8. Levy, Y. and J. Krikun. 1979. Effect of vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations. New Phytol. 85:25–31.
9. Levy, Y., J.P. Syvertsen, and S. Nemec. 1983. Effect of drought stress and vesicular-arbuscular mycorrhiza on citrus transpiration and hydraulic conductivity of roots. New Phytol. 93:61–66.
10. Maronek, D.M., J.W. Hendrix, and J. Keirnan. 1982. Mycorrhizal fungi and their importance in horticultural crop production. Hort. Rev. 3:172–213.
11. Mosse, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. Annu. Rev. Phytopath. 11:171–196.
12. Nelson, C.E. and G.R. Safir. 1982. Increased drought tolerance of mycorrhizal onions due to improved phosphorous nutrition. Planta 154:407–413.
13. Newman, E.I. 1966. A method of estimating the total length of a root in a sample. J. Appl. Ecol. 3:139–145.
14. Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55:158–160.
15. Reid, C.P.P. 1979. Mycorrhiza and water stress. The soil-root interface. Academic Press, New York.
16. Sands, R. and C. Theodorou. 1978. Water uptake by mycorrhizal roots of radiata pine seedlings. Austral. J. Plant Physiol. 5:301–309.
17. Safir, G.R., J.A. Boyer, and J.Q. Gerdeman. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. Plant Physiol. 49:700–703.
18. Strong, M.E. and F.T. Davies, Jr. 1982. Influence of selected vesicular-arbuscular mycorrhizal fungi on seedling growth and phosphorus uptake of *Sophora secundiflora*. HortScience 17(4):620–621.
19. Wall, L.L. and C.W. Gehrke. 1975. An automated total protein nitrogen method. J. Assn. Anal. Chem. 58:1221–1226.

J. Amer. Soc. Hort. Sci. 109(2):213–218. 1984.

Biochemical Markers for *Carica papaya*, *C. cauliflora*, and Plants from Somatic Embryos of Their Hybrid

G.A. Moore[1] and R.E. Litz[2]

Fruit Crops Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611

Additional index words. isozyme, papaya

Abstract. **Isozyme markers for glutamate oxaloacetate transaminase (GOT), superoxide dismutase (SOD), peroxidase (PER), and malate dehydrogenase (MDH) were identified for *Carica papaya* L. and the related but sexually incompatible *C. cauliflora* Jacq. These markers were used to determine the nature of somatic embryos derived from papaya ovules cultured on modified Murashige and Skoog (MS) medium 65 days after controlled pollination with *C. cauliflora*. Zymograms of plantlets from somatic embryos contained bands specific to either *C. papaya* or *C. cauliflora* (PER, GOT) and a unique band not present in the zymogram of either species (PER). Zymograms of somatic embryo-derived plantlets were distinctively different from those of either of the *Carica* species for all the enzyme systems examined. Evidence from isozyme markers indicates that somatic embryos produced from cultured papaya ovules following pollination with *C. cauliflora* may be hybrids. The isozyme banding patterns of 60 plantlets derived from somatic embryos from the same ovule were very uniform and suggest genetic uniformity among the regenerated plantlets.**

The papaya, *Carica papaya*, is one of the most widely grown fruits in the tropics. In recent years, a serious disease caused by papaya ringspot virus (PRV) has affected adversely papaya production in many parts of the world. Efforts to identify sources of resistance to PRV and to transfer this resistance to papaya have been reported (2, 8). Conover and Litz (3) found that PRV resistance in some papaya accessions from South America was conferred by a complex of genes. Monogenic resistance to PRV, which is conferred by a dominant gene, has been identified in 3 other *Carica* species, *C. stipulata* Badillo, *C. cauliflora*, and *C. pubescens* Lenne & Loch (8, 9). All of the PRV-resistant species are sexually incompatible with papaya.

Khuspe et al. (11) reported the successful *in vitro* culture of immature zygotic embryos from crosses between *C. papaya* and *C. cauliflora*. Litz and Conover (13) observed that polyembryony occurred in cultured papaya ovules derived from the same interspecific cross. The embryogenic response was highly dependent upon the maternal genotype and could not be induced in some papaya types (15). The somatic embryos could be induced to germinate and regenerated plants have been established in the greenhouse (14). These plants closely resembled the maternal papaya parent. However, because *in vitro* polyembryony occurred only rarely, histological studies were ineffective in determining the anatomical origin of the somatic embryos. If the regenerated plants are interspecific hybrids, they could be used to transfer PRV resistance from *C. cauliflora* to papaya.

Biochemical markers such as isozymes have been used widely to identify both sexual and somatic hybrids (1, 5, 6, 23, 24). Isozyme bands have been used also to characterize the amount or kind of variability present in plant populations produced through

Received for publication 12 Mar. 1983. Florida Agricultural Experiment Stations Journal Series No. 4573. This work was supported in part with funds provided by USDA Cooperative Agreement No. 58-7B30-9-116 and by the Rockefeller Foundation. The authors wish to thank Vicki P. Vaughan for her excellent assistance with the illustrations. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Assistant Professor.
[2]Assistant Professor, Institute of Food and Agricultural Sciences, Agricultural Research and Education Center, Homestead, FL 33031.

both *in vivo* and *in vitro* methods (6, 7, 18). Little work has been done to identify biochemical markers for papaya. Jindal and Singh (10) investigated the total protein electrophoretic banding patterns of male and female papaya plants at various stages of development. Isozyme banding patterns of mature fruits and leaves of some papaya cultivars were reported by Tan and Weinheimer (22).

The current study was undertaken to: 1) establish biochemical markers for *C. papaya* and *C. cauliflora*; 2) determine if somatic embryos derived from cultured polyembryonic papaya ovules after pollination with *C. cauliflora* are maternal or zygotic in origin; and 3) characterize plantlet populations from such cultures for variability in isozyme patterns as evidence for somaclonal variation.

Materials and Methods

Ovule culture. Pistillate flowers of dioecious plants of *C. papaya* were enclosed in glassine bags 2–3 days prior to flowering. Pollen from staminate plants of *C. cauliflora* was applied directly to the stigmas of open papaya flowers and the flowers were rebagged. Young fruits were harvested 65 days after pollination and were surface-sterilized with 1% sodium hypochlorite for 30 min. Ovules were removed aseptically from bisected fruit following a sterile, distilled water rinse and were cultured on sterile MS (17) medium that had been modified accordingly: half strength major salts and chelated iron, 60 g/liter sucrose, 400 mg/liter glutamine, and 8 g/liter Difco Bacto agar (14). Filter-sterilized coconut water (20% v/v from freshly harvested, 8–10 cm long, immature coconuts) was added to the autoclaved medium. Ovular callus was removed from polyembryonic cultures and was subcultured on the same medium for 1 month, after which it was transferred to liquid medium without coconut water in order to induce efficient somatic embryogenesis. Plantlets were regenerated following the procedure of Litz and Conover (14) by transfer of mature embryos to medium with benzylamino purine (BA) and naphthaleneacetic acid (NAA). The pH of all media was adjusted to 5.7 with 0.1N HCl or KOH prior to autoclaving at 1.1 kg/cm^2 and 120°C for 15 min. Liquid cultures were maintained in 125-ml Erlenmeyer flasks on a rotary shaker at 100 rpm. All cultures were grown under a 16-hr photoperiod (1.2 klx) at 25°.

Sampling of plant materials. Sixty germinated plantlets derived from somatic embryos from a single ovule were sampled for electrophoresis and isozyme staining. All sampled plantlets had well-defined radicles and cotyledons and ranged from 10–30 mm in length. Germinating zygotic embryos were dissected also from seeds of open-pollinated *C. papaya* and *C. cauliflora*. Ten germinating embryos (10–30 mm) of each species were sampled. For each sample, the entire germinating embryo or plantlet was weighed and ground with 0.1 ml chilled extraction solution for each 100 mg of tissue.

Leaf tissue was taken from *C. papaya* and *C. cauliflora* seedlings which were maintained in the greenhouse. Twenty seedlings of each species were sampled at 2 separate times, when they were about 10 and 20 cm tall. Leaf tissue was taken also from 4 staminate and 5 pistillate mature, field-grown *C. papaya*. In each case, the petioles and large veins were removed from the leaves before weighing and grinding. The leaf tissue was ground with 2 ml of chilled extraction solution per gram tissue. All operations after sample collection were done at 4°C. Preliminary experiments with several extraction solutions and buffers showed that the best solution could be obtained with 10% sucrose dissolved in distilled water. A small amount of insoluble polyvinylpyrrolidone was added to each sample as it was ground. Each sample was centrifuged at 13,000 g for 5 min after grinding for 2–3 min. The samples were then divided into 100 µl portions and either used immediately after preparation or kept frozen at −5° for periods of up to 1 week.

Electrophoresis and isozyme staining. The apparatus used to form and run the slab acrylamide gels was that of Driedger and Blumberg (4). Gels were prepared from a stock solution of 28.38% by weight acrylamide and 1.62% by weight of methylenebis-acrylamide. The gels were 7.5% acrylamide, 0.375 M Tris-HCl (pH 8.8), 0.025% by volume of NNNN-tetramethylethylenediamine (TEMED), and 0.025% by weight of ammonium persulfate. The electrode buffer was 0.025 M Tris base and 0.194 M glycine, pH 8.3. Bromphenol blue was included as a tracking marker. Gels were run at 4°C and 35 mA for 3 hr or until the tracking dye reached the bottom of the gel. Staining was for PER, MDH, GOT, or SOD. The PER stain used was that of Smith et al. (21), with 5 µl of sample loaded on the gels. The MDH stain used was that of Scandalios (19), the GOT stain was that of Shaw and Prasad (20), and the SOD stain was that of Moore and Collins (16). For these stains, 10 µl of each sample was loaded on the gels.

Each sample, for each enzyme studied, was run at least twice on a separate gel and in a random slot position. All gels were scored for band migration as compared to that of the tracking dye (R_f = distance from origin to band/distance from origin to front) and the intensity of individual bands was scored visually.

Results

Production of somatic embryos. Globular somatic embryos developed from papaya ovular callus in large numbers following transfer to coconut water-free medium. Germination of the somatic embryos occurred following transfer to medium with BA and NAA (Fig. 1). A low percentage of germinating somatic embryos demonstrated certain morphological anomalies such as hypocotyl branching, polycotyledony, fused cotyledons, fasciation, and loss of apical dominance. Most of these anomalies disappeared with advancing age. Regenerated plants closely resembled the maternal parent papaya.

PER isozyme banding patterns. PER zymograms for papaya seedlings, *C. cauliflora* seedlings, and plantlets from the somatic embryos are shown in Fig. 2 and 3. The PER banding pattern of germinating papaya embryos, papaya seedlings of both sizes, and mature papaya leaves of both sexes were similar. The zymograms of *C. cauliflora* germinating embryos and seedlings were also similar, but distinctly different from those of papaya. Banding patterns of plantlets from the somatic embryos contained a unique PER band, band 2 (R_f 0.44), not found in either papaya or *C. cauliflora* samples. The somatic embryo PER banding pattern also contained 2 bands (band 5, R_f 0.14; band 4, R_f 0.36) present in papaya but absent in *C. cauliflora* zymograms and 1 band (band 7, R_f 0.03) found in *C. cauliflora* zymograms but not in papaya.

MDH isozyme banding patterns. Papaya, *C. cauliflora*, and the somatic plantlet samples each had unique banding patterns (Fig. 2 and 4) when gels were stained for MDH. Samples of germinating papaya embryos contained MDH bands 1 (R_f 0.38), 2 (R_f 0.32), and 4 (R_f 0.25). MDH patterns of 10- and 20-cm-tall papaya seedlings and mature leaves also contained these bands and many of the samples also contained band 3 (R_f 0.28). Two of 20 papaya seedling samples contained a lightly staining band at R_f 0.15. Lightly staining bands at R_f 0.15 and 0.04 were present in some of the germinating embryos and seedlings of *C.*

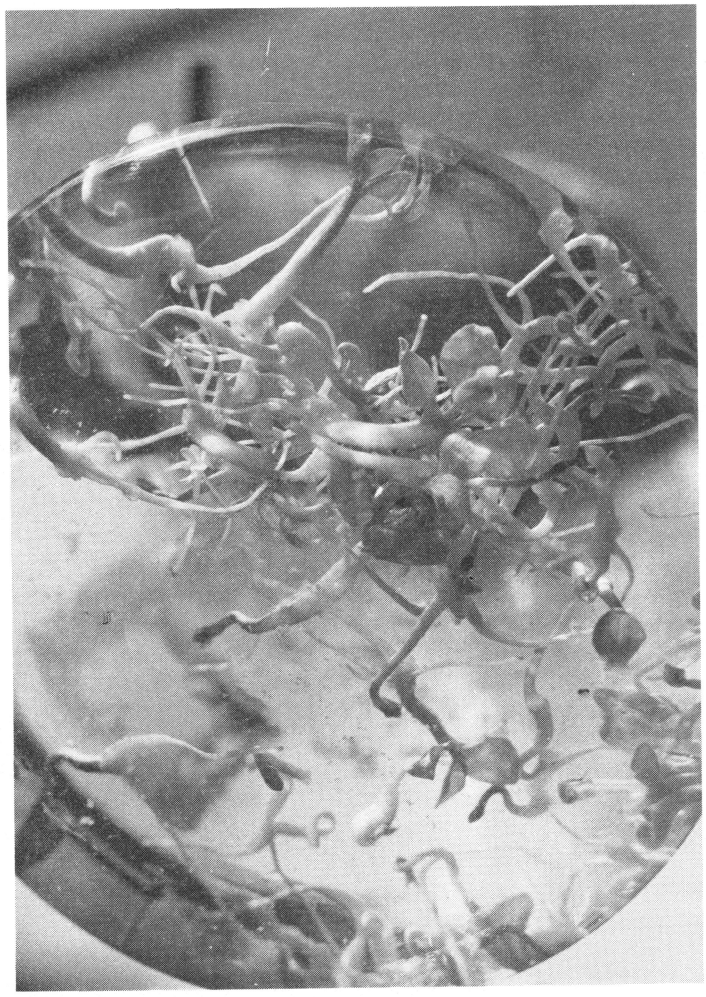

Fig. 1. Germinated *Carica* somatic embryos in modified Murashige and Skoog medium.

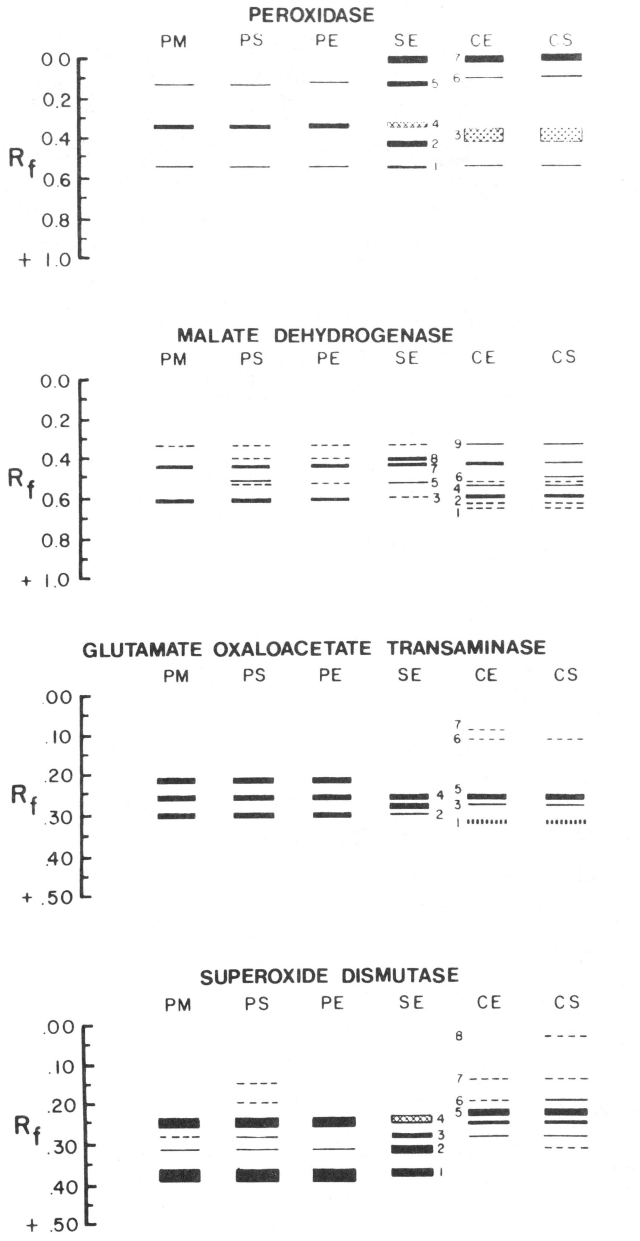

Fig. 2. Zymograms of *Carica papaya*, *C. cauliflora*, and plantlets from somatic embryos (derived from a single ovule of *C. papaya* pollinated with *C. cauliflora*) stained for PER, MDH, GOT, and SOD. PM = mature *C. papaya* plants; PS = 10- and 20-cm-tall *C. papaya* seedlings; PE = germinating embryos extracted from seeds of *C. papaya*; SE = plantlets from somatic embryos; CE = germinating embryos extracted from the seeds of *C. cauliflora*; CS = 10- and 20-cm-tall *C. cauliflora* seedlings.

cauliflora as was a more intensely staining band, band 6 (R_f 0.20). Six of 20 *C. cauliflora* seedling samples had MDH patterns which contained a lightly staining band 2. Bands 3, 4, and 5 (R_f 0.22) always were present in *C. cauliflora*. The MDH zymograms of plantlets from the somatic embryos contained bands 1, 2, 3, and 4. Band 4 was more lightly staining than the others and in a few cases appeared to be absent.

GOT isozyme banding patterns. Mean R_f values for each band detected in the GOT isozyme banding patterns for all of the materials sampled are shown in Fig. 2 and 5. Papaya samples contained band 2 (R_f 0.30), 4 (R_f 0.26), and 5 (R_f 0.22). The maturity of the material sampled had no effect on the GOT banding patterns observed. Zymograms of *C. cauliflora* germinating embryos and seedlings consisted of 2 major bands, bands 1 (R_f 0.32) and 4. GOT band 3 (R_f 0.28) was usually present although it stained much more lightly. A lightly staining band at R_f 0.09 was found only in the patterns of the smallest germinating embryos, and the staining intensity of a band of R_f 0.11 decreased as the maturity of the sampled material increased. The GOT zymograms of the somatic plantlets contained band 2 in common with papaya, band 3 in common with *C. cauliflora*, and band 4 in common with both species.

SOD isozyme banding patterns. Bands found in the SOD zymograms are illustrated in Fig. 2 and 6. Several minor lightly staining bands were observed in the isozyme patterns of all the material except the leaves of mature papaya plants. Zymograms of all samples contained a major band (band 7 R_f 0.45), and all appeared to contain band 3 (R_f 0.61), although this band stained very lightly in the zymograms of the somatic plantlets. The SOD pattern of the somatic plantlets contained an intensely staining band at R_f 0.41 (band 8), which was present also as a minor band in a few papaya germinating embryo and seedling zymograms.

Summary of findings from isozyme banding patterns. The isozyme bands in the 4 enzyme-staining systems examined in this experiment could be used as biochemical markers for papaya and *C. cauliflora* since each species had a distinctive banding pattern for each system. The zymogram of each species was

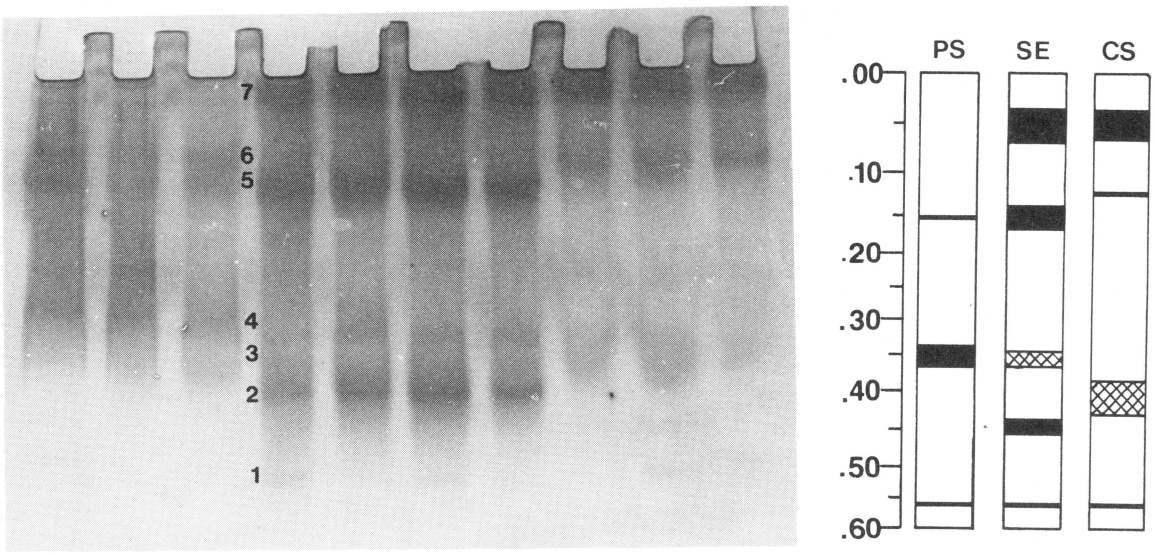

Fig. 3. Acrylamide gel stained for PER. Samples include: lanes 1–3, 10-cm-tall *C. papaya* seedlings, lanes 4–7, individual somatic plantlets, and lanes 8–10, 10-cm-tall *C. cauliflora* seedlings. Origin is at the top of the photograph; anode is at the bottom. The schematic diagram on the right depicts patterns for *C. papaya* seedlings (PS), plantlets from somatic embryos (SE), and *C. cauliflora* seedlings (CS).

constant, with the exception of a few bands, regardless of the developmental stage (germinating embryo, potted seedling, or, in the case of papaya, mature plant in field). No differences in banding patterns were observed in leaf samples from mature staminate and pistillate papaya plants. The plantlets from somatic embryos which arose from a single polyembryonic papaya ovule after pollination with *C. cauliflora* had isozyme banding patterns which differed from those of papaya and *C. cauliflora* in all 4 enzyme systems.

Discussion

These results indicate that somatic embryos from cultured papaya ovules derived from interspecific hybridization between *C. papaya* and *C. cauliflora* are probably of zygotic origin. Other researchers (5, 6, 24) have found that both sexual interspecific hybrids and somatic hybrids from protoplast fusion have isozyme banding patterns that are frequently the sum of those of the parents. The zymograms also may include hybrid bands not present in either parent and some parental bands may be missing in the hybrid zymogram. Zymograms of the somatic plantlets in some cases contained bands specific to either papaya or *C. cauliflora* (PER, GOT) or a unique band not seen in the zymogram of either species (PER) in this experiment. The somatic plantlets had zymograms which were distinctly different from those of either *Carica* species in all 4 enzyme-staining systems. It is unlikely that the differences in banding patterns between the somatic plantlets and either parental species are due to physiological factors such as *in vivo* vs. *in vitro* culture since the zymograms of both species changed very little with developmental stage. Because of the difficulty in hybridizing papaya

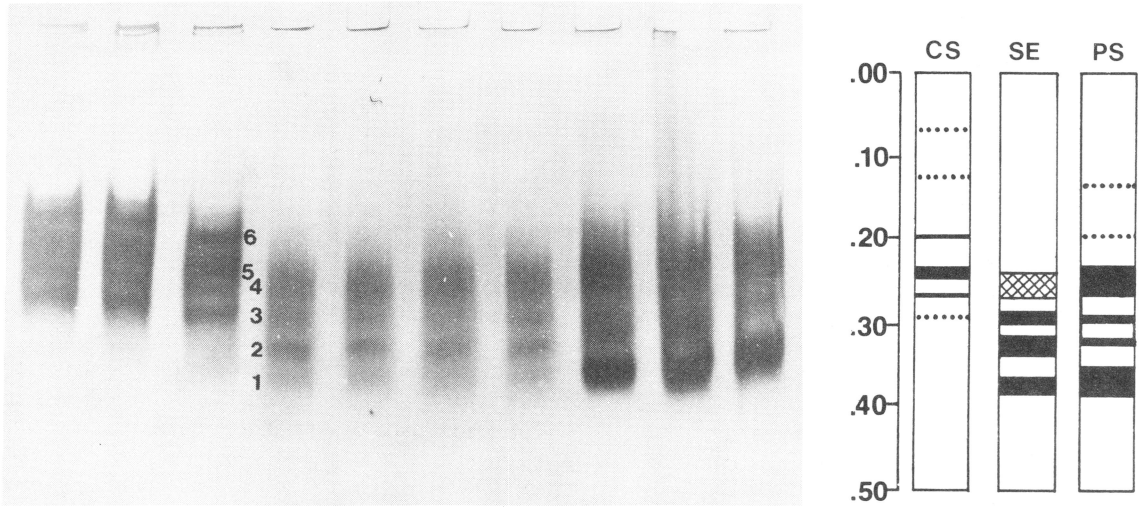

Fig. 4. Acrylamide gel stained for MDH. Samples include: lanes 1–3, 10-cm-tall *C. cauliflora* seedlings; lanes 4–7, somatic plantlets; lanes 8–10, 10-cm-tall *C. papaya* seedlings. Origin is at the top of photograph; anode is at the bottom. The schematic diagram at right depicts patterns for *C. cauliflora* seedlings (CS), plantlets from somatic embryos (SE), and *C. papaya* seedlings (PS).

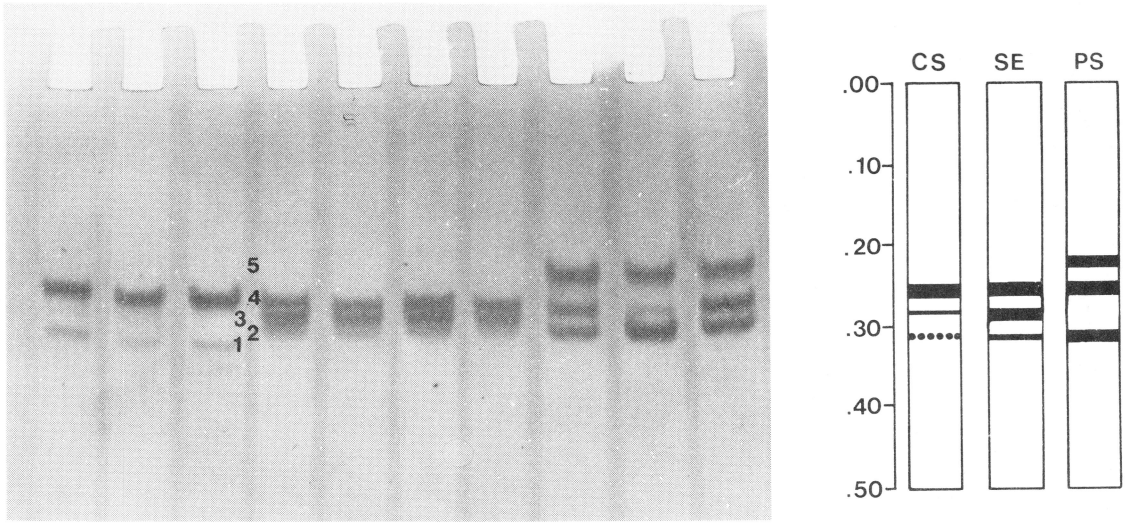

Fig. 5. Acrylamide gel stained for GOT. Samples include: lanes 1–3, 3 *C. cauliflora* seedlings, lanes 4–7, 4 somatic plantlets, and lanes 8–10, 3 *C. papaya* seedlings. Origin is at the top of the photograph; anode is at the bottom. The schematic diagram at right depicts patterns for *C. cauliflora* seedlings (CS), plantlets from somatic embryos (SE), and *C. papaya* seedlings (PS).

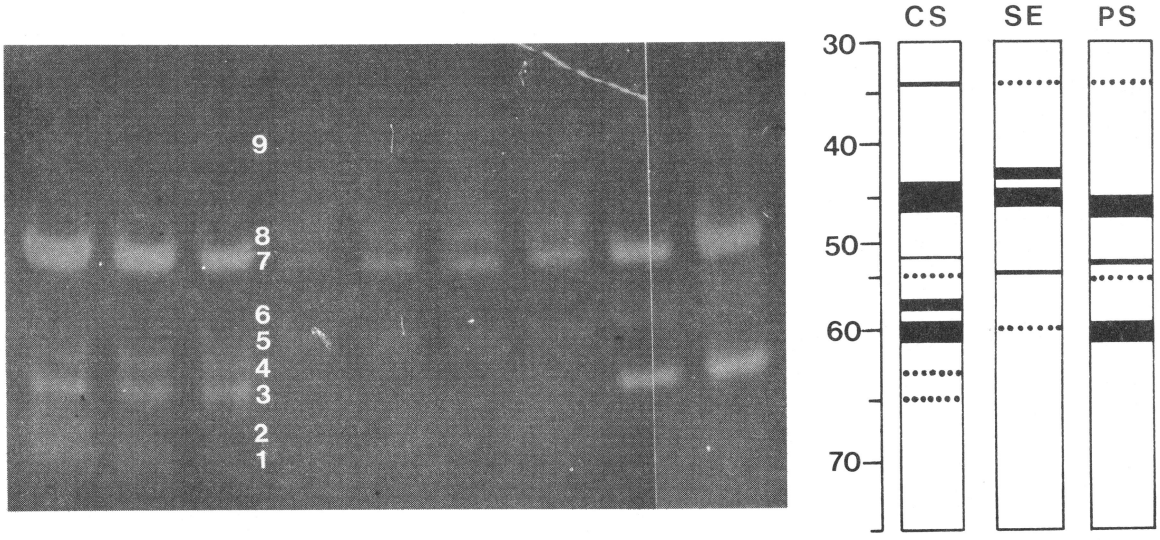

Fig. 6. Acrylamide gel stained for SOD. Samples include: lanes 1–3, 3 *C. cauliflora* seedlings, lanes 4–7, somatic plantlets, and lanes 8–9, 2 *C. Papaya* seedlings. Origin is at the top of photograph; anode is at the bottom. The schematic diagram at right depicts patterns for *C. cauliflora* seedlings (CS), plantlets from somatic embryos (SE), and *C. papaya* (PS) seedlings.

with PRV-resistant *C. cauliflora*, ovule culture appears to be a useful method for enhancing the efficiency of embryo rescue by increasing the number of available hybrid embryos.

Heinz and Mee (7) found that in *Saccharum* species hybrid clones, plants regenerated from callus of both chromosomally stable and chromosomal mosaic plants, more often showed variability in isozyme banding patterns (31.0% and 80.9%, respectively) than in morphological traits (12.1% and 34.8%, respectively). Only qualitative differences, i.e., the presence or absence of individual bands, were scored, although differences in band intensity were noted also. The isozyme banding patterns of the 60 plantlets derived from somatic embryos were extremely uniform in this experiment. The few differences noted were in the presence or absence of intensity of staining of a few minor bands and these differences could be due to differences in total protein concentration in the samples. This similarity in isozyme banding patterns indicates either that the somatic plantlets were genetically uniform or that the 4 enzyme systems studied were ineffective for detecting any somaclonal variation that might have resulted from plant cell culture (12). However, PER and GOT stains were very effective in detecting variability in *Saccharum* (7).

Literature Cited

1. Chien, Y.C., K.N. Kao, and L.R. Wetter. 1982. Chromosomal and isozyme studies of *Nicotiana tabacum–Glycine max* hybrid cell lines. Theor. Appl Genet. 62:301–304.
2. Conover, R.A. 1976. A program for development of papaya tolerant to the distortion ringspot virus. Proc. Fla. State Hort. Soc. 89:229–231.
3. Conover, R.A. and R.E. Litz. 1978. Progress in breeding papayas with tolerance to papaya ringspot virus. Proc. Fla. State Hort. Soc. 91:182–184.

4. Driedger, P.E. and P.M. Blumberg. 1978. Design and construction of a simple and inexpensive slab gel electrophoresis apparatus. Anal. Biochem. 87:177–186.
5. Evans, D.A., L.R. Wetter, and O.L. Gamborg. 1980. Somatic hybrid of *Nicotiana glauca* and *Nicotiana tabacum* obtained by protoplast fusion. Physiol. Plant 48:225–230.
6. Gates, P. and D. Boulter. 1979. The use of seed isoenzymes as an aid to the breeding of field beans (*Vicia faba* L.). New Phytol. 83:783–791.
7. Heinz, D.J. and G.W.P. Mee. 1971. Morphologic, cytogenetic, and enzymatic variation in *Saccharum* species hybrid clones derived from callus tissue. Amer. J. Bot. 58:257–262.
8. Horowitz, S. and H. Jimenez. 1967. Cruzamientos interspecificos e intergenericos en Caricaceae y sus implicaciones fitotecnicas. C.I.A. Venezuela. Agron. Trop. 17:323–343.
9. Jimenez, H. and S. Horowitz. 1958. Cruzabilidad entre especies de *Carica*. C.I.A. Venezuela. Agron. Trop. 7:207–215.
10. Jindal, K.K. and R.N. Singh. 1976. Electrophoretic changes in soluble proteins during vegetative and floral development of male and female papaya plants. Biochem. Physiol. Pflanzen. 5:301–307.
11. Khuspe, S.S., R.R. Hendre, A.F. Mascarenhas, and V. Jagannathan. 1980. Utilization of tissue culture to isolate interspecific hybrids in *Carica* L., p. 198–205. In: P.S. Rao, M.R. Heble, and M.S. Chadha (eds.). Plant tissue culture, genetic manipulation and somatic hybridization of plant cell. Bhabha Atomic Research Center, India.
12. Larkin, P.J. and W.R. Scowcroft. 1981. Somaclonal variation—a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60:197–214.
13. Litz, R.E. and R.A. Conover. 1981. *In vitro* polyembryony in *Carica papaya* L. ovules. Z. Pflanzenphysiol. 104:285–288.
14. Litz, R.E. and R.A. Conover. 1982. *In vitro* somatic embryogenesis and plant regeneration from *Carica papaya* L. ovular callus. Plant Sci. Lett. 26:153–158.
15. Litz, R.E. and R.A. Conover. 1983. High frequency somatic embryogenesis from *Carica* suspension cultures. Ann. Bot. 51:683–686.
16. Moore, G.A. and G.B. Collins. 1982. Identification of aneuploids in *Nicotiana tabacum* by isozyme banding patterns. Biochem. Genet. 20:555–568.
17. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473–497.
18. Orton, T.J. 1980. Haploid barley regenerated from callus cultures of *Hordeum vulgare* x *H. jubatum*. J. Hered. 71:280–282.
19. Scandalios, J.G. 1969. Genetic control of multiple molecular forms of enzymes in plants: a review. Biochem. Genet. 3:37–39.
20. Shaw, C.R. and R. Prasad. 1980. Starch gel electrophoresis of enzymes—a compilation of recipes. Biochem. Genet. 4:297–320.
21. Smith, H.H., D.E. Hamill, E.A. Weaver, and K.H. Thompson. 1970. Multiple molecular forms of peroxidases and esterases among *Nicotiana* species and amphiploids. J. Hered. 61:203–212.
22. Tan, S.C. and E.A. Weinheimer. 1976. The isoenzyme patterns of developing fruit and mature leaf of papaya (*Carica papaya* L.). Sains Malaysiana 5:7–14.
23. Tanksley, S.L. and R.A. Jones. 1981. Application of alcohol dehydrogenase allozymes in testing the genetic purity of F_1 hybrids of tomato. HortScience 16:179–181.
24. Wetter, L.R. 1977. Isoenzyme patterns in soybean—*Nicotiana* somatic hybrid cell lines. Molec. Gen. Genet. 150:231–235.

J. Amer. Soc. Hort. Sci. 109(2):218–221. 1984.

Pentaploid Blueberries from 6*x* × 2*x* Crosses

Ronald G. Goldy[1] and Paul M. Lyrene
Fruit Crops Department, University of Florida, Gainesville, FL 32611

Additional index words. *Vaccinium* sp., interspecific hybridization, fertility

Abstract. **Three pentaploids produced from hybridizing hexaploid *Vaccinium ashei* Reade and diploid *V. darrowi* Camp were analyzed for fruit set, number of seeds/fruit, seedlings/pollination, pollen grains/sporad, 2*n* gamete production, pollen germination, leaf area and berry weight, color, scar, and firmness. The pentaploids were intermediate between the parental species in fruit size and leaf area. There was high variation in fertility and in fruit quality among the pentaploid hybrids.**

Cultivated blueberries are primarily tetraploid or hexaploid. The cultivated tetraploids include highbush (*V. corymbosum* L. and *V. australe* Small) and lowbush (*V. angustifolium* Ait.). The cultivated hexaploid is the rabbiteye (*V. ashei*). However, desirable horticultural traits exist in plants at all 3 of the common chromosome numbers found in *Vaccinium* (1), with plants of each chromosome number possessing traits not found in others (7). For this reason, heteroploid crosses are pursued actively despite low success rates. Moore (8) lists several successful interspecific crosses made under controlled conditions. Some of these crosses have been repeated and the progenies evaluated by several blueberry breeders. Homoploid hybrids have led to the release of several cultivars (8), but only 3 heteroploid hybrids have been released (10, 11, 12).

Crosses between tetraploid and hexaploids, especially between the 4*x* ($2n = 48$) highbush and the 6*x* ($2n = 72$) rabbiteye, are of greatest interest since commercial cultivation of blueberries is limited primarily to these groups. Direct hybridization between the 2 levels results in 5*x* ($2n = 60$) plants that have not yet proven useful in further breeding. Therefore, production of synthetic 6*x* highbush-types or 4*x* rabbiteye-types have been proposed to facilitate gene exchange between the 2 groups (2, 3). The procedure that was first pursued was development of 4*x* rabbiteye-types from 6*x* × 2*x* crosses (2, 3).

Darrow et al. (2, 3) and Sharpe and Sherman (9) have used various 6*x* × 2*x* crosses in breeding. Darrow et al. (2, 3) crossed *V. ashei* with the southern lowbush 2*x* species, *V. tenellum* Ait., to produce 4*x* rabbiteye-types which were crossed then to high-

Received for publication 5 Aug. 1983. University of Florida Agricultural Experiment Station Journal Series No. 5049. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Present address: Dept. of Horticultural Science, North Carolina State Univ., Raleigh, NC 27650.