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A Stenospermocarpic, Seedless *Vitis vinifera* x *Vitis rotundifolia* Hybrid Developed by Embryo Rescue

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***Abstract*. Hybridizations between seedless *Vitis vinifera* L. genotypes and *V. rotundifolia* (Michx.) were made specifically to introgress the seedless trait into the disease-resistant background of *V. rotundifolia*. The seedless, gynoecious P79-101 was hybridized with three *V. rotundifolia* parents, producing a total of 102 ovules. From these ovules, 44 embryos developed, producing 20 plants. Isozyme analyses and morphological traits confirmed that 19 of the plants were hybrids. Sixteen were planted in an experimental vineyard at California State Univ. Fresno. One seedling, C41-5, produced seedless fruit that appeared to be stenospermocarpic based on fruit and aborted seed size. Fruit weight was slightly less than that of 'Thompson Seedless' (stenospermocarpic) and at least twice that of parthenocarpic fruit of 'Black Corinth' and C41-7, a seeded hybrid with many parthenocarpic fruit. Aborted seeds of C41-5 were larger than, but not significantly different from, those of 'Thompson Seedless', while parthenocarpic fruit from 'Black Corinth' and C41-7 had aborted seeds that were smaller than those of C41-5. Seed weight of C41-7 averaged almost 10 times that of aborted C41-5 seeds. This is the first report of a stenospermocarpic, seedless hybrid of *V. vinifera* x *V. rotundifolia*.**

The major grape cultivars grown for wine, table grapes, and raisins are derived from *Vitis vinifera*, a species susceptible to diseases and pests. However, *V. vinifera* has very good bunch and fruit qualities, such as large clusters and large berries, and good flesh and skin texture, and many seedless cultivars exist. Seedless fruit development can be parthenocarpic (fruit develops without fertilization) or stenospermocarpic (fertilization occurs but embryo development soon stops) (Pearson, 1933; Stout, 1936). *Vitis rotundifolia* is native to the southeastern United States, is very disease- and pest-resistant, and is adapted to warm climates. However, *V. rotundifolia* cultivars have small clusters, and the berries are usually seeded, with thick skins that separate from the tough flesh and pronounced aromatic flavors. Seedless *V. rotundifolia* were unknown until the recent development of 'Fry Seedless' (U.S. Plant Patent 7296). 'Fry Seedless' appears to be parthenocarpic, given its very small aborted ovules and small berry size. Snyder (1940) reported that parthenocarpy was not transmitted to the F_1 generation.

Hybridization of *V. vinifera* with *V. rotundifolia* has been performed for over a century (Goldy, 1992; Jelenkovic and Olmo, 1968; Olmo, 1971, 1986; Patel and Olmo, 1955; Reisch and Pratt, 1996). *Vitis vinifera* x *V. rotundifolia* crosses have been more successful than the reciprocal (Dearing, 1917; Jelenkovic and Olmo, 1986; Patel and Olmo, 1955). A more recent publication by Lu and Lamikanra (1996) revealed a barrier to *V. vinifera* pollen tubes near the stigma in the styles of *V. rotundifolia*. This has complicated the procedure for introgressing seedlessness into *V. rotundifolia* because the *V. vinifera* plant used as the female parent had to be seeded in order to produce seedlings. When seeded *V. vinifera* genotypes with no or one parent with seedlessness in their background were hybridized, an average of 2.2% and 1.3% seedless progeny were obtained, respectively (Loomis and Weinberger, 1979). Even when both seeded parents had seedless progenitors, an average of only 1.8% seedless progeny were obtained. However, when seeded genotypes were pollinated with pollen from seedless ones, an average of 23.7% seedless progeny were obtained, making the production of seedless types much more efficient. The development of embryo rescue techniques now allows the recovery of plants from seedless grapes, allowing their use as female parents (Emershad and Ramming, 1984; Emershad et al., 1989). Seedless x seedless crosses have produced 45% to 85% seedless offspring (Ramming et al., 1990). Hybrids from seedless *V. vinifera* x seeded *V. rotundifolia* were obtained via embryo rescue and their hybrid origin verified by isozyme analysis (Goldy et al., 1988). In this report we document the first stenospermocarpic, seedless F_1 hybrid from *V. vinifera* x *V. rotundifolia* developed by Goldy et al. (1988).

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Materials and Methods

In 1986, a number of pollinations were made between seedless *V. vinifera* genotypes as the female parent and *V. rotundifolia* as the male parent. The resulting ovules were excised 6 weeks after pollination, and cultured using procedures reported by Goldy et al. (1988). Three plants from P79-101 x Fla. H17-66 and 13 plants from P79-101 x 'Welder' were planted in the field at California State Univ., Fresno, in 1989 from the embryos cultured in Fresno. Isozyme analysis and leaf morphology (Goldy et al., 1988) showed that all seedlings were hybrids, except for one from the cross P79-101 x 'Welder'. The nonhybrid nature of this seedling was also verified by its morphological characteristics in the field. The seedlings first flowered in 1991 and two (C41-5 and C41-7) set fruit. Both were derived from crossing P79-101 x 'Welder'. Since then a total of five seedlings have set fruit. Fruit from the hybrids was compared with open-pollinated fruit from the stenospermocarpic cultivars Thompson Seedless and Flame Seedless, and the parthenocarpic cultivar Black Corinth for berry size and seed development in 1993 and 1999. Three replications of 10 berries of each genotype were analyzed for individual berry weight and weight of the largest seed or aborted seed in each berry. Pollen grains of C41-5 were germinated on 1% agar with 20% sucrose at 27 °C. C41-5 flowers were emasculated and pollinated with *V. vinifera*, 'DOVine', B40-208, C49-24, and *V. rotundifolia*, 'Nesbitt' and 'Carlos', or left open-pollinated. This was done to provide every chance for embryo development. Ovules were collected ≈6 weeks after bloom for microscopic observations of embryos and for embryo rescue using the medium of Emershad et al. (1989) solidified with agar. This was done to determine if C41-5 would produce embryos in the aborted seeds, thereby verifying that it was stenospermocarpic.

Results and Discussion

C41-5 had fruit that appeared to be stenospermocarpic and C41-7 had mixtures of seeded and parthenocarpic fruit. Both mature and overripe fruit of C41-5 was smaller than those of 'Thompson Seedless' and 'Flame Seedless' (Table 1). Parthenocarpic berries from 'Black Corinth' and C41-7 were half the size of stenospermocarpic fruits of C41-5 at maturity (Fig. 1). This is similar to observations made on the first crop of C41-5 and seeded C41-7 berries, which averaged 2.1 and 2.5 g respectively. The aborted ovules in the parthenocarpic fruit of 'Black Corinth' and C41-7 were barely noticeable, and smaller than those of C41-5 (Table 1). C41-5 had aborted seeds that were similar in size to those of 'Thompson Seedless' and 'Flame Seedless' (Fig. 2). The smaller size of aborted seeds for the sample of mature C41-5 fruit was due to two replications of fruit having seeds weighting <1.4 mg, similar to aborted seeds found in parthenocarpic fruit, and the third sample hav-

Table 1. Average berry and seed weight (n = 30) for open-pollinated fruit of *Vitis vinifera* x *V. rotundifolia* hybrids (C41-3, C41-5, C41-7, C41-24), 'Thompson Seedless', 'Flame Seedless', and 'Black Corinth' in 1993 and 1999.

	1993		1999	
Genotype	Mean berry wt (g)	Mean wt of largest seed/aborted seed in each berry (mg)	Mean berry wt (g)	Mean wt of largest seed/aborted seed in each berry (mg)
		Parthenocarpic		
Black Corinth	0.49 e[y]	0.53 b	0.63 d	0.00
C41-7	0.47 e	0.00	0.61 d	0.27 c
C41-3	---	---	1.60 b	0.43 c
C41-24	---	---	0.94 c	0.03 c
		Stenospermocarpic		
C41-5 (mature)	1.13 d	6.97 b	1.80 b	6.90 c
C41-5 (overripe)	2.01 c	11.77 b	---	---
Thompson Seedless	2.91 b	4.33 b	2.48 a	7.97 c
Flame Seedless	3.60 a	13.80 b	2.53 a	7.67 c
		Seeded		
Black Corinth			2.69 a	38.10 b
C41-7	2.43 bc	86.30 a	2.92 a	76.37 a

[z]Mean separation within columns by one-way ANOVA and Tukey's HSD mean separation, $P \leq 0.01$.

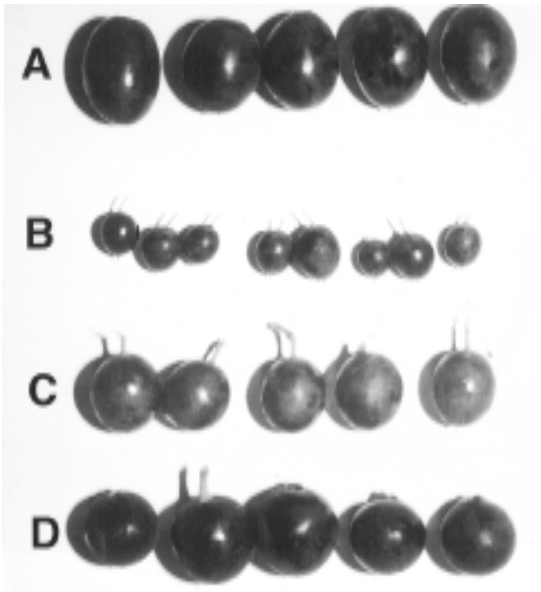

Fig. 1. Fruit of (**A**) seeded berries from C41-7, (**B**) parthenocarpic berries from C41-7, (**C**) mature berries from C41-5, and (**D**) overripe berries from C41-5. Bar = 1 cm.

Fig. 2. Aborted seed of (**a**) 'Thompson Seedless', (**b**) 'Flame Seedless', (**c**) C41-5, and seed of (**d**) seeded 'Black Corinth' and (**e**) C41-7. Bar = 1 cm.

ing aborted seeds weighing 19.4 mg. Since C41-5 bloomed later than the surrounding *V. vinifera* plants and had pollen germination that ranged from 0% to 3%, the very small aborted seeds probably resulted from the lack of fertilization. In 1999, the weight of aborted seeds of C41-5 did not differ from that of those of the stenospermocarpic cultivars Flame Seedless and Thompson Seedless (6.90, 7.67, and 7.97 mg, respectively). Seed weight in C41-7 was almost 10 times that in C41-5. This is similar to observations made on the first crop, in which C41-5 and C41-7 seeds averaged 15.1 and 86.4 mg, respectively. Since C41-5 berries were only slightly smaller than stenospermocarpic fruit and had aborted seeds larger than those of parthenocarpic genotypes but similar to those stenospermocarpic genotypes, we believe the seedless fruit of C41-5 are stenospermocarpic. Histological examination of aborted seeds of C41-5 from the various pollinations 6 weeks after bloom showed no embryos. Ovules cultured for embryo rescue 6 weeks after bloom also failed to develop embryos, perhaps because of the imbalance of chromosome number and homology in the *V. vinifera* x *V. rotundifolia* hybrid (Patel and Olmo, 1955). However, the continued growth of the ovules could have resulted from stimulation by fertilization, as occurs in stenospermocarpic berries.

P79-101 fruit is dark blue to purple and 'Welder' is green-bronze. C41-5 (Fig. 3) apparently inherited its purple to dark purple color from P79-101 (Barritt and Einset, 1969), as did all the other seedlings in this family. Fruit of C41-5 is oval to round in shape, more closely resembling P79-101, and has a slight muscadine flavor inherited from 'Welder'. The leaves and shoots of C41-5 are intermediate between those of its parents in shape and texture. Based on our observations, C41-5's seedlessness appears to be stenospermocarpic in nature. To our knowledge this is the first stenospermocarpic, seedless *V. vinifera* x *V. rotundifolia* hybrid to be reported.

In 1999, fruit from C41-3 and C41-24, derived from P79-101 x Fla H17-66 and P79-101 x 'Welder', respectively, were collected and measured (Table 1). Fruit of C41-3 were similar in weight to those of C41-5, and significantly larger than the parthenocarpic fruit from 'Black Corinth' and C41-7. However, the aborted seeds were similar in size to those in parthenocarpic fruit of 'Black Corinth' and C41-7. No seeded fruit have been observed on C41-3. However, in 1999 one cluster had three large fruit that averaged 4.4 g. Two of the fruit each contained one aborted seed that averaged 6 mg, similar to those of C41-5, 'Flame Seedless', and 'Thompson Seedless'. C41-3 is apparently parthenocarpic with occasional stenospermocarpic fruit. C41-3 has red fruit. C41-24 fruit is smaller than C41-3 and C41-5 fruit but larger than parthenocarpic fruit of 'Black Corinth' and C41-7 (Table 1). C41-24 had no seeded fruit. The aborted seeds were very small, only slightly larger than those found in 'Black Corinth'. Thus C41-24 also appears to be parthenocarpic.

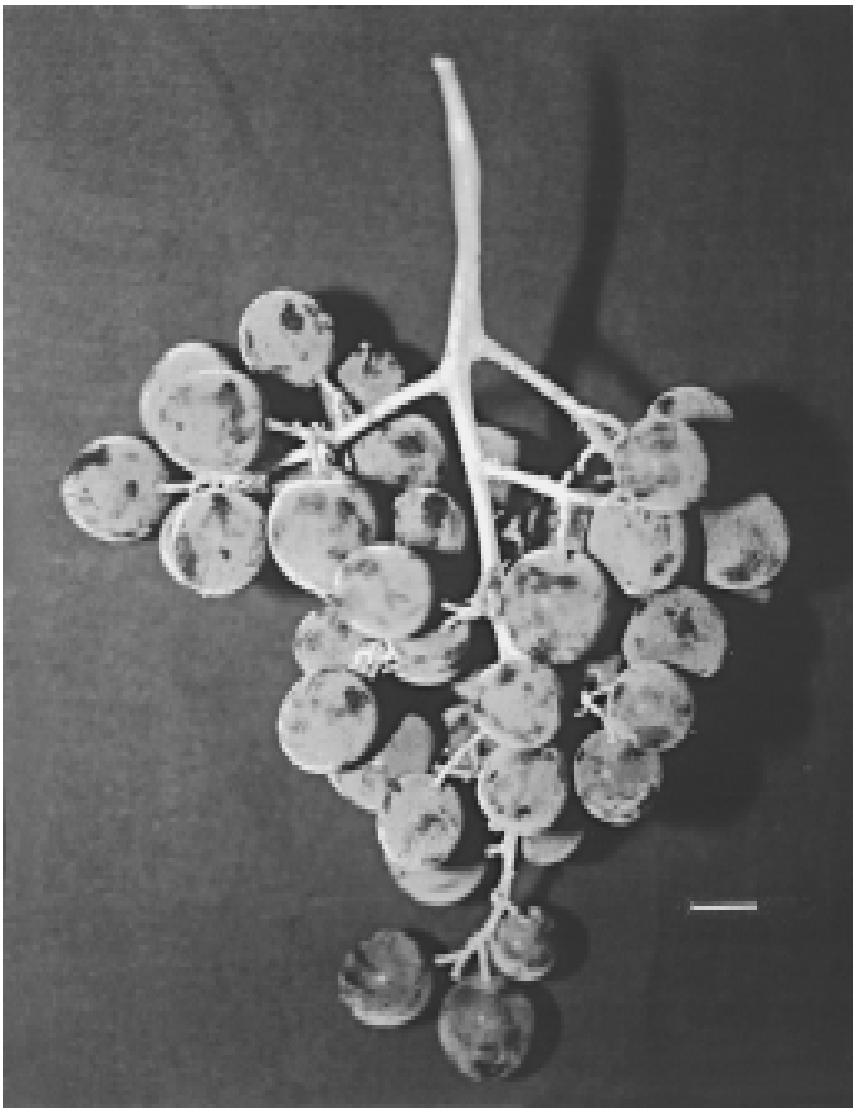

Fig. 3. Cluster of C41-5. Bar = 1 cm.

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