Clonal Propagation in Papaya

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Papaya (*Carica papaya L.*), a member of the family Caricaceae, is a commercially significant fruit crop widely cultivated in tropical regions. Introduced to India in the 16th century by Portuguese explorers, papaya gained substantial economic importance in the country during 20th century. Papaya is highly regarded as a nutraceutical fruit due to its rich content of essential nutrients, including vitamin A, β-carotene, vitamin C, folate, lycopene, dietary minerals, and fibre, which contribute to the prevention of diabetes, cancer, and cardiovascular diseases (Retuta *et al*., 2012). Beyond its fruit, papaya is also a valuable source of the proteolytic enzyme papain, widely utilized across various industries, including meat processing, chewing gum production, wool pre-shrinking, natural silk degumming, and in the pharmaceutical, cosmetic, brewing, and leather sectors (Kabir *et al*., 2016).

Papaya is a dicotyledonous, polygamous and diploid species and it exhibits three primary sex forms: male, female, and bisexual (hermaphrodite). The species is broadly classified into two groups *viz*., dioecious and gynodioecious based on the prevalence of sex forms. Dioecious cultivars produce both male and female plants, while gynodioecious cultivars produce female and bisexual plants. While gynodioecious varieties are advantageous as all plants are capable of fruit production, their productivity can be hindered by poor fruit set in bisexual plants, particularly under high-temperature. Additionally, these varieties are highly susceptible to Papaya Ring Spot Virus (PRSV) (Thirugnanavel *et al*., 2015). In contrast, dioecious varieties show greater tolerance to PRSV but suffer from a 1:1 segregation of male and female plants when propagated by seed.

Seed propagation

Papaya is traditionally propagated through seeds and the sex of the seedlings cannot be determined through morphological characteristics until flowering. Sex determination in papaya seedlings is not feasible through morphological markers, necessitating the maintenance of excess seedlings per planting pit until flowering for sex identification and subsequent thinning. Although molecular markers for early sex determination have been identified (Leela *et al*., 2018), their large-scale application remains commercially unviable. Therefore, the practice of sowing 5-6 seeds per nursery bag and maintaining 4-6 plants per pit in the main field until flowering is recommended to maximize the chances of achieving an optimal female plant population in dioecious papaya cultivars. This practice increases the overall cost of cultivation due to the need for additional labour and resources for thinning. In addition, papaya cultivation through seed propagation faces challenges due to its inherent heterozygosity, the production of non-true-to-type plants, and susceptibility to papaya ring spot virus.

Clonal propagation

Clonal propagation or vegetative propagation from selected clones has become an attractive alternative in papaya cultivation. In the recent years, asexual propagation utilizing various *in vitro* and *ex vitro* techniques such as cuttings, grafting, and tissue culture, has been increasingly employed in papaya cultivation. Vegetative propagation methods like cuttings and grafting are well-established in other fruit trees, with the primary aim of preserving the desirable traits of the mother plant while also promoting earlier fruit production. These techniques offer a reliable means of maintaining genetic uniformity and enhancing cultivation efficiency.

(i) Cuttings

The initial step in propagation by cuttings involves selection of mother plants and determination of appropriate size of the cuttings. In papaya, shoot production in mother plants varies depending on the cultivar and occurs irregularly throughout the year and hence, necessary pruning is needed to ensure a sufficient supply of cuttings. Cuttings between 12.0 and 30 cm in length and approximately 2.5 cm in diameter are found for optimal root development in papaya (Oliveira, 2018). In addition, treating the cuttings with Indole-3-butyric acid (IBA) at the base resulted in successful rooting of 75% to 95% of the cuttings (Allan and Carlson 2007). Papaya plants propagated from cuttings produced fruit earlier than those grown from seeds.

Grafting

Approach grafting or inarching of promising papaya hybrids onto *Carica cauliflora,* a wild species resistant to Papaya Ring Spot Virus (PRSV-P) was found to delay viral symptom expression in papaya plants. Terminal wedge grafting showed superior success rate (100%) compared to chip budding (85%) and side grafting (75%), with strong subsequent field performance. A grafted Tainung No. 2 and No. 5 papaya plants were shorter than ungrafted counterparts, showed no incompatibility between scion and rootstock, and produced higher yields. The performance of grafted papaya plants under field conditions demonstrates significant advantages over seedling-propagated plants, particularly in terms of precocity, vigour, and yield efficiency. Grafted papayas exhibit early flowering at a reduced height, and studies in Brazil have shown that these grafted plants consistently outperform seedlings in terms of productivity (Allan, 2009). The application of plant growth regulators *viz*., BA and GA_3, combined with techniques such as decapitation and selective leaf removal, can effectively overcome apical dominance in papaya and stimulate the production of graftable side shoots. Grafted plants exhibited dwarfism, early flowering at lower heights, and earlier harvests compared to seedling plants, though both showed no differences in fruit quality.

Micropropagation

In papaya, micropropagation has become a prominent focus in recent research efforts, aiming to enhance propagation efficiency and genetic uniformity. The efficiency of in *vitro* propagation techniques is influenced by several factors, including plant species, genotype, explant source, environmental conditions, culture medium composition, and the hormonal balance of growth regulators. Auxins and cytokinins, particularly Naphthalene Acetic Acid (NAA) and Benzylaminopurine (BAP), are commonly utilized in the induction and regeneration of adventitious buds in papaya (Setargie *et al*., 2015). The papaya variety 'Rajshahi Red' exhibited the highest shoot proliferation when cultured on MS medium containing 0.5 mgL^{-1} BAP and 0.1 mgL^{-1} NAA. Higher concentrations of auxins and cytokinins were found to suppress shoot proliferation. In papaya cv. Maradol, the greatest shoot length and highest number of leaves were achieved on MS medium supplemented with 0.5 mgL^{-1} NAA and 1 mgL^{-1} BAP. Increasing TDZ concentrations led to a reduction in callusing, while BAP at 2 mgL^{-1} induced callus formation in immature embryo explants after TDZ exposure.

Somatic embryogenesis represents a promising alternative for clonal plant development, as it allows for the initiation of numerous embryos from somatic or zygotic cells under *in vitro* conditions. The bipolar nature of somatic embryos facilitates the formation of complete plants with shoot and root systems, effectively bypassing the *in vitro* rooting stage in micropropagation and reducing associated costs. In papaya, somatic embryos have been successfully derived from immature zygotic embryos, leaf tissues, and young hypocotyl tissues. Furthermore, somatic embryos provide a viable platform for genetic transformation, enabling the incorporation of PRSV resistance traits. *S*omatic embryos can be generated by incubating immature zygotic embryos in half-strength Murashige and Skoog (MS) medium with full-strength vitamins, supplemented with 2,4-D, L-glutamine, myo-inositol, adenine sulphate, gelrite, and sucrose (Chong *et al*., 2007). These embryos on transfer to a maturation medium containing phloroglucinol resulted in well matured embryos. Shoot and root regeneration were achieved in MS medium + 3% sucrose + 0.8% agar and ½ MS medium + 2 mg L^{-1} indole-3-butyric acid respectively. *In vitro* plantlets were then acclimatized in peat moss soil.

Conclusion

Vegetative propagation of papaya faces several challenges, limiting its widespread commercial use. One significant hurdle is the low multiplication rate, which restricts the production of large quantities of uniform planting material. Additionally, propagation through methods like cuttings and grafting carries the risk of transmitting sap-transmissible diseases, particularly Papaya Ring Spot Virus (PRSV), which can devastate crops and lead to significant economic losses. Micropropagation is also challenging due to poor responses during subculturing. Furthermore, repeated subculturing can lead to a decline in the viability of shoot cultures, making it difficult to maintain healthy, proliferating material. Hence, these factors are to be addressed through future research for promoting commercial

application of vegetative propagation in papaya cultivation.

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