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Micropropagation of Minor Fruit Crops of India: A Review

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ABSTRACT

Micro-propagation refers to the technique of *in vitro* multiplication of plants in a short duration using any plant parts (nodes, leaves, flowers, seeds *etc*.) under aseptic conditions. It is advantageous over vegetative propagation and its application in horticultural aspects. It is well-proven method of surplus production of elite identical plants in a controlled environment which are uniform, stable, disease-free, true-to-type and are independent of any seasonal restrictions or limitations. India is homeland to diverse of significant but minor fruit crops such as Indian gooseberry, (*Emblica officinalis* Gaertn.), Karonda (*Carissa carandas* L.), Bael (*Aegle marmelos* Corr.), Jamun (*Syzygium cuminii* L.), Jackfruit (*Artocarpus hetrophylous* L.) which bear high nutritional, medicinal, therapeutic values and of great commercial importance (medicinal, food and cosmetics). Due to a paucity of desirable planting materials, the commercial production process for these crops is restricted. Micropropagation has the potential to significantly increase the number of new cultivars or genotypes of such fruit crops. The objective of this review study is to compile existing research work done on the micropropagation of these underutilized fruit crops.

Key words: Bael, *In vitro,* Indian gooseberry, Jackfruit, Jamun, Karonda, Micro-propagation, Minor fruit.

India with second largest producer of fruits in world is also considered as homeland to a wide range of potentially valuable yet underutilised fruit crops. which bear high nutritional, medicinal and therapeutic values and can be grown even on neglected marginal conditions. Underutilized fruit crops include- aonla, bael, karonda, jamun, jackfruit *etc*. These crops can be cultivated successfully with minimal inputs under distressing extreme temperature conditions. Minor fruits contain a range of vital nutrients, vitamins, and minerals, as well as bioactive compounds that have been attributed to antioxidant effects against various free radicals. Besides these advantages these minor fruit crops are not extensively cultivated and also geographically as well as quantitatively their consumption and commercial trade are limited as compare to major fruits. Underutilized fruits are essential part of traditional foods that is among rural as well as tribal communities and have requisite potential to ensure food security and also to cope-up with poverty. 'Biodiversity International' recommended that underutilized fruits can be used as alternative sources to fight against hidden hunger. Due to a scarcity of suitable planting material, commercial production of these crops is limited. Micropropagation has the potential to significantly increase the number of important cultivars of these fruit crops.

Breeding and selection experiments are extremely difficult method due to the long-life cycle. Numerous biotic and abiotic stress factors substantially reduce fruit tree productivity. Conventional methods are insufficient to overcome these issues, necessitating the urgent integration of plant biotechnology approaches for fruit tree improvement. Micro-propagation refers to an artificial technique of producing genetically identical or clonal plantlets *in vitro* under aseptic condition with defined nutrient medium using tissue culture methods. This technique is independent of seasonal constraints. It is an important component of nursery, propagation as well as orchard industries as it provides uniform, stable, disease-free, true-to-type, elite propagules and qualitative plantlets with rapid multiplication. Plant tissue culture technology has been commercially applied for microbe-free plants (Parmessur *et al.,* 2002).

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Applications of micropropagation in fruit crops are as follows:

a) *Ex situ* conservation of minor fruits can be done using *in vitro* culture.
b) Using micropropagation, a small amount of plant tissues is enough to produce millions of clones of fruit crops annually. Producing an equal number of plants using traditional methods would take a long time.
c) Disease free plants can be generated using tissue culture particularly 'meristem tip culture'.
d) Yield attributes can be increased.
e) Qualitative attributes can be improved using tissue culture.

f) Management of large number of plants in limited laboratory area.
g) Ensuring favourable growing condition in limited laboratory area.
h) In vitro stock proliferation (rapid replication of a cell, component, or organism) can be performed any time in a year.
i) It is possible to interchange plant material quickly across international borders without the risk of disease transmission through 'Introduction' process. This strategy reduces the amount of time required for quarantine.
j) Economy of time and seed rate.

➢Indian gooseberry (*Emblica officinalis* Gaertn.)

Aonla is an important indigenous hardy minor fruit with commercial importance originated in tropical south-eastern Asia, particularly in Central and Southern India and also grown at foot hills of Himalayas as well as in Peninsular India. Various active tannins composition principles (Emlicannin A, Emblicannin B, Puniglucon and Pedunculagin) have been identified in addition to strong antioxidants, which contribute for numerous health benefits. (Rastogi, 1993; Rao *et al.,* 1985). Fruits of aonla are richest source of vitamin C (600 mg/100 g) after Barbados cherry and source of valuable minerals, riboflavin, calcium, Iron, phosphorus, nicotinic acid, tryptophane, lysine and methionine, and lysine as well as bearing herbicidal properties due to presence of high anti-ascorbic value (Anonymous, 1960). Aonla is becoming more popular in India due to its hardiness, adaptability for cultivation in a variety of agroclimatic conditions, and its wide use in the pharmaceutical, nutraceuticals, cosmetics as well as post-harvest processing industries. The greatest constraint to the establishment of Indian gooseberry orchards is the lack of uniform planting material. The rate of success of vegetative propagation varies between 25 and 80 per cent depending on the genotype (Ram, 1982). Aonla can be grown through seed materials as well or through 'Inarching' (grafting). However, because of cross-pollination, seed does not produce a true-to-type plant. Conventional method of vegetative propagation of aonla is at slow rate and also season dependent. Furthermore, such plants take longer time to bear fruit. Also due to erect branching habit a large number of branches are not available close to ground surface ground level for approach grafting purpose. Another constraint in field multiplication and field survival is unavailability of the desired number of shoots for vegetative propagation such as grafting or budding (Mishra and Srivastava, 1999). However, standardized micropropagation technology can be used for mass replication of true to type plantlets. The two most critical parameters that influence the performance of any micropropagation system are *in vitro* bud induction and shoot proliferation.

Mishra and Pathak (2001) conducted an experiment-*in vitro* shoot proliferation in aonla (*Emblica officinalis* Gaertn.) cv. Narendra aonla-7 by selecting axillary shoots having one node as explant. Before preparing the explant for *in vitro*, the shoots were clipped to 1-2 cm length and determinate shoots attached with nodes were removed, leaving 0.5 cm from the base. The cut end of the explant was sealed with melted paraffin wax to prevent oxidative browning and contamination *in vitro* and this technique has been termed as 'explant waxing'. This method has been reported to have an 80 per cent success rate in establishing cultures. MS medium (Murashige and Skoog, 1962) which was supplemented with 0.8% agar, 3% sucrose, 0.4 mg/l kinetin and 1.0 mg/l GA3 were used for inoculation of shoot. The pH of media was maintained at 5.7 and cultures were kept at 25±2°C temperature, 50-55% RH and 2000 lux of florescent tube light illumination with 16/8 hour of light and dark cycling. The observation of the experiment reveals that aonla shoots collected between August-November had better culture establishment, bud induction, and microshoot growth than shoots collected between April-July. Moderately hard (10-15 nodes) and slightly green shoot of aonla and exhibited highest *in vitro* bud induction and proliferated much more than the soft (1-10 nodes) or extremely hard, brown (20-30) nodal segments. Treatment of the explant with Bavistin (1.0%) and Chloramphenicol (0.1%) for 240-260 minutes, followed by treatment with $HgCl_2$ for 8 minutes under aseptic conditions, was used to control *in vitro* contamination (Mishra, 1997). A determinate stem with leaves arises early after *in vitro* bud induction, bearing solely flowers. Indeterminate shoots, on the other hand, are required for future multiplication. Therefore, the role of GA3 in the elongation of indeterminate shoots is significant. Under the presence of 4.33 µM GA3 + 13.9 µM Kinetin + 342.11 µM Glutamine high proliferation of shoot (13.33 shoots/culture) were obtained (Mishra *et al.,* 2006). Several other reports on aonla micropropagation involving a callus phase and seedling explant are present. However, this mechanism cannot be employed for true-to-type plant multiplication, but it can be useful for investigation of genetic transformation. *E. officinalis* callus cultures were used to produce high frequency plantlet regeneration (Verma and Kant, 1999).

➢Karonda (*Carissa carandas* L.)

Karonda (*Carissa carandas* L.) is an indigenous hardy, evergreen, sprawling semi vine spiny shrub of apocynaceae family. It thrives well throughout the tropical and subtropical climates. It is susceptible to heavy rainfall and waterlogged conditions. It produces berry-sized fruits which are sour and astringent in taste making it unsuitable for fresh consumption and therefore can be processed into various excellent quality products such as nakal cherry, jam, jelly, jam, syrup, squash, sauce (ripe fruits) and chutneys and pickles (unripe fruits) which are of high demand in the national as well as international market. The fruits are rich in vitamin C (1.6-17.9 mg/100 g), protein (1.1-2.25%) as well as abundant minerals such as iron (39.1 mg/100 g), calcium (21 mg/100 g) and phosphorus (38 mg/100 g) (Anonymous, 1950; Anonymous., 1979; Kumar and Singh, 1993) and also dried fruits are one of the richest source of iron content (39.1 mg/100 g). The roots are used to treat intestinal worms, scabies,

and itch and as an anthelmintic, stomachic, and antiscorbutic substance (Warrier *et al*., 1993). Karonda plant's wood is white, firm and smooth and can be used for making combs and spoons. The plants can be trained to grow into a strong hedge and can be used as live fencing around fruit orchards. Wide application of karonda required large number of plants per unit area. In order to increase the area under cultivation of this vital underutilised crop, an alternate approach for quick and large-scale propagation is urgently needed. Rai and Misra (2005) reported that during different seasons, shoot tips from mature *Carissa carandas* cv. Pant Sudarshan plants were cultured on Murashige and Skoog's (MS) basal media supplemented with benzyl adenine (BA) and indole butyric acid (IBA). The 1.5 cm long explants taken in the spring season (February-March) had the highest sprouting rate, followed by those obtained in the summer season (April-June). On MS basal media supplemented with 3.0 mg l1 BA, shoot proliferation was highest. The optimum micro-shoot rooting was found in 1/2 MS with 0.8 mg l1 IBA and 0.2 mg l1 naphthalene acetic acid (NAA). The rooted seedlings were successfully acclimatised in a potting mixture of vermiculite, sand and soil (1:1:1). Dey *et al*. (2017) also conducted an experiment on various cuttings of Carissa carandas which were treated with different concentrations of sucrose (2%, 4% and 6%) as well as with IBA (7500 ppm, 8000 ppm and 8500 ppm) and observed that in a short period of time, different levels of sucrose and IBA had a significant impact on the success, survival, and rooting of Karonda (*Carissa carandas* L.) cuttings. The optimum treatments for commercial vegetative multiplication of Karonda by stem cuttings were recommended is application of IBA @ 8000 ppm followed by 4% sucrose.

➢ Bael (*Aegle marmelos* Corr.)

Bael (*Aegle marmelos* Corr.) is deciduousa and ancient medicinal indigenous fruit tree of India belonging to family rutaceae with chromosomes number (2n=36). Ripe fruit of bael is rich in minerals, vitamins and fibres as well as act as tonic, restorative, laxative and good for heart and brain. Pulp of fruit are rich in 'psoralen' and 'marmelosin'. Hard shell, muciliganeous texture and numerous seeds of bael fruit make it difficult to eat out of hand and therefore, can be processed into diversified value-added products such as bael candy, preserve, sherbet and powder. Bael is traditionally propagated via seed, which is sown in June and matures into seedlings after 1 year and are not true to type. Also, bael seeds have short viability and highly prone to insect attack. Root sucker propagation is slow and cumbersome (Anonymous, 2003). Excessive exploitation and indiscriminate collection of wild populations of bael species has maximized the pressure on wild sources, resulting in the extinction of this plant, which is now listed as vulnerable in a few Indian states (Ravikumar and Ved, 2000). *In vitro* propagation of bael has been described by employing 'axillary bud multiplication' (Arumugam and Rao, 1996; Hossain *et al*., 1994a; Ajithkumar and Seeni, 1998) and by leaf explants (Islam *et al.,* 1993) and also by nucellar callus technique (Hossain *et al.,* 1994 b).

➢ Jamun (*Syzygium cuminii* L.)

Jamun is an important hardy medicinal fruit crop of Indo-Malayan region which can be easily grown in neglected and marshy areas. In folk medicine and the pharmaceutical industry, the jamun has garnered significantly more attention than in any other discipline. Fruits contain a wide range of anti-oxidant chemicals, including polyphenolic compounds, flavonoids, carotenoids and vitamins, all of which are essential for human health by reducing oxidative stress and inhibiting macromolecular oxidation (Kubola *et al.,* 2011). It contains both nutritive as well as therapeutical value. It is a rich source of iron and is used to treat diabetes, heart disease and liver disease. The seed powder of jamun fruit quickly reduces the amount of sugar in urine.

Micropropagation of Jamun using juvenile tissue (Roy *et al*., 1996), using seedling explants (Yadav *et al.,* 1990 and Jain and Babber, 2000) has been developed to produce large-scale true-to-type planting material. Jamun (*Syzygium cuminii* L.) can be propagated *in vitro* by employing single nodal explants from seedlings. Shoot proliferation was better in single nodal explants cultured on half strength MS with 2 mg/l BAP + 3 per cent sucrose + 3 per cent activated charcoal. On 1/4 strength MS with 3 per cent sucrose and 2.5 mg/l IBA, the shoot lets were successfully rooted. After six weeks of hardening on vermiculite (32.5 percent survival), the rooted shoot lets were planted in polybags and transported to the field (Chaudhary *et al*., 2013).

➢Jackfruit (*Artocarpus hetrophylous* L.)

Jackfruit (*Artocarpus hetrophylous* L.) belongs to family moraceae with chromosome number 2n=56 of Indian origin. It is also called as poor man's food and regarded as staple food in African countries such as Uganda. Ripe fruits are utilised as table fruits, whereas immature fruits are used as vegetables. Rind and perigones are high in pectin, making them ideal for making jelly. Mature but unripe fruits can also be used to make chips and papad. Jackfruit seeds are a good source of starch and are used in a variety of cuisines. Due to high heterozygosis, propagation of jackfruit is not widely accepted. Tissue culture technique could be utilised for jackfruit multiplication to retain true to type quality fruit throughout the year. Additionally, jackfruit cultivation is typically confined due to a scarcity of superior cultivars and desirable planting materials. A tissue culture approach using apical bud cultures (Amin and Jaiswal, 1993), plants Shoot tip or nodal explant (Roy *et al.,* 1990; Azam and Rahmatullah, 2009; Miro and Acedo, 2015) has been devised for rapid vegetative multiplication of jackfruit. For the multiple shoot development of jackfruit, healthy (disease free) and juvenile shoot tips were employed as explant and cultured in Murashige-Skoog (MS) media supplemented with varied concentrations of (0 mg/L, 1 mg/L, 2 mg/L, 3 mg/L, and 4 mg/L) of plant growth regulators *i.e*., BAP (6-benzylea minopurine). When MS media was supplemented with

2 mg/L BAP, shoot regeneration was significantly improved. The percentage of shoot proliferation increased as the subculture was increased (up to the tenth maximum). These shoots were subsequently cultured on half strength MS medium supplemented with 0 mg/L, 1 mg/L, 2 mg/L, 3 mg/L and 4 mg/L IBA (Indole-3-butyric acid), with the number of roots/explants, root length and early root induction being maximum in the 2 mg/L IBA containing medium (Ashrafuzzaman *et al.,* 2012).

CONCLUSION

Considering all the above things provided we can easily adopt given methodologies in nursery and orchard industry to produce annually millions of clones of minor fruit crops which are true to type, stable, elite, uniform and disease-free.

Conflict of interest: None.

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