

Elite female tissue culture papaya 'Dawn Delight'

Papaya is generally propagated using seeds where the seedling population could segregate into female, male, and hermaphrodite plants depending on the genetic background of the parental line. Tissue culture allows the selective rapid multiplication of desired plants and preferred sex forms. An elite papaya line 'Dawn Delight' has been identified and is now clonally multiplied through tissue culture. 'Dawn Delight' consists of only female plants with the parthenocarpic fruit setting which avoids the need for the pollinizer male or hermaphrodite plants. In the absence of pollination, fruits become seedless displaying very slow ripening with extended keeping quality of 38 days. Fruits are ovoid-oblong, medium size, weighing about 1.0 kg±250 g. 'Dawn Delight' offers a uniform field stand of dwarf female plants with continuous bearing and higher yields. Seedless fruits with extended keeping quality and firm red flesh add brand value and export potential for this line. 'Dawn Delight' forms an ideal candidate for terrace cultivation due to its dwarf stature. Although micropropagation of papaya has been reported in literature decades ago, commercial multiplication through tissue culture is demonstrated for the first time in this country.

PAPAYA (*Carica papaya* L.; Family Caricaceae) forms an important fruit crop in India. It is also valued as a vegetable, medicinal and industrial crop, often grown as a homestead or pure crop. India is the world leader in papaya production with about 1.2 lakh hectares area constituting about 40% of global production. Major growing states include Gujarat, Telangana, Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Madhya Pradesh, Bihar, and West Bengal. Papaya is a rich source of vitamin A, vitamin C, riboflavin, carbohydrates, calcium and other minerals. Traditional cultdivars were of yellow flesh with caricaxanthin, which are now being replaced by red flesh varieties rich in lycopene. Ripe fruits are used mainly for table purpose and for processed products such as jam, juice, etc.

Botanically papaya is a polygamous species. In nature, it used to be predominantly dioecious with separate male and female plants. The crop shows varied sex forms, of which dioecious and gynodioecious (female and hermaphrodite plants in the population) form the common types. Dioecious varieties (example: Pusa Delicious, Pusa Nanha, Co-1) warrants planting 3-4 seeds per hill followed by culling of excess male plants retaining about 80-90% female plants. This demands high labour, seeds and other investments. Newer varieties are gynodioecious (eg: Red Lady, Arka Prabhat) having hermaphrodite and female trees often displaying considerable difference in plant stature, fruit size and shape between the two sex forms. In general, consumers prefer smaller size fruits of around

1 kg or less to suit the nuclear families.

Tissue Culture (TC) forms a very powerful tool for the rapid clonal propagation of horticultural crops. This demands a combination of superior genotype and a strong micropropagation technology. In this respect, an elite female papaya selection 'Dawn Delight' has been identified, and a commercial micropropagation scheme has been worked out with the supply of TC plants to the growers.

Development of 'Dawn Delight' papaya line

'Dawn Delight' ('DD') papaya has become a reality from the dedicated efforts at Thomas Biotech to develop a superior indigenous papaya line with medium size red-flesh fruits possessing good quality attributes. 'DD' was selected from the F2 segregating population of F1 papaya 'Red Lady', which is a widely grown gynodioecious hybrid involving a combination of female and hermaphrodite plants. This selection is exploited with the concept that gynodioecious sex type in papaya forms a more stable form than hermaphrodite type, and the parthenocarpic fruit setting potential bypasses the need for pollen-source plants leading to seedless fruits. The methodology followed for the identification and selection of this line has been described in the publication: 'Dawn Delight': An Elite Micropropagated Female Papaya Line with Parthenocarpic Fruit Setting and Extended Shelf Life (HortScience; <https://doi.org/10.21273/HORTSCI16218-21>). This line is being maintained *in vitro*, multiplied through commercial

TC technology and made available to the Indian growers.

TC technology

A robust micropropagation protocol has been developed for 'DD' papaya which is covered by the Indian patent application at Chennai, India (No. 202041037562: "Tissue culture-based method of propagation of *Carica papaya* plant"). This involves culture initiation from seedling shoot-tips or field axillary buds, multiple shoot induction in a proliferation medium, and rooting induction in a rhizogenesis medium followed by hardening and plant establishment *ex vitro*.



In vitro/ micropropagation steps: Culture establishment in culture tubes (A), multiplication in proliferation medium (B), and rooting induction in rhizogenesis medium (C).

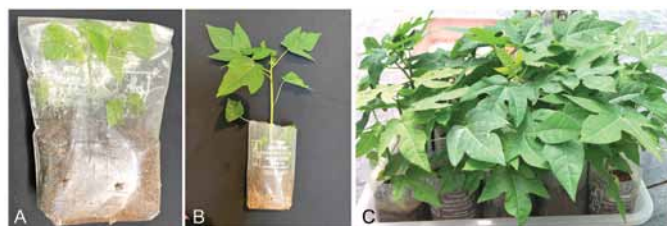
Unlike seed-derived plants which takes just about 6-7 weeks from sowing to field planting, TC plants take extra time (5-6 months) from *in vitro* rooting induction phase (3-4 weeks) to the final field planting stage, which includes primary hardening (8-15 weeks) and secondary hardening (6-8 weeks) which together with the technological and infrastructure requirements make it relatively more expensive. *In vitro* rooted or rooting-induced plantlets are first established in net pots or in small polybags as part of primary hardening. Growth is generally very slow in the early stages and it takes about 2-4 months for root development and primary hardening depending on the vigour or health status of *in vitro* plantlets generally giving 40-60% success. For secondary hardening, primary hardened plants with 4-5 small leaves are employed, and this step is generally accomplished with 390% success. It is proposed to outsource this step of secondary hardening with nursery men or even farmers to avoid the distant



Primary hardening phase for TC papaya: Plants established in net-pots in portrays with protection from desiccation (A), view of primary hardened plants in net pots (B, C).

transportation of delicate plants, and hence this aspect is described in more detail below. Further, this saves the space and freight, and facilitates the supply of TC papaya plants at reduced costs.

For secondary hardening, polybags of 6" height and 4" width are generally employed. These bags are filled to half the height with the planting mix (red soil, sand and vermi-compost or peat moss, 1:1:1). After drenching the planting mix with the fungicide, mix Carbendazim 12% + Mancozeb 63% (SAAF) @ 2 g/l, primary hardened plants from net-pots are scooped out carefully and planted in the centre of the bag followed by drenching with a fungicide solution. After a mist-spray of water, the top of polybags is folded and closed with a stapler pin at one side. These closed bags or 'sachets' are incubated under shaded condition away from direct sunlight and rainfall, or indoors with supplemental light. Later on, one side of the polybag is opened to allow the new growth to come out. Once the plant produces 2-3 new leaves outside the bag, the top part is opened fully. The *in vitro* formed leaves and those emerge inside the closed bags are very delicate. The open bags should be kept covered with polythene sheet or net-cloth to protect from dehydration. The plants could be maintained in the same bags with additional nutritional supply till field planting. It is essential to ensure insect free conditions to protect the plants from viral infection during the hardening phase. It is advised to keep the plants in a well lit area to avoid lanky tall growth.

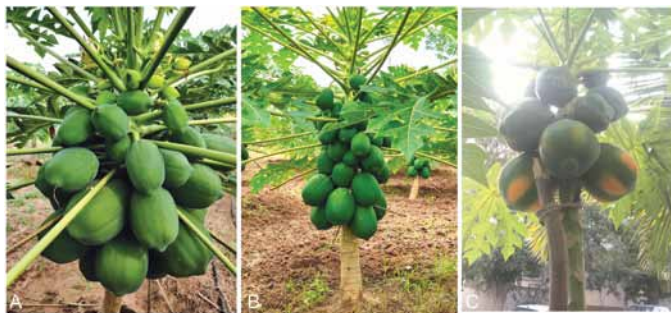


Secondary hardening (A) and plants ready for field planting/ supply to growers (B, C)

Plants of 8-10 inches (20-25 cm) tall are generally supplied to the farmers. Leaves formed under *in vitro* conditions and those developed during the initial stages of hardening might not survive the harsh outside conditions. It is natural for such leaves to turn yellow and drop off when the plants are shifted out from the shaded-polyhouse conditions. It is advised to keep the plants in the open for about 10-14 days to acclimatize to the local growing conditions before field planting. Papaya plants are very delicate, and transporting the hardened plants to the distant locations needs great care and attention for packaging.

Description of field plants and fruits

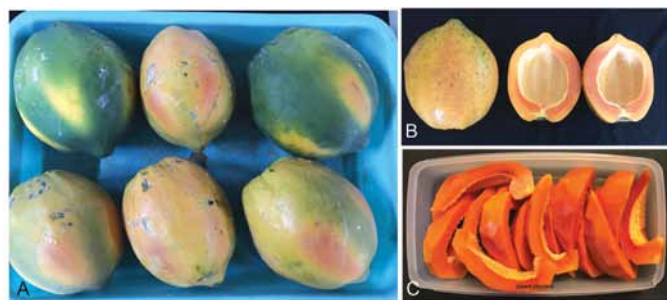
TC field plants appear relatively uniform with close-set nodes and dwarf stature. Flowering commences by 3 months after planting, and covers the whole population by 3-4 months. Fruit setting normally occurs within 30-60 cm from ground level. 'DD' plants generally display one fruit per node, but occasionally branched inflorescence with multiple flowers are observed. Fruits undergo gradual enlargement for 3 months and thereafter enter maturation



Bearing plants of 'TC-DD' in the field (A, B) and a pot-grown plant (C)

phase. Flowering and fruiting appear almost continuous. Generally 30-60 fruits were observed at a time in different growth stages, and occasionally, >100 fruits in well nourished conditions.

'DD' yields medium size fruits of one type (ovoid-oblong). The parthenocarpic fruit setting potential avoids the need for pollination. 'DD' plants are fully fertile and seed set may occur if any pollen source is there nearby. Such seeded fruits often grow larger with thicker pulp and faster ripening compared to seedless fruits. To get the full advantage of TC female line, DD is recommended as a pure crop. By 4 months after flowering, fruits show ripening with 3-4 orange streaks. Ideal stage of harvest is indicated by 4-6 streaks. Fruits generally weigh 0.750-1.0 kg. Occasionally larger and smaller fruits are also observed. In some plants, the initial fruits appeared bigger weighing 32 kg. Fruit size settles down to the preferred medium or small size thereafter. At maturity, fruits are about 12-15 cm long and 10-12 cm wide with 12-15 mm thick flesh and 12-14°B TSS.



Fruits of TC papaya 'Dawn Delight' in ripening phase (A), ripe fruit with seedless flesh (B), and sliced fruits with firm red flesh (C)

Fruits take about 7-10 days for complete external yellow-orange colour development after harvesting. Ripe fruits show orange-red flesh with a moderate central cavity. Occasionally, fruits display accelerated ripening in one side which corresponds to the stem-facing side of fruits. One added benefit of seedlessness in 'DD' is slow ripening contributing to extended shelf life retaining firm and crisp flesh. Half-fruits wrapped in cling-film or polythene could be kept at ambient temperature overnight or refrigerated without reduction in quality.

Cultivation practices

TC-DD plants of about 8-10 cm height with 10-12 nodes

and 4-6 healthy terminal green leaves are suitable for field planting. TC-DD is recommended for open field cultivation with full sunlight. Under low-light/shaded conditions, plants grow taller and lanky with long internodes and fewer fruits.

Papaya is highly sensitive to water logging. It is important to ensure good drainage. In high rainfall areas, raised beds of 10-12 cm height and 60-75 cm width, or raised mounds may be adopted to minimize the collar/foot-rot damages. There are three major planting seasons, namely monsoon (June-Aug/Sept), autumn (October-November), and spring (February-March). Planting could be done round the year if proper care and protection are given depending on local weather conditions.

A spacing of 2 × 2 m with about 800 plants per acre (2000 plants/ha) is generally recommended. Higher density cultivation with spacing of 1.8 × 1.8 m enhances the gross returns, but yield per plant is better with wider spacing without shade effects. For land preparation, dig-up or plough well and level the soil. Prepare pits of 45 × 45 × 45 cm, or trenches 45 cm wide and 45 cm deep with a JCB. Ensure irrigation facilities (drip or conventional) before planting. Apply 10 kg well decomposed farm yard manure (FYM), or compost and 50 g superphosphate per pit. Cover the pit/trench with top soil, mix well and apply 250 g neem cake, 250 g bone meal and 1 kg vermicompost/cocopeat at the planting spot. Use of biocontrol agents (*Trichoderma* and *Pseudomonas*) at the planting spot is advocated. Water the plants adequately one day before transplanting and do the planting keeping the shoot base at the ground level. Planting too deep could induce foot-rot disease. Provide shade with green twigs/coconut/areca leaves or 50% green shade nets, which may be removed after one week. Soil drenching with about 200 ml of SAAF 2 g/l is recommended to avoid soil-borne fungi/foot-rot. Water the plants as required.

A combination of organic and inorganic fertilizers is advised as TC-DD plants generally show continuous fruiting. Apply 200 g each of N, P₂O₅ and K₂O per plant per year for high yields. Alternatively, use about 10 g 20:20:20 NPK mixture per plant one month after planting, and about 25 g per plant after 3, 6, 8, and 10 months of planting. After six months, apply additionally about 1-2 kg vermin-compost. After 12 months, open a 15-20 cm trench about 15-20 cm away from base and apply 2-3 kg compost/FYM and 500 g neem cake plus 50 g NPK per plant. Thereafter, repeat the fertilizer application at 25-50 g NPK per plant after every 2-3 months. Ensure irrigation after fertilizer application.

'TC-DD' crop shows an economic life span of 18-24 months beyond which fruit size shows some reduction, and the harvesting becomes difficult due to tree height. The yield per plant is generally estimated at 50-100 fruits, and with good management practices 100-200 fruits (100 kg/plant).

Management of pests and diseases

Mealy bugs and mites are encountered as pests which can normally be managed with neem oil application (3 ml/l). Mealy bug infection appears as deformation of terminal leaves with cottony white insects underneath the leaves.

Physical removal of insects with cotton buds dipped in neem oil, or the application of Sefina[®] (Afidopyrogen 50G/L DC) @1.5-2.0 ml/l or Agas[®] (Diafenthiuron 50% WP) underneath the leaves with a repeat spray after 7-10 days could offer good control of this pest. Mites are controlled effectively with wettable sulfur spray (2 g/l) underneath the leaves.

Major fungal diseases include foot-rot caused by *Phytophthora*, *Pythium*, or *Rhizoctonia* spp. Ensure good soil drainage to avoid the collar-rot. Soil drenching with SAAF[®] or Ridomyl[®] at 2 g/l could check the pathogen entry from soil. Leaf and fruit spot incited by *Asperisporium caricae* is a problem affecting the marketability. It is controlled with the spraying of *Bacillus subtilis* + *Pseudomonas fluorescens* each at 2 g/l water, or Roko[®] (Thiophanate Methyl 70% WP at 1 g/l water). If any fungicide is employed, ensure sufficient waiting period before fruit harvest/consumption.

Currently, the major factor limiting papaya cultivation in India forms, viral diseases, namely, Papaya Ring Spot Virus (PRSV) and Papaya Leaf Curl Virus. Of these, PRSV is highly devastating affecting yields, fruit quality and marketability. TC-DD displays tolerance to PRSV, but in extreme disease prone areas, early stage infection could impart high crop losses. Several management practices and products are prescribed for PRSV control in papaya; the details are not described here. To ensure a good crop, protected cultivation of papaya is now recommended for which TC-DD forms a good candidate on account of the dwarf stature and the parthenocarpic fruit setting potential.

Uses

'DD' papaya forms an ideal choice for table purpose

fresh fruits with extended shelf life. Papaya is a common breakfast fruit where the cut fruits are normally picked with fork or tooth-picks for which DD offers the good flesh texture for proper holding. With the firm seedless flesh, 'DD' fruits are ideal for canning and dehydration besides products like juice and jam.

Tropical fruits are generally not suitable for refrigeration. Conversely, the seedless fruits of 'DD' could be refrigerated well (wrapped with paper or in refrigeration bags) for a several days unlike the seeded fruits of 'Red Lady' which displayed faster flesh softening. Even the cut fruit pieces could be refrigerated for 1-2 days in a closed container without significant deterioration in quality. The seedless nature of 'DD' contributes to small-medium fruit size, slow ripening, and extended keeping quality, and with undisturbed seedless flesh, it forms a superior papaya line with high marketing and export potential.

'Dawn Delight' forms an ideal candidate for terrace cultivation with its dwarf stature and the non-dependence on pollen sources for fruit setting. Papaya performance is always better when planted in field soil. However, the use of bigger grow-bags ($\geq 16 \times 16$ ') or 50 litre plastic drums could offer a good crop. This also makes good use of terrace space towards organic or chemical free cultivation.

For further interaction, please write to:

Dr Pious Thomas, Thomas Biotech & Cytobacts Centre for Biosciences (TBCCB), Amruthahalli, Bengaluru 560 092, Karnataka. *Corresponding author e-mail: pioust@gmail.com

Varieties of vegetable crops for release and notification

Dolichos bean (Bush): VRBSEM 3-Days to first picking: 80–85 days, Peak fruiting period: December to March. DYMV disease free in cropping period with temperature tolerance up to 35°C. Yield: 360 q/ha in five pickings. Identified for cultivation in Zone VI (Rajasthan, Gujarat, Haryana and Delhi), Zone VII (Madhya Pradesh, Maharashtra and Goa) and Zone VIII (Karnataka, Tamil Nadu, Kerala and Puducherry).



Indian (Dolichos) bean/Sem: Arka Neelachal Pushti, is high yielding, pole type, round podded variety. The pods are rich in protein (4.61 g/100 g) and micronutrients (iron: 15 ppm, zinc 37 ppm). Duration of crop is 95 days from sowing to green harvest. Rabi season is ideal for its cultivation in Eastern India. Average yield is 24 tonnes/ha in 120 days.

French bean (Bush): VRFBB 91 is bush-type, early, short duration, flowers at 32–35 days after sowing. Pods are green and bright, fleshy, tender, straight, cylindrical and free from parchment. Bears about 20–25 pods/plant. Resistant to sclerotinia rot (*Sclerotinia sclerotiorum*). Tender pod yield potential is 125–150 q/ha. Recommended for Zone I: Humid Western Himalayan Region (Jammu and Kashmir, Himachal Pradesh and Uttarakhand).



Source: ICAR Annual Report 2021-22