

The zygotic origin of hybrids from Thompson Seedless grape, *Vitis vinifera* L.

by

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Die zygotische Herkunft von Hybriden der Rebsorte Thompson Seedless (*Vitis vinifera* L.)

Zusammenfassung: Um die zygotische Herkunft von Nachkommen der stenospERMOKARPEN Sorte Thompson Seedless abzusichern, wurden aus *in vitro* kultivierten Samenanlagen Pflanzen herangezogen und eine Reihe von Merkmalen analysiert. Die Sämlinge aus unkontrollierter Bestäubung oder aus gezielter Kreuzung von Thompson Seedless unterschieden sich von der Muttersorte entweder in Beerenfarbe, Samengröße, Aroma oder Isoenzymmuster. Diese Befunde bestätigen, daß die durch *in vitro*-Kultur der Samenanlagen von Thompson Seedless erzeugten Pflanzen in der Regel zygotischen Ursprungs sind. Die Embryokultur aus Samenanlagen ist eine wertvolle Technik zur Erzeugung von Hybriden aus stenospERMOKARPEN Genotypen; sie erlaubt die Kreuzung mit anderen kernlosen Sorten und ermöglicht viele bisher nicht realisierbare Kreuzungskombinationen.

Key words: table grape, raisin, parthenocarpy, seedlessness, berry, colour, seed, flavour, isoenzyme, tissue culture, embryo culture, breeding.

Introduction

Seedless grapes have long been recognized. In 1898, MÜLLER-THURGAU observed that some seedless grape cultivars were characterized by berries containing small, soft seeds without embryos or endosperm compared to other seedless cultivars in which the ovules did not enlarge at all. He noted that pollination was required for fruit set (PRATT 1971). STOUT (1936) used the term stenospERM to designate the seedless types in which fertilization occurs, embryo and endosperm start to develop, but then abortion of the seed occurs.

Seedless grapes are valued for their use as fresh table grapes or for drying into raisins. Of all the grapes shipped for the fresh market from California in 1986—89, 73 % were seedless (CALIFORNIA TABLE GRAPE COMMISSION 1989). Over the past 15 years, the acreage of seedless grapes for fresh consumption or drying has increased by 79,741 acres and the seeded grape acreage has decreased by 15,317 acres (CALIFORNIA CROP AND LIVESTOCK REPORTING SERVICE 1977; CALIFORNIA AGRICULTURAL STATISTICS SERVICE 1989). Almost all raisins produced in California are from seedless genotypes except for Muscat of Alexandria which accounts for less than 0.2 % of the crop (THE RAISIN INDUSTRY NEWS Oct. 22, 1987). The consumption of seedless grapes has been increased by the introduction of new cultivars such as Flame Seedless. The production of this single cultivar alone has increased from 1,121 t to 137,073 t in the last 11 years (1978—1989) (CALIFORNIA TABLE GRAPE COMMISSION 1983, 1989). This indicates the importance and desirability of new seedless grape genotypes.

In the past, conventional breeding techniques used seeded grapes as female parents. Seeded grapes were hybridized with pollen of seedless types to develop progeny with seedless fruit. However, the proportion of seedless progeny obtained by this method was low and averaged only 21—24 % (LOOMIS and WEINBERGER 1975). It has always been the dream of grape breeders to hybridize seedless genotypes directly. This concept has only recently become a reality with the successful rescue of embryos from seedless grapes (EMERSHAD and RAMMING 1984; SPIEGEL-ROY *et al.* 1985). Plants have been produced from embryos of Thompson Seedless, Flame Seedless and Perlette. SRINIVASAN and MULLINS (1980) were the first to culture unfertilized grape ovules and produced somatic embryos from nucellar callus. When an ovule is subjected to artificial culture, the origin of the embryoid plant is in question. This paper reports results verifying the zygotic origin of plants obtained from embryo culture of Thompson Seedless ovules.

Materials and methods

In 1982, ovules were collected from open pollinated Thompson Seedless fruit grown in the U.S. Department of Agriculture, Agriculture Research Service's cultivar collection at Fresno, CA. The flowers could have been pollinated by any of a number of cultivars nearby that have seeds as well as colored fruit. Ovules were cultured using the embryo rescue techniques of EMERSHAD *et al.* (1989).

In 1983, Thompson Seedless was hybridized with Concord (presumed *V. labrusca* hybrid containing *V. vinifera*) and Venus (hybrid containing *V. labrusca*) (MOORE and BROWN 1977). Concord and Venus were chosen as pollen parents because they have dark blue fruit and pubescent shoots which could serve as markers. The ovules obtained were cultured as above and the acclimated plants were planted in the field for evaluation.

Results and discussion

In 1982, of the 1680 open pollinated ovules from Thompson Seedless that were cultured, 94 embryos developed and these were sub-cultured. From these embryos, 93 plants resulted and were planted in the field in 1983. Only 19 plants had enough fruit (50-berry sample) in either 1985 or 1986 to evaluate. Plants that resembled Thompson Seedless might be assumed to originate from somatic tissue of the female parent Thompson Seedless. However, if plants bearing seeded fruit occurred, they would have developed from the zygote of Thompson Seedless, not from somatic tissue. Only in very rare instances would the seeded plants have occurred as somatic mutations. 7 of the 19 seedlings were seeded. The fresh weight (FW) of seeds ranged from 45.1 to 70.0 mg. Seed traces from progeny bearing seedless fruit ranged from 0 to 13.8 mg. Thompson Seedless seed traces average 6 mg.

Fruit of all seedlings would be expected to be white and seedless like Thompson Seedless if the plants originated from somatic tissue. This was not the case as 10 of the seedlings had colored fruit and of the remaining 9 white-fruited seedlings, 6 had seeded fruit. Of the 3 seedlings that bore white seedless fruit, 2 had vestigial traces too small to weigh, making them smaller than Thompson Seedless traces and, therefore, different. Only 1 plant had fruit color and seed trace sizes similar to Thompson Seedless.

In 1983, 180 ovules of Thompson Seedless × Concord and 60 ovules of Thompson Seedless × Venus were cultured. One flask of 30 ovules of Thompson Seedless × Con-

cord became contaminated (Table 1). Embryos developed only from ovules of Thompson Seedless × Concord in which 27 % of the ovules developed embryos during *in vitro* culture. From these embryos, 4 plants developed and 3 fruited in the field. Table 2 shows a comparison of the traits of Thompson Seedless, Concord, and the F₁ hybrids. The F₁ seedlings truly are hybrids, having fruit with 'slip skins' which is definitely acquired from the pollen parent. The leaves have pubescence similar to Concord the pollen parent, although not as pronounced. The putative Thompson Seedless × Concord seedling and the parents were tested using isozyme techniques to determine their

Table 1

The number of ovules cultured, embryos sub-cultured and plants produced from the crosses Thompson Seedless × Concord and Thompson Seedless × Venus

Anzahl der kultivierten Samenanlagen, der subkultivierten Embryonen und der Pflanzen, die aus den Kreuzungen Thompson Seedless × Concord und Thompson Seedless × Venus hervorgingen

Observations	TS × Concord	TS × Venus
No. emasculations	1 231	1 715
No. ovules cultured	180 ¹⁾	60
No. ovules with embryos	40 ²⁾	0
Av. embryo length (mm)	1.03	—
No. plants	4	—

¹⁾ One flask (30 ovules) was contaminated.

²⁾ 9 ovules contained multiple embryos, 1 embryo was growing out of ovule.

Table 2

Description of Thompson Seedless (TS), Concord, and F₁ hybrids

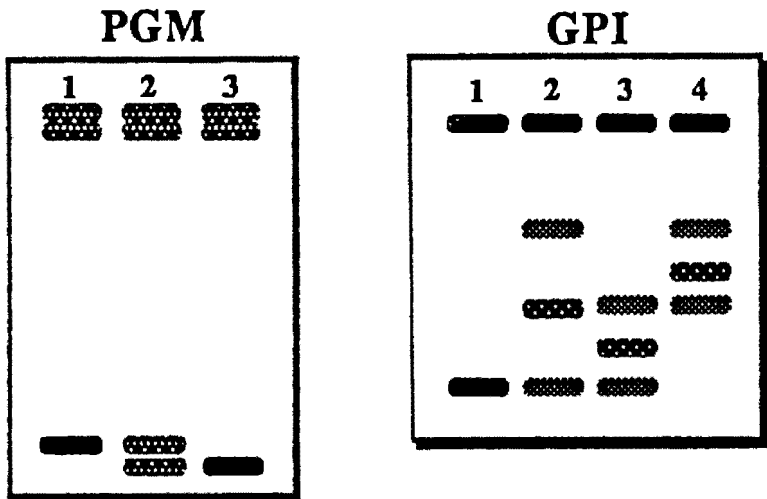
Beschreibung der Sorten Thompson Seedless, Concord und ihrer F₁-Hybriden

Trait	TS	A29-65	A29-66	A30-12	Concord
Cluster size (g)	454	178	320	45	160
Cluster shape	conical winged	conical to cylindrical winged	cylindrical to conical winged	conical winged	cylindrical winged
Berry wt (g)	1.58	2.6	3.08	1.19	2.51
Berry shape	oval/ovate	round	round	round	round
Berry color	white	white	dk blue	white	dk blue
Skin	adheres to flesh	separates from flesh	separates from flesh	separates from flesh	separates from flesh
Flesh	firm	tough	tough	soft	tough
Concord flavor ¹⁾	—	+	++	+	++
Seeded/seedless ²⁾	—	— large	+	— large	+
Avg. seed wt (mg)	6	15	98	39	62

¹⁾ + = present; — = absent.

²⁾ — = seedless; + = seeded.

hybrid status (DURHAM *et al.* 1987; GOLDY *et al.* 1988; CHAPARRO *et al.* 1989). Staining for phosphoglucumutase (PGM) indicated that Concord was homozygous for a fast migrating allele (FF) at the PGM-2 locus, Thompson Seedless was homozygous slow (SS), and the seedlings were heterozygous (FS), carrying both parental alleles (Fig.). Glucose phosphate isomerase (GPI) also gave positive identification of the hybrids. There appears to be two loci encoding GPI and it has a dimeric structure. Thompson Seedless and Concord are homozygous for the same allele at the fast locus as are the hybrids. Concord is homozygous for a slow allele and Thompson Seedless is heterozygous for a fast migrating allele and for an allele that migrates at an intermediate rate at the slow locus. The fruiting hybrids show intermediate patterns for allele migration at the slow locus compared to the parents.



Isozyme banding patterns for Concord (lane 1 for PGM and GPI), Thompson Seedless (lane 3 for PGM and lane 4 for GPI) and their hybrids (lane 2 for PGM; lane 2 = A29-66, lane 3 = A25-48, A29-65, A30-12 for GPI).

Bandenmuster der Isoenzyme der Sorten Concord (Spur 1: PGM und GPI), Thompson Seedless (Spur 3: PGM; Spur 4: GPI) und ihrer F₁-Hybriden (Spur 2: PGM; Spur 2 = A29-66, Spur 3 = A25-48, A29-65, A30-12 für GPI).

All the hybrids had some level of labrusca flavor indicating the plants were of hybrid origin. REYNOLDS *et al.* (1982) state that the labrusca flavor is made up of many chemical components and is difficult to breed against, suggesting it is controlled by a dominant factor/s. Methyl anthranilate, a major flavour component of labrusca and two other volatile esters are suggested to be controlled by three dominant complementary factors.

Fruit of the F₁ Thompson Seedless × Concord seedlings was either white or black. BARRETT and EINSET (1969) proposed the genotype Bbrr for the black fruited Concord and bbrr for Thompson Seedless. Using this hypothesis, 1/2 of the progeny should be colored (black) and 1/2 white. Colored fruit in the F₁ show the transmission of the colored (black) gene that could only come from the pollen parent, assuring that the plants developed are zygotic. All fruiting F₁ hybrids also had large seed traces indicating they did not originate from somatic tissue of Thompson Seedless.

Summary

Seedlings from open pollination and controlled crosses of Thompson Seedless were different from the parent in either fruit color, seed size, flavor or isozyme pattern. These results confirm that plants produced by *in ovulo* embryo culture of Thompson Seedless are usually zygotic in origin. This is a useful tool for producing hybrid plants from stenospermic seedless genotypes, allowing the hybridization of seedless by seedless and permitting many crosses previously unattainable.

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