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Production of Transgenic Hybrid Papaya Seed in Hawaii

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Summary

The University of Hawaii released its first transgenic papaya cultivars on May 1, 1998. The original inbred transgenic line, red-fleshed 'SunUp', was derived by transformation of 'Sunset' with a papaya ringspot virus (PRSV) protein coat gene construct (Fitch et al., 1992) which confers resistance to PRSV virus infection. A second cultivar, F_1 hybrid 'UH Rainbow', was produced by crossing 'SunUp' with 'Kapoho Solo'. 'Kapoho Solo' is a yellow-fleshed variety which is the preferred type for export. 'UH Rainbow' is yellow fleshed and carries one copy of the transgene; it is PRSV resistant after the seedling stage. Hawaii Agriculture Research Center has produced the first commercial hybrid papaya seed in the United States, under contract with the Papaya Administrative Committee. The 'UH Rainbow' plantings in May 1998, are also the first commercial plantings of transgenic papaya. Commercial production from the first plantings is expected in May 1999.

Introduction

The papaya industry in Hawaii has been severely impacted by the spread of PRSV, an aphid-borne virus which causes ringspotting, leaf and fruit distortion, yellowing and eventual tree death. The primary growing area in the Puna district of the Island of Hawaii saw a 50 percent reduction in yield in the five years following the first occurrence of PRSV in 1992. In Puna, the yellow-fleshed 'Kapoho Solo' is the preferred cultivar for export because of its firmness and relatively long shelf life. 'Kapoho Solo' is highly susceptible to PRSV, and there are no papaya genotypes known to be naturally immune to PRSV.

The University of Hawaii (UH) collaborated with Cornell University and the Upjohn Company (Fitch et al., 1992) to transform papaya with the gene for the PRSV protein coat. 'Sunset' papaya was successfully transformed with the PRSV protein coat gene and was found to be immune to PRSV as a result. A homozygous transgenic line was developed and named 'SunUp' papaya. 'Kapoho Solo' was also transformed but plants were susceptible to PRSV. To fill the urgent need for a Kapoho-type papaya cultivar in Puna, a hybrid was made by crossing 'SunUp' and 'Kapoho Solo'. The F_1 hybrid 'UH Rainbow' has yellow flesh and is intended for the same markets as Kapoho. 'SunUp' and 'UH Rainbow' papaya varieties were released to the Papaya Administrative Committee (PAC) by the UH on May 1, 1998. Bulk quantities of seed of 'UH Rainbow' were required to establish orchards in the virus-impacted growing areas. HARC, in an arrangement with PAC and the UH, was contracted to produce the hybrid transgenic seed.

Materials and Methods

Production plan. The five acre seed production field was located at the Kauai Agricultural Research Center of the University of Hawaii, located in the Wailua region of Kauai. The site is about 800 ft elevation and receives about 100 inches of rainfall a year, distributed throughout the year. No irrigation was necessary. The soil pH tested around 5.3 before liming. The prior

crop history was quite varied, with small experimental plots, grass and forested areas.

The papaya seed production was originally scheduled to be done on the Island of Oahu, at a site fairly isolated from sources of PRSV infection. However, PRSV was found in the vicinity just before the project was due to begin. The College of Tropical Agriculture and Human Resources cooperated with HARC in relocating the site to the Kauai Agricultural Research Center.

The seed yield assumptions used for production were 150 seeds per fruit, with one fruit harvested per week per tree, and 3,500 trees total on five acres. The total yield target for three years was 27 million seeds, or about 850 lb of dry seed. Due to a shortage of breeder seed, it was necessary to use both sexes of both parent varieties, raising the risk of accidental sib pollination.

Nursery and field planting procedures. Plants were started in December through April in a greenhouse with screened sides. Planting was staggered to accommodate the fact that Kapoho starts flowering two months later than 'SunUp'. Seeds were germinated in pots of vermiculite and then transplanted to 3-inch square pots in Sunshine Mix #4. Some of the seeds were also direct-sown in Speedling trays. Seedlings were fertilized weekly by drenching with a solution of 1-T all-purpose Miracle-Gro plant food (15-15-15 N, P and K, plus minor elements) per gallon of water. The plants in the nursery were drenched with Ridomil before transplanting to the field to prevent *Phytophthora* and *Pythium* root rots.

The land was cleared by UH staff, disked and rotovated to a smooth seed bed. Before final tillage, 2 tons/acre of agricultural lime were applied to four acres, and 1 ton of dolomite was applied to the fifth acre. Plastic woven weed mat (36 inches wide) was installed as the rows were bedded up.

Holes were cut in the weed mat and the seedlings were transplanted into the holes, setting them slightly deeper than they grew in the nursery. Seedlings were watered by hand at planting time, and sprayed prophylactically twice with Ridomil fungicide at 10-14 day intervals to prevent root rot. About 2 oz of 16-16-16 granular fertilizer was scattered around each new transplant.

Field cultural practices. Plants were fertilized monthly with 16-16-16 until flower buds became visible, increasing the dosage gradually to about 4 oz per tree in the final application. Thereafter, trees were fertilized bimonthly with 1-1.5 lb of 10-20-20 granular fertilizer per tree. Rates were adjusted according to rainfall and season, the lower rate being used in the cooler months and during dry periods.

To prevent aerial fungal diseases, a regular program of Dithane spray was applied beginning in the ninth month. Initial interval was monthly, but an outbreak of *Phoma* made it necessary to spray biweekly. The biweekly spray was continued throughout the production period.

Leafhoppers became evident after about eight months of growth. Malathion was used with the Dithane spray to control the leafhoppers, successfully for about four months. Gradually, the leafhoppers became resistant to Malathion, and to Pyrellin. An experimental use permit was obtained to spray Provado (imidacloprid) insecticide. The Provado was extremely effective and allowed the trees to recover completely, although a significant number of 'SunUp' trees were lost before the leafhoppers could be brought under control. The leafhopper population remained very low for several months after the Provado spray.

Pollination procedures. Pollination was done by hand, using female buds that were mature but not yet open. The pollen was from mature, unopened buds from the hermaphrodite trees. Where possible, purely staminate flowers were taken for pollen, because the anthers are more accessible in the absence of a stigma. The entire staminate bud was harvested, then carefully opened at the time of use. By peeling away the individual petals, the staminate flower can be used as a brush to apply pollen to the

stigma. The staminate buds were sometimes stored in a refrigerator in a plastic bag until needed, or up to a week after harvest. Storage for at least a day is preferable, because the anthers dehisce more completely for greater ease of pollination after storage. To avoid mistakes and cross-contamination, only one direction of the cross was made in a given work session. Immediately after pollination, female flowers were covered with paper cones made by shortening paper drinking cups, and a string tag was placed around the peduncle to mark the flower. The cones remained on the flower through the receptive period, and later shed as the fruit swells.

Harvest and seed handling. Fruit was harvested by hand when quarter-to-half ripe and held in closed wooden bins for about five days to fully ripen and soften for easier seed removal. The fruits were then cut in half and the seeds were scooped out into 5-gallon plastic pails with trash bag liners. The seeds, with sarcotestae still attached, were then shipped by air freight to the HARC Experiment Station on Oahu.

Final seed cleaning was done using a Robot Coupe commercial food processor with a dull blade to avoid cutting the seeds. The food processor cuts the sarcotestae from the seeds without damaging the seed. The debris was removed from the seeds by straining in a soil sifting screen and by floating off the macerated sarcotestae in a pail of water. The seed was allowed to drain in a mesh bag, then spread on spun-bonded floating row cover which was spread over screen elevated about 1 inch above lab benches in an air-conditioned room. Oscillating fans were used to hasten the drying. After several days on the benches, the seed was bagged in woven plastic bags for shipment.

A sample of about 1,000 seeds was reserved from each seed lot. The seeds were tested for germination by pre-treating with a 30-minute soak in 1 M $KN0_3$ solution, then planted immediately in community pots of clean vermiculite. The pots were kept in a greenhouse at ambient temperatures for germination. Seedlings were counted and removed as they germinated. Pots were held for five weeks to accommodate slow sprouting seeds, but the great majority germinated within three weeks.

Mean germination rates were 76.6% and 67.8% for seed from ‘Kapoho Solo’ and ‘SunUp’ female trees, respectively. Individual seed lots ranged from 52-97% germination with Kapoho as female parent, and 55-94% with ‘SunUp’ as female parent. Germination rates were highest in the first months of harvest. The lower germinating samples were produced at the peak of the stress period from leafhopper.

Seed purity, i.e., occurrence of accidental parental varieties, is an important issue for papaya handlers, because the parents are of different fruit flesh color and shelf life. The occurrence of any pink flesh fruits (‘SunUp’) in an order for yellow flesh fruit can cause the order to be canceled. Also, ‘SunUp’ has a much shorter shelf life than Kapoho, so must be handled more carefully. The occurrence of an occasional Kapoho plant in a ‘UH Rainbow’ field is not as serious, because ‘UH Rainbow’ and Kapoho fruit are similar in appearance. Kapoho, however, is susceptible to PRSV.

Purity of seed from Kapoho females was tested by excising 96 randomly selected embryos and assaying for GUS activity (Jefferson, 1987). Embryos that turn blue are Rainbow hybrid because they carry the GUS indicator gene from the ‘SunUp’ pollen parent. All of the seed lots tested (first 20 lots at the time of this report) were >98% pure based on the GUS indicator. This test cannot be used on the Rainbow seed from ‘SunUp’ females because the female parent donates the GUS gene to all of the seeds, hybrid or not.

The dried seed was shipped by FedEx to the PAC office in Hilo. The PAC handled packaging and distribution of the seed to commercial growers.

Results

The original yield estimates used turned out to be much too low. Actual seed count per fruit

was typically from 800-1,200 seeds, and there were several flowers per tree per week. Consequently, the estimated production was accomplished in two years instead of three. The trees had grown too tall for easy pollination by the time hand pollination was stopped in the 18th month. The total yield for the project was 958 lb, or 12.7% over the minimum yield target. The high seed set demonstrates the value of hand pollination; females flowers that were left unpollinated frequently failed to set fruit, in spite of being surrounded by hermaphrodite trees. The rainy environment of the production site may have been a contributing factor to the low natural fruit set.

It is apparent that future seed production plots can be greatly scaled down in acreage and duration and still achieve the same yield goal. It is estimated that a one acre plot, intensively managed, can produce about 600 lb of dried seed in a 28-30 month crop cycle.

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