate, Solapur, Maharashtra.
- Sharma J, Sharma K K and Jadhav V T. 2012. Diseases of pomegranate. *Diseases of Fruit Crops*, pp 181–224. Misra A K, Chowdappa P, Sharma P and Khetrapal R K. (Eds). Indian Phytopathological Society, New Delhi. India.
- Sheela V L and Nair S R. 2001. Growth, flowering and yield potential of tissue culture banana (Musa AAB cv. Nendran). *Journal of Tropical Agriculture* **39**: 1–4.
- Shwartz E, Glazer I, Bar–Ya'akov I, Matityahu I, Bar–Ilan I, Holland D and Amir R. 2009. Changes in chemical constituents during the maturation and ripening of two commercially important pomegranate accessions. *Food Chemistry* **115**: 965–73.
- Singh N V, Babu K D, Maity A, Sharma J and Pal R K. 2017. Elite planting material in pomegranate : a key to productive orchard. (In) *Souvenir, 2nd National Seminar–Cum–Farmers' Fair on Pomegranate for health, growth and prosperity*, ICAR–NRCP and Society of Advancement of Research on Pomegranate (SARP), Solapur, Maharashtra, April 28–30, pp 84–93.
- Smith P M. 1976. Minor Crop. *Evolution of Crop Plants*, pp 301–24. Simmonds NW (Ed). Longman, London.
- Tehraniyar A, Zarei M, Nemati Z, Esfandiari B and Vazifeshenas M R. 2010. Investigation of physico–chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. *Scientia Horticulturae* **126**: 180–85.