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Accelerated In Vitro Breeding Creates Improved Designer Papaya

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Summary

Papaya (*Carica papaya* L) has very limited genetic variability that hampers conventional breeding. Therefore, we developed, novel, high yielding, more flavoursome designer Skybury papaya lines with high Brix through accelerated in vitro breeding. Skybury farm changed to clonal papaya cultivation, which allowed us to achieve continuous improvement through somaclonal selection from the 300,000 plus papaya grown annually. We developed a high throughput somatic embryogenesis system for Skybury papaya. Induced genetic variability among the embryogenic cell lines, regenerated variant papaya through non-GM method, conducted large scale field trials and recovered unique variants with better agronomic and fruit qualities. We also generated several mutant lines free of dreaded Papaya Sticky Diseases (PSD) caused by the Papaya Meleira Virus (PMeV). We developed a rapid and reliable molecular diagnostic test to screen for PSD and verified the PSD free status of several of the mutant lines using this kit. We field trialled PSD free lines on a large scale to select PSD free lines with improved characteristics in a brief 5 year period compared to other breeding options. Our results confirm that accelerated in vitro breeding approach is the best way for rapid

papaya crop improvement in the non-GM agriculture system of Australia. Gene editing technology like CRISPR technology can be handy for further rapid crop improvement (disease resistant e. g. phytophthora resistant) papaya in future.

INTRODUCTION

Skybury farms located in the Tropical Queensland (17° 00' 21.456" S and 145° 20' 18.24" E) is a major, diversified farm producing Papaya, Coffee, Avocados, Lemon, Lime, Oranges, Banana and Passion fruit. The unique Skybury Red Papaya is a market leader with over 50% of the Australian market share. Skybury strives for excellence in all areas of innovative farming, value addition like the award-winning Papaya Vodka and coffee liquors, and marketing tropical fruits. Skybury engages in applied research using conventional breeding as well as biotechnology tools to achieve accelerated crop improvement.

Skybury Red Papaya is a unique hybrid line with high yield and sweet, red flesh, a pleasant aroma and nice texture. It's a vigorous papaya with fast growth and strong thick stem. A potential drawback is the characteristic fruit skin which is not shiny as in other papaya varieties with low sugar levels and strong papaya aroma. Being a hybrid that segregates drastically into all sorts of papaya – ranging from red to-pink to yellow flesh, only cloned Skybury plants can make a stable plantation. Papaya crop improvement is mainly achieved through a conventional breeding approach in Australia (Nandawan et al. 2016), India (Mitra and Dinesh, 2016), Malaysia (Sekeli et al. 2018), Brazil (Pereira et al. 2019) and Mexico (Beans, 2020). However, low genetic variability among members of the genus *Carica* is hampering rapid development of improved papaya hybrids (Da Silva et al. 2007). This is the cited reason for the lack of new improved papaya varieties delivered by the large Australian papaya crop improvement program that has received millions of $ from the Papaya levy fund. On top of this delay in crop improvement, new disease (Papaya Sticky Disease- PSD) became a significant problem for papaya production in recent years. Therefore, Skybury decided to take a more pragmatic approach than conventional breeding to produce a designer papaya that is free of PSD and with better productivity and fruit characteristics through in vitro breeding.

In vitro breeding refers to the crop improvement achieved using in vitro methods. Plants cells are totipotent, meaning a cell is capable of developing into all kinds of tissues and a whole new plant eventually. There exists some variability among the somatic cells (1/ 1,000,000 or so). Therefore, it is possible to capitalise on this variation within the cell population of a plant species to achieve crop improvement through in vitro breeding, provided, high frequency regeneration in vitro can be achieved from cell cultures of the species. Callus based regeneration through organogenesis or preferably through somatic embryogenesis can aid in vitro breeding. Somatic embryogenesis is preferred over organogenesis because of the single cell origin of somatic embryos which reduces potential formation of chimera as in the case of regeneration through organogenesis from callus.

Callus, Cell culture

Callus culture of papaya was initiated from one year old leaf explants of quality assured, superior papaya trees. Friable, rapidly proliferating callus (**Fig. 1**) developed on semisolid MS medium supplemented with Kinetin (1-2 mg/l), IBA and NAA (0.1- 0.5 mg/l). Rapidly proliferating, white, friable callus made an excellent cell suspension culture when transferred to liquid MS medium with similar hormone combinations.

Figure 1 Proliferating friable callus.

Cell cultures were maintained on a shaker (125 rpm) under dark conditions at 25°C. Very fine cell suspension with little clumps and mostly isolated cells or aggregates of 2-3 cells after three to four subcultures at monthly interval. Papaya cell cultures showed a short, 2-day lag phase followed by lag phase of four weeks, then plateaued to the stationary phase (**Fig. 2**).

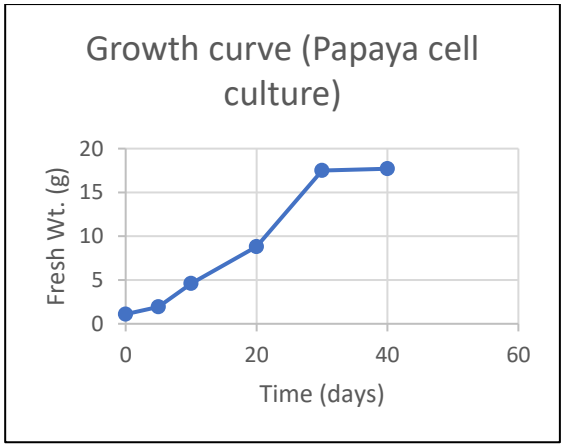

Figure 2. Growth curve (Papaya cell culture).

Cell plating and Somatic embryogenesis

Plated at 10^4 cells per 10 cm Petri dish produced microcolonies in 3-4 weeks when maintained at 25°C in the dark. Microcolonies developed a decent callus mass that became embryogenic upon prolonged incubation (8-12 weeks) in ¼ MS medium + full MS vitamin, fortified with 500 mg/l Glutamine, 60 g/l sucrose and 1-2 mg/L 2,4-D (**Fig. 3**).

Figure 3. Embryogenic callus.

Somatic embryos germinated to produce complete plants under light (2000 lux) on MS medium supplemented with 20 g/l sucrose and no hormones (**Fig. 4**).

Figure 4. Somatic embryo germination.

Field trials

Key to the success of an invitro breeding program is the large-scale field trial to pick up the useful variants coming out of the in vitro system. Somatic embryo derived plants were micropropagated (30-50 plantlets) and used in the replicated, multiplot field trials. Undesirable variants such as albinos and other visual phenotypes with distorted leaves and spindly stem were rouged out at the in vitro stage or in the early nursery stage. However, many other variants like non-flowering, non-fruiting, stunted types couldn't be identified until the plants are grown to the bearing stage. Similarly, some variants with potential (e. g. hyper vigorous, with excellent fruiting) may turn out to be not ideal if the fruit qualities like shelf life, flavour, flesh colour, texture and brix are not favourable. In our experience, sucking pests like mites and caterpillars loved and appreciated some potential variants with little sap, a good characteristic from picking and packing perspective.

A large population of cloned papayas (300,000 plus) maintained at Skybury farm allowed us to make use of somaclonal variation (culture induced variations) for rapid papaya crop improvement. Although, somaclonal variants are of rare occurrence, we could locate 1-2 superior variants annually from the 300,000+ clonal papaya. Somaclonal selection has aided Skybury to achieve continuous crop improvement over the years.

Induced variations for rapid crop improvement

Gamma rays induced genetic variation is a very useful non-GM tool for rapid crop improvement (Andrew-Peter-Leon et al. 2021). Callus cultures were subjected to gamma radiation to induce variations. Plants were regenerated from gamma treated cell mass through somatic embryogenesis. These variants were tested for Papaya Sticky Disease (PSD) using the molecular diagnostic kit developed specifically to identify PMeV that causes PSD in papaya. Interestingly, gamma radiation at

70 Gy eliminated most of the PMeV and doses Gy 80 and above completely eliminated PMeV (Fig. 5). Some of the superior gamma treated variants selected through the field trial also demonstrated excellent fruit qualities like better shelf life, red flesh colour, texture, flavour and Brix ≥ 17. A great advantage of having an excellent cloning facility at Skybury is that all the improved selections can be rapidly cloned to develop an improved plantation within a year.

Conclusion

- In vitro breeding approach is not hampered by the low genetic variability among *Carica papaya* that hampers conventional breeding.
- In vitro breeding technology can reduce the time needed to generate improved papaya varieties from classic 9-12 years to under 5 years.
- In vitro breeding can be further accelerated by combining with mutagenesis.

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