In Vitro Cultures and the Development of New Fruit Varieties©

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INTRODUCTION

Numerous cultivars of stone fruits and grapes have been developed in the breeding programs at the Agricultural Research Service's Fresno laboratory. Evaluation of grapes began in the early 1900s and breeding of table grapes and raisins commenced shortly thereafter. In the mid-1950s, *Prunus* breeding efforts began to enhance the few available varieties of stone fruit. Breeding efforts in both stone fruits and grapes could be considered traditional and without the use of in vitro cultures until the mid-1970s when embryo culture techniques were first employed. In vitro embryo and ovule cultures are now used routinely to improve the efficiency of the breeding efforts. Micropropagation is also employed in *Prunus* to provide clones of elite germplasm for experimentation and for the dispersion of new varieties.

BREEDING PROGRAMS

***Vitis* Breeding Efforts.** Numerous grape varieties were introduced into the San Joaquin Valley in the early 1900s. It was apparent to industry representatives that recommendations were needed to provide producers with information stating which grape varieties would produce the highest quality or largest yields in the California environments. Our current research location in Fresno was originally established to evaluate the many new grape varieties and make the necessary recommendations. The variety 'Sultanina' (syn., 'Thompson Seedless') was easily identified as a well-adapted and productive grape for the table and for drying purposes (Husmann, 1932).

Breeding of both table and raisin grapes commenced in 1923 and from the onset, seedlessness was an important characteristic (Snyder and Harmon, 1952). Since the seedless condition resulted in embryo abortion, breeding for new seedless vines was possible only through crosses between seeded and seedless individuals. Pollination of seeded mother vines with seedless males led to a generally low frequency of seedless vines in the subsequent generation.

In-ovulo embryo culture was first attempted with grapes at the Fresno location in the late 1970s. The goal was to hybridize two seedless vines directly and to rescue the resulting hybrid embryos prior to abortion. A higher frequency of seedless individuals could be recovered in the F_1 as compared with traditional seeded × seedless hybridizations (Ledbetter and Burgos, 1994). Nutrient medium suitable for the culture of immature *Vitis* embryos was adapted initially from in-ovulo culture of *Gossypium* embryos (Stewart and Hsu, 1977). Experimentation has led to several changes in medium composition over the years in order to enhance embryo development and improve the efficiency of the culture procedure (Cain, et al., 1983; Emershad and Ramming, 1984; Emershad, et al., 1989). Utilization of in-ovulo embryo culture techniques at the Fresno lab has led to the introduction of two varieties in *Vitis*. 'Dovine' was named and released for propagation in 1989 as a dry-on-the-vine raisin grape. More recently, a white mid-season seedless table grape named 'Princess' was introduced in 1998.

In Vitro Cultures in *Prunus*. In vitro ovule and embryo cultures are necessary in *Prunus* to develop very early ripening varieties. Embryo immaturity in early season fruit is a limiting factor in *Prunus*. This contrasts with the abortion of the developing hybrid embryo in *Vitis* seedless × seedless cultures. Fruit flesh of the earliest ripening varieties matures far sooner than the embryo inside. Traditional stratification of the seed and sowing in the greenhouse flats leads to an extremely small percentage of the sown seed actually producing viable plants for field transplantation. In vitro cultures offer a chance for these immature embryos to develop further and become capable of establishment as growing seedlings for new variety development.

Embryo culture was first used in *Prunus* at the Fresno location in the mid-1970s. Employed medium at that time was that developed by Smith et al. (1969) at Rutgers University. Initial results were successful and demonstrated thoroughly the benefits in using embryo culture to attain earlier ripening peaches and nectarines for selection in the breeding program. Continued breeding with this early ripening germplasm has led to still earlier ripening fruits. Similar to the situation in *Vitis*, an in-ovulo embryo culture technique was necessarily developed and utilized as a prelude to the standard embryo culture procedure for embryos ranging in size from 1 to 5 mm (Ramming, 1985). Improvements in medium composition were necessary in order to successfully culture these small embryos (Ramming, 1990). Comparisons of the effects of nutrient media on embryo growth and plant development have been made with various commercially available products in early maturing apricots (Burgos and Ledbetter, 1993) as well as with early season nectarine, peach, and plum (Emershad and Ramming, 1994). While embryo culture has been used in the breeding efforts of all four of these germplasm types, to date only peach and nectarine varieties have been named and released that are the product of embryo culture. In the mid-1980s 'Mayfire' and 'Goldcrest' were introduced and became the San Joaquin Valley's earliest ripening commercial nectarine and peach, respectively. More recently, 'Spring Baby' peach and 'Crimson Baby' nectarine were named and released for commercial propagation.

Micropropagation plays a supporting role in the development of new *Prunus* germplasm. Proliferating in vitro cultures of *Prunus* can provide explants for in vitro studies and can be an alternative for establishing rooted clones of elite materials or hard-to-root germplasm. In our Fresno laboratory, we have found Quoirin and Lepoivre (1977) nutrient medium to be an excellent proliferation medium for a wide variety of *Prunus* germplasm. Establishment of sterile germplasm in vitro has proven far more difficult than growth of clonal material during the proliferation phase. Useful sterilization procedures for field- and greenhouse-grown plant materials have been developed and presented by Rajashekar et al. (1995).

Micropropagation has been used in our Fresno laboratory in *Prunus* rootstock development. In vitro screening techniques have been developed to identify elite germplasm that tolerate a high osmotic in vitro environment. Candidate clonal plum rootstocks have been screened (Ledbetter et al., 1996) and a wide genetic assortment of seed-propagated peach, peach-almond, and almond germplasm have also been subjected to mannitol-modified nutrient media (Ledbetter et al., 1998). Significant differences in net in vitro growth were evident in both studies relative to specific *Prunus* accessions or germplasm types. Routinely, micropropagation facilitates shadehouse grafting studies by providing uniform clonal rootstock

accessions for experimental tree production. Without the availability of micropropagation, many elite rootstock accessions would prove very difficult to root by traditional horticultural means.

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