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INTROGRESSION OF SEEDLESSNESS FROM BUNCH GRAPES INTO MUSCADINE GRAPES

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Abstract. **Two distinguished types of grape vines, bunch (*Euvitis* Planch) and muscadine (*Muscadinia* Planch) grapes, are cultivated commercially. Bunch grapes are grown world-wide while the cultivation of muscadine grapes is limited to Florida and other southeastern states. A major problem for the muscadine grape industry is the lack of a seedless commercial cultivar. On the other hand, seedlessness has been well known in bunch grapes. Therefore, it would be extremely beneficial to the grape industry in the South to develop seedless muscadine grapes. Possible routes to introduce seedlessness from bunch grapes to muscadines were investigated in this study. Pollen tube study revealed that it is difficult to use muscadines as the female parent when crossing with bunch grapes because of prefertilization barriers. Instead, seedlessness can be transferred to muscadines by using seedless bunch grapes as the female parent combined with embryo rescue.**

Wild and cultivated grapevines belong to the genus *Vitis* in the family *Vitaceae*. The genus *Vitis* is divided into two subgenera: *Euvitis* Planch (with common name bunch grape) and *Muscadinia* Planch (with common name muscadine grape) (Olien, 1990; Winkler et al., 1974). The former has 38 chromosomes while the later has 40 chromosomes. *V. vinifera* of *Euvitis* is the predominant commercial species grown world-wide although many other species have been described in this section. Only three species have been identified in the subgenus *Muscadinia*; *V. rotundifolia* is the only species with commercial value.

Muscadine grapes (*V. rotundifolia*) are native to the Southeastern United States and characterized by very good disease and pest resistance as well as a unique flavor. The absence of seedlessness is the major obstacle for wide acceptance of muscadine grapes in the fresh fruit market. On the other hand, seedlessness due to stenospermocarpy has been well known in bunch grapes. In order to develop a seedless muscadine grape variety, the source of seedlessness must be the bunch grape. There are two possibilities: muscadine as seed parent crossed with seedless bunch grapes or vice versa. Hybrids have been difficult to obtain when muscadine grapes were used as female parents (Bouquet, 1980; Chaparro et al., 1989; Olmo, 1971; Olmo, 1986). On the other hand, hybrids were readily produced when bunch grapes were used as seed parents crossed with muscadine grapes. Few breeders have made crosses using seedless bunch grape as the female parent, however, since special facilities and extra effort are required for embryo rescue in order to recover the hybrids. These difficulties have seriously hampered the progress toward developing seedless muscadine cultivars.

Although most grape breeders agree that muscadine grapes are difficult to use as the seed parent when crossing with bunch grapes, there has been no report about the barriers for gene flow from bunch grapes into muscadine grapes by sexual means. It is not clear if any of the *in vitro* culture techniques such as embryo rescue will help the production of the interspecific hybrids when muscadine is used as the seed parent. On the other hand, although more than a dozen hybrids were produced through embryo rescue when seedless bunch grapes were used as female (Goldy et al., 1988), the efficiency of hybrid recovery was extremely low. Therefore, the feasibility of using this method to obtain seedless muscadine grapes is uncertain. Indeed, it is still not clear what the most efficient route to introgress seedlessness from bunch grapes to muscadines is. The purpose of this investigation is to understand the incompatibility of muscadine × bunch grape and determine the most efficient way to incorporate seedlessness from bunch into muscadine grapes.

Materials and Methods

Emasculation and pollination. Emasculation was made a day before pollination for the perfect flower grape cultivars, and paper bags were used to cover the flower clusters immediately after emasculation. No emasculation was needed for those muscadine cultivars with female flowers only ('Fry', 'Jumbo', and 'Summit'). For these clusters, opened flowers were removed and the clusters were subsequently bagged a day before pollination. A glass rod was used for transferring pollen to stigmas.

Seed production and pollen tube study. Fifteen cross combinations, which included seven muscadine female parents and three bunch grape pollen parents were used for the study of hybrid productivity between muscadine (female) × bunch grapes (Table 1). Nine cross combinations, including three muscadine cultivars ('Fry', 'Jumbo', and 'Summi') as female,

Table 1. Berry and seed production of muscadine (female) × bunch grapes.

Cross combinations	Flowers	Berries	%	Seeds
Fry × Orlando seedless	250	5	2.0	18
Fry × Thompson S.L.	450	3	0.7	10
Higgins × Orlando S.L.	850	4	0.5	15
Higgins × Flame S.L.	900	20	2.2	77
Higgins × Thompson S.L.	900	6	0.7	22
Jumbo × Orlando S.L.	350	7	2.0	25
Jumbo × Flame S.L.	400	0	0	0
Jumbo × Thompson S.L.	400	2	0.5	8
PK Hunt × Orlando S.L.	1400	14	1.0	53
PK Hunt × Flame S.L.	900	10	1.1	39
PK Hunt × Thompson S.L.	750	3	0.4	12
Summit × Orlando S.L.	400	1	0.3	1
Summit × Thompson S.L.	400	1	0.3	1
Dixie × Orlando S.L.	650	4	0.6	16
Welder × Orlando S.L.	1225	43	3.5	170

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and three bunch grape cultivars ('Blanc du Bois', 'Orlando Seedless', and 'Thompson Seedless') as pollen parent, were used for the pollen tube study. Five pollinated flowers in each cross were collected 8 hours, 1 day, 2 days, and 3 days after pollination and fixed in FAA (1 formalin : 1 acetic acid : 8 alcohol). The fixed pistils were stored at 4°C until microscope examination. The pistils were examined under a fluorescent microscope after aniline blue staining. The number of flowers with pollen tubes, and the point which the pollen tubes had reached in the style were recorded.

Ovule and embryo culture. Twelve cross combinations, including four bunch grapes and three muscadine cultivars, were used for the embryo rescue study (Table 3). 'Flame Seedless', 'Perlette' and 'Thompson Seedless' were pollinated in Fresno, California, and 'Orlando Seedless' was pollinated in the vineyard at Florida A&M University. Berries were harvested 6 weeks after pollination, and those obtained from pollinations in California were shipped to FAMU in dry ice and refrigerated at 4°C upon receipt. Berries were surface sterilized with 70% ethanol and 50% liquid bleach (with 0.1% Tween 20), and then rinsed three times with sterile distilled water. Ovules were dissected from the berries; half of them were transferred to solid medium, and the other half were transferred to liquid medium. Thirty ovules were put in each petri dish containing solid medium. Twenty ovules were placed on a filter paper bridge in each baby food jar with liquid media. The medium for grape ovule culture was developed by Ramming and Emershad (1990), with 0.7% agar added to make the solid medium. Ovules were cultured on the media for 2 to 3 months. Embryos were then cut out under a dissecting microscope and transferred to 20 × 150 mm glass tubes containing woody plant media (McGown and Lloyd, 1981).

Results and Discussion

Barriers to muscadine × bunch grapes. When muscadines were used as seed parent pollinated with bunch grape pollen parents, the number of berries compared to total pollinated flowers was extremely low (Table 1), with the highest being 3.5% ('Welder' × 'Orlando seedless'), while most were less than 1%. Such a low percentage of fruit setting makes the breeding program very inefficient. Understanding the possible causes of the low fruit set is necessary in order to find ways to overcome the incompatibility and increase the production of hybrids.

In the interspecific crosses of muscadine (female) × bunch (male), pollen could hydrate and germinate on the stigma surface. Pollen tubes could also penetrate the stigma without obstacles. However, most of the bunch grape pollen tubes were arrested in the style near the stigma of muscadine grapes. Very few pollen tubes were found at the bottom of the styles and the ovules. The result indicated that the failure of seed set in the muscadine × bunch crosses resulted from prefertilization barriers.

Embryo rescue may help to recover hybrids if the failure of seed set is a result of embryo abortion. However, ovule and embryo culture will not help to overcome the incompatibilities of muscadine × bunch grapes revealed in this study since the barriers occurred before fertilization. Overcoming the prefertilization barriers should therefore be the first step in obtaining hybrids when muscadines are used as female parent.

Embryo rescue to recover hybrids of seedless bunch × muscadine grapes. Unlike the muscadine as female pollinated by bunch grape pollen, the muscadine pollen tubes were healthy in the style of the bunch grape and grew all the way down to the bottom of the style and entered the ovule. Subsequently, much better berry set was obtained when bunch grapes were used as the seed parent (Table 2). 'Orlando Seedless', a Florida cultivar derived from crosses between *V. vinifera* and native American species, is the only seedless bunch grape cultivar resistant to Pierce's disease. Besides 'Orlando Seedless', three other well-known seedless cultivars with *V. vinifera* genotype, 'Flame Seedless', 'Perlette', and 'Thompson Seedless', were also pollinated with muscadine cultivars 'Carlos', 'Noble', and 'Welder'. Approximately 3000 flowers were pollinated in each cross combination. Like 'Orlando Seedless' × muscadine cultivars, 10% to 20% of the pollinated flowers set fruits (data not shown). After dissecting, we found that some berries contained no seed trace (ovule) or very tiny seed traces which could not be used for culture, while the others had two to three relatively large seed traces. The number of seed traces which could be cultured was approximately the same as the number of berries obtained (Table 2). Embryos were dissected after ovules were cultured 2-3 months in either liquid or solid media. An average 11.3% of the ovules yielded embryos (Table 3). No difference was found between liquid and solid medium regarding the percentage of embryos being recovered. The embryos were then transferred to a woody plant medium in 20 × 150 mm glass tube and about 70% of these embryos have germinated.

Table 2. Berries and ovules obtained from Orlando Seedless (female) × muscadine grapes.

	Pollinations	Berries	Berries/ Flowers (%)	Ovules	Ovules/ Berries
Carlos	1256	95	7.5	85	0.89
Noble	1256	176	14.0	157	0.89
Welder	1235	73	9.4	73	0.63
AA 12-3	1416	246	17.4	226	0.92
AA 6-48	512	52	10.2	70	1.35
Total	5675	642	11.3	611	0.95

Table 3. Embryos recovered from seedless bunch grapes by muscadines.

Crosses	Ovules	Embryos	Embryo/ovules (%)
Orlando Seedless ×			
Carlos	134	21	15.7
Noble	248	27	10.9
Welder	73	12	6.7
Flame Seedless ×			
Carlos	84	5	6.0
Noble	236	29	12.3
Welder	260	55	21.2
Perlette ×			
Carlos	166	22	13.3
Noble	370	23	6.2
Welder	60	7	11.7
Thompson Seedless ×			
Carlos	?	33	?
Noble	229	18	7.9
Welder	178	12	6.7
Total	2038	231	11.3

This result indicates that embryo rescue is an effective and feasible method to produce muscadine-bunch hybrids for seedless muscadine cultivar development. The rate of embryo recovery is much higher than the previous report (Goldy et al., 1988) and similar to those of intraspecific crosses of *V. vinifera* (Barlass et al., 1988).

Due to the barriers of prefertilization, berry set was very low when muscadine was used as female parent crossed with bunch grapes. Moreover, it is not clear that the small number of seeds obtained from these crosses were true hybrids or were from contaminated muscadine pollen. A further study using morphology, isozyme, or DNA markers is needed to verify the status of these hybrid seeds.

Since barriers occur before fertilization for muscadine × bunch crosses, introgression of seedlessness to muscadine is not practical if there are no feasible methods to overcome the prefertilization barriers. We therefore believe that using seedless bunch grapes as the female parent combined with embryo rescue is the better way to introgress seedlessness from bunch grapes to muscadine grapes, despite the extra facilities and effort required. A disadvantage of using seedless bunch grapes as female parents is that muscadine pollen must be stored for one year since the blooming season of muscadine grapes is normally one month later than for bunch grapes. For unknown reasons, hybrid productivity is reduced when stored pollen is used even though the germination rate is still the same (J. Harris, CFREC, IFAS, Leesburg, personal communication).

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SUCROSE, ABSCISIC ACID AND METHYLGLYOXAL BIS-(GUANYLHYDRAZONE) AFFECT GRAPE SOMATIC EMBRYOGENESIS

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Abstract. **The effects of sucrose, abscisic acid (ABA) and methylglyoxal bis-(guanylhydrazone) (MGBG) on grape (*Vitis vinifera* L. cv. Thompson Seedless) somatic embryogenesis was examined by subculturing somatic embryos and embryogenic cells to somatic embryo maintenance medium (EMM) containing either 60, 90, 120, 150, or 180 g/liter sucrose; 0, 1, 10 or 100 M μABA; or 0, 0.1, 1 or 10 mM MGBG. The number of cotyledonary stage somatic embryos resembling zygotic embryos was increased by culturing embryogenic cells on EMM with 120 g/liter sucrose for 2 to 3 months. No difference was detected between the control (60 g/liter) and the other sucrose treatments. ABA (10-100 μM) reduced precocious germination of cotyledonary and torpedo stage somatic embryos without reducing embryo viability. The same ABA concentrations reduced secondary embryo production among cotyledonary stage embryos but not torpedo stage embryos. ABA inhibited plant formation from cotyledonary stage somatic embryos, but promoted plantlet regeneration from torpedo stage embryos at the 1 μM concentration. Adding 1 or 10 mM MGBG to EMM inhibited the growth of grape embryogenic cells and somatic embryos. The number of cotyledonary stage somatic embryos resembling zygotic embryos was reduced 88% to 100% when embryogenic cultures were incubated on EMM with 1 or 10 mM MGBG, respectively, for 3 months.**

Somatic embryos have historically been used for clonal propagation of elite lines (Attree et al., 1990), or to obtain genetically engineered plants following infection with *Agrobacterium tumefaciens* (Chee, 1990; Delbreil et al., 1993) or bombardment with DNA-coated tungsten particles (Cao et al., 1992; Vasil et al., 1992). Somatic embryos have also been used as "synthetic seeds", either encapsulated in alginate (Redenbaugh et al., 1987) or fluid-drilling gel (Kitto et al., 1991), dehydrated naked (Gray, 1989; Gray et al., 1987), or dehydrated following encapsulation (Kitto and Janick, 1985a,b; Kim and Janick, 1989; Janick et al., 1989).

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