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Seedless-Seedless Grape Hybridization via *In-Ovulo* Embryo Culture

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Summary

The effect of berry sampling time and genotypic difference in stenospermocarpic seedless grapes on the recovery of viable embryos through *in ovulo* embryo culture was examined. In 5 open-pollinated seedless cultivars, berry weight increased consistently as the fruit developed, but the ovule length did not correspondingly increase, being either constant or shrinking. The recovery rate of viable embryos significantly increased as the days after anthesis proceeded, especially on the 40th day. During the culture, polyembryony was occasionally observed in some ovules. At the micropylar end of the ovule, most multiple embryos emerged adventitously from the hypocotyl-root axis of zygotic embryo. In 5 open-pollinated selections and 6 cultivars, véraison ranged from 49 to 66 days after anthesis. Varietal difference in the recovery of viable embryos from ovules sampled at véraison was significant, ranging from 0 to 24.7%. In a total of 20 crosses between seedless accessions, the recovery rate of viable embryos significantly varied from 2.7 to 47.0%. The rate of plant recovery *in vitro* ranged from 0.6 to 24.9%. F_1 progenies produced through *in ovulo* embryo culture in seedless x seedless crosses segregated as follows; completely seedless : perceptible seed traces : seeded = 24 : 56 : 21.

Key Words: embryo rescue, grape, ovule culture, seedless, stenospermocarpy.

Introduction

Seedless table grapes are preferred by consumers in much of the world, especially in the USA (Gray et al., 1990) and Japan (Bessho et al., 1998). Two types of seedlessness are known to occur in cultivated grape, *Vitis* spp. (Pearson, 1933; Stout, 1937; Pratt, 1971): i) parthenocarpy, in which fruit set does not require fertilization; and ii) stenospermocarpy, in which fertilization occurs but the fertilized embryos are underwent various degrees of ovule abortion, resulting in almost none to perceptible seed traces. Due to their potentially large berry size, stenospermocarpic grapes are commercially introduced to an increasing extent than are parthenocarpic ones (Clingelerffer, 1998). The use of parthenocarpic grape cultivars as pollen parents results in a few seedless F_1 progenies (Stout, 1939; Sato et al., 1994)

Breeding of stenospermocarpic seedless grapes by conventional methods is limited by several problems: i) seedless cultivars can be used only as pollen parents; ii) the frequency of seedless progeny in crosses of seeded x seedless is generally low (Loomis and Weinberger, 1979; Spiegel-Roy et al., 1985): iii) genetic control of seedlessness has not yet been clearly defined (Reisch and Pratt, 1996; Roytchev, 1998). *In ovulo* embryo culture provides an attractive alternative to the conventional breeding method by allowing recovery of progeny from abortive ovules in seedless x seedless crosses (Cain et al., 1983; Emershad and Ramming, 1984; Spiegel-Roy et al., 1985; Gray et al., 1990; Tsolova, 1990; Wang et al., 1990; Gribaudo et al., 1993). According to Ramming (1990), selected seedless parents may be hybridized directly without a genetic bridge (seeded cultivar) to combine each desirable trait in addition to shortening the breeding cycle at least 5 years. However, general agreement has not yet been obtained among investigators with respect to berry sampling time, media composition, and culture period.

Our breeding program at Fukuoka Agricultural Research Center, Japan which was activated in 1974, has seedlessness as its primary objective. Since then, using the conventional methods of seeded x seedless crosses, no favorable accessions with complete seedlessness have been selected but some promising parental lines with perceptible seed traces were obtained ('F 1851', 'F 4016': 'Super Hamburg' x 'Himrod'; 'F 4075': 'Italia' x'Bronx Seedless'). From 1988 to 1995, we have performed *in ovulo* embryo culture to increase the frequency of completely seedless seedlings. Our method may contribute to an understanding of genetic factors

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controlling stenospermocarpic seedlessness. The present research has been undertaken with the following aims: i) to study the effect of berry sampling time on embryo recovery; ii) to compare genotypic difference to the extent of embryo/ovules development; iii) to examine the segregation of seedless progeny in seedless x seedless crosses.

Materials and Methods

Sixteen diploid seedless grape accessions were used: *Vitis vinifera* L. ('Pusa Seedless', 'Ruby Seedless', 'Thompson Seedless', 'Monukka', 'CG 102295'); *V.* x *labruscana* Bailey ('Aki Seedless', 'Bronx Seedless', 'Himrod', 'Prima Seedless', 'Romulus', 'Suffolk Red', 'Akitsu No.2', 'Akitsu No.3', 'F 1851', 'F 4016', 'F 4075'). On the basis of degree of seed development (Sato et al., 1994), each berry was recorded as follows: score 0; completely seedless, score 1; perceptible seed traces, score 2; seeded. The berries were taken from i) 5 open-pollinated cultivars at 20, 30, and 40 days after anthesis in 1988, ii) 5 open-pollinated selections and 6 cultivars at véraison in 1989. From 1988 to 1995, a total of 20 controlled crosses among seedless grape cultivars was made.

In-ovulo embryo culture was performed following a slight modification of the procedure described by Gray et al. (1990). The berries were surface-sterilized by 1.0% sodium hypochlorite for 15 min., then rinsed three times with sterile water. After ovules were aseptically excised, 20 were placed in a 100-ml culture flask containing 20 ml of autoclaved culture medium (pH 5.8) (Fig. 1A). All media contained 3% (w/v) sucrose and 0.7% (w/v) agar, and all cultures were grown at 25 ± 2 °C under 16-hr photoperiod with ca. 3000 lx cool-white fluorescent illumination. Ovules were cultured on Nitsch's medium (Nitsch and Nitsch, 1969) with 10 μM indoleacetic acid (IAA) for 14 ± 1 week, after which they were dissected and subjected to embryo culture.

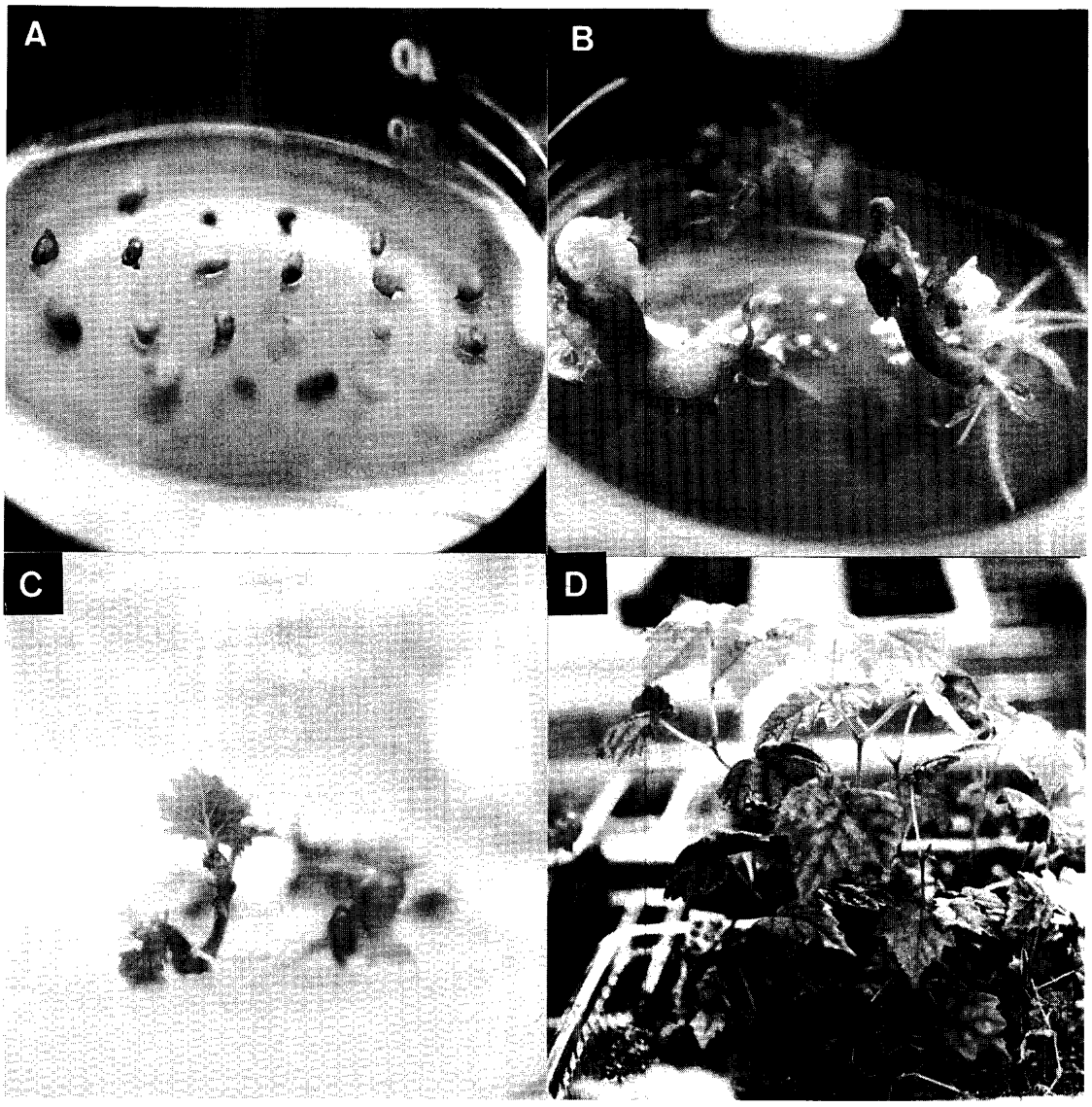

Fig. 1. *In ovulo* embryo culture of open-pollinated grape cultivar, 'Aki Seedless'.
A: Ovules on Nitsch's medium, pH 5.8. B: Germination of embryos excised from cultured ovule.
C: Shoot growth from embryos (viable embryo). D: Plantlets under acclimatization.

Excised embryos were placed on 1/2 strength MS medium (Murashige and Skoog, 1962) containing 5 μM zeatine and subcultured to induce germination (Fig. 1B). According to Cain et al. (1983), embryos in which cotyledons expanded and became green were considered "viable" (Fig. 1C). Subsequently, shoots from viable embryos were multiplied on MS medium with 3 μM benzyladenine (BA). After division of branching shoots, each shoot was rooted and subcultured on 1/4 strength MS medium with 0.01 μM α-naphthaleneacetic acid (NAA). Four to five rooted plantlets were acclimatized in a polyethylene container (12 x 17 x 6 cm) containing vermiculite (Fig. 1D). The container was covered with a glass plate. Following a gradual decrease in relative humidity to 60%, acclimated plantlets were transferred in 0.5-liter pots, and established in the vineyard and subjected to progeny testing.

Results

The effect of berry sampling time on recovery rate of viable embryos through *in ovulo* embryo culture was tested by using five open-pollinated seedless cultivars (Table 1). The linear increase in berry weight was recorded up to 40 days after anthesis when 80% of the calyptra had fallen, whereas ovule length did not increase linearly; it either stopped growing or shrank. 'Bronx Seedless' attained the highest berry weight (2.22 g) and ovule length (2.68 mm) on the 40th day, whereas 'Suffolk Red' had the lowest ovule length (1.37 mm) and berry weight (1.49 g). As days after anthesis proceeded, the recovery rate of viable embryos (%) significantly increased with the exception of 'Suffolk Red'. All cultivars had viable embryos on the 40th day; the respective rates for 'Aki Seedless', 'Bronx Seedless', 'Himrod', 'Prima Seedless', and 'Suffolk Red' were 35.8, 11.7, 12.5, 10.0, and 6.4%. In addition, the berries became progressively soft in 'Bronx Seedless', 'Himrod', and 'Prima Seedless' after the 40th day.

During the culture, multiple embryos occasionally differentiated in some ovules of 'Aki Seedless' and 'Himrod', principally at the micropylar end of the ovule, on the hypocotyl-root axis of a zygotic embryo (Fig. 2). These embryos, derived from cultured ovule, were placed on the germination medium; only the most vigorous embryo was subcultured. Data are thus based on the recovery of a single embryo per ovule.

Ovules of the 5 open-pollinated selections and 6 cultivars were excised approximately at véraison when over 50% of berries per bunch had softened (Table 2). The véraison stage ranged from 49 ('F 4016') to 66 ('Monukka') days after anthesis. Viable embryos were frequently obtained in a selection of 'Akitsu No.3' (24.7%: 'Steuben' x 'Himrod') and 'Akitsu No.2' (23.7%: 'Muscat Bailey A' x 'Romulus'), followed by 'Romulus' (11.0%) and CG 102295 (10.5%). Other accessions varied from 4.7 ('Bronx Seedless') to 8.6% ('F 4075'). No viable embryos were recovered from cultured ovules of 'Monukka'.

There were significant differences in the percent embryo recovery obtained from crosses between seedless accession (Table 3). Percentages of the viable embryos ranged from 2.7 to 47.0%. In the culture of

Table 1. Effect of berry sampling date on recovery of viable embryos from open-pollinated seedless grape accessions.

Accession (score)[z]	Days after anthesis[y]	Weight of berry (g)	Length of ovule (mm)	No. of ovules cultured	No. of viable embryos (%)
Aki Seedless (0)	20	0.61	1.54	140	3 (2.1c)[x]
	30	0.98	2.01	140	19 (13.6b)
	40	2.00	1.70	120	43 (35.8a)
Bronx Seedless (0)	20	0.84	2.25	137	4 (2.9b)
	30	1.25	2.15	140	4 (2.9b)
	40	2.22	2.68	128	15 (11.7a)
Himrod (0)	20	0.61	1.54	140	3 (2.1b)
	30	0.98	2.01	133	2 (1.5b)
	40	2.00	1.70	136	17 (12.5a)
Prima Seedless (0)	20	0.79	2.15	140	4 (2.9b)
	30	1.55	2.13	139	9 (6.5b)
	40	1.99	2.40	140	14 (10.0a)
Suffolk Red (0)	20	0.69	1.56	116	4 (3.5a)
	30	1.11	1.53	140	6 (4.3a)
	40	1.49	1.37	140	9 (6.4a)

[z] Score 0; completely seedless.

[y] 80% of calyptra fallen.

[x] Mean separation within columns by Scheffe's multiple range test, $p = 0.05$.

Table 2. Production of viable embryos by culturing ovules obtained from open-pollinated seedless grape accessions.

Accession (score)[z]	Days after anthesis[y]	No. of ovules[x] cultured	No. of viable embryos (%)
Akitsu No. 3 (0)	57	166	41 (24.7a)[w]
Akitsu No. 2 (0)	55	152	36 (23.7a)
Romulus (0)	50	82	9 (11.0b)
CG 102295 (0)	53	143	15 (10.5b)
F 4075 (1)	55	266	23 (8.6b)
Pusa Seedless (0)	61	158	13 (8.2b)
Thompson Seedless (0)	55	139	11 (7.9b)
F 4016 (1)	49	183	14 (7.7b)
Ruby Seedless (0)	59	189	14 (7.4b)
Bronx Seedless (0)	51	149	7 (4.7b)
Monukka (0)	66	160	0 (0c)

[z] Score 0; completely seedless, Score 1; perceptible seed traces.
[y] 80% of calyptra fallen.
[x] Ovules were sampled at véraison.
[w] Mean separation within a column followed by Scheffe's multiple range test, $p = 0.05$.

'Aki Seedless' ovules from five pollen parents, viable embryos were 47.0 ('Prima Seedless'), 44.7 ('Bronx Seedless'), 27.0 ('Himrod'), 15.3 ('F 4075'), and 12.1% ('F 1851'). In culturing 'Akitsu No. 2' ovules, derived from four pollen parents, the recovery values were 47.0 ('Himrod'), 29.7 ('Prima Seedless'), 25.8 ('Bronx Seedless'), and 17.0% ('Aki Seedless'). Reciprocal crosses of 'Himrod' with three cultivars yielded viable embryos ranging from 3.2 ('Bronx Seedless') to 10.4% ('Prima Seedless'); they averaged 5.9% when 'Himrod' was used as a pollen parent. In contrast, when 'Himrod' was used as a female parent, 10.4 to 11.9% viable embryos were obtained. As for other cross combinations, embryo recovery of 'Thompson Seedless' x 'F 4016' (16.4%) and 'F 4075' x 'CG 102295' (22.4%) was relatively high; that of 'Suffolk Red' x 'F 4016' (3.6%), 'Bronx Seedless' x 'F 4016' (2.7%), and 'F 1851' x 'F 4075' (3.5%) was rather low. The rate of plant recovery *in vitro* ranged from 0.6 ('Bronx Seedless' x 'Himrod') to 24.9% ('Aki Seedless' x 'Prima Seedless').

Out of 545 seedlings obtained from *in ovulo* embryo culture, 275 seedlings were transplanted in the vineyard, of which 73% fruited in 1994 (Table 4). In the cross of seedless x seedless, the average of 34 fruited F_1 pro-

Table 3. Production of viable embryos and plants by culturing ovules obtained from seedless × seedless crosses.

Cross (score × score)[z]	No. of ovules[y] cultured	No. of viable embryos (%)		No. of plants established *in vitro* (%)	
Score 0 × Score 0					
Aki Seedless × Prima Seedless	177	83	(47.0a)[x]	44	(24.9)[w]
Aki Seedless × Bronx Seedless	273	122	(44.7a)	65	(23.8)
Aki Seedless × Himrod	289	78	(27.0bc)	24	(8.3)
Akitsu No. 2 × Himrod	245	115	(47.0a)	57	(23.3)
Akitsu No. 2 × Prima Seedless	696	207	(29.7b)	86	(12.4)
Akitsu No. 2 × Bronx Seedless	395	102	(25.8bc)	56	(14.2)
Akitsu No. 2 × Aki Seedless	666	113	(17.0d)	61	(9.2)
Himrod × Prima Seedless	200	31	(15.5d)	10	(5.0)
Himrod × Suffolk Red	336	52	(15.5d)	9	(2.7)
Himrod × Bronx Seedless	311	37	(11.9e)	15	(4.8)
Prima Seedless × Himrod	77	8	(10.4e)	4	(5.2)
Suffolk Red × Himrod	127	5	(4.0f)	3	(2.4)
Bronx Seedless × Himrod	157	5	(3.2f)	1	(0.6)
Score 0 × Score 1					
Thompson Seedless × F 4016	67	11	(16.4d)	3	(4.5)
Aki Seedless × F 4075	452	69	(15.3d)	30	(6.6)
Aki Seedless × F 1851	332	40	(12.0e)	10	(3.0)
Suffolk Red × F 4016	416	15	(3.6f)	3	(0.7)
Bronx Seedless × F 4016	450	12	(2.7f)	6	(1.3)
Score 1 × Score 0					
F 4075 × CG 102295	509	114	(22.4c)	57	(11.2)
Score 1 × Score 1					
F 1851 × F 4075	57	2	(3.5f)	1	(1.8)

[z] Score 0; completely seedless, Score 1; perceptible seed traces.
[y] Ovules were sampled at véraison.
[x] Mean separation within a column followed by Scheffe's multiple range test, $p = 0.05$.
[w] No. of plants established *in vitro*/No. of ovules cultured × 100%.

Table 4. Segregation of seedless progenies in the seedless × seedless crosses.

Cross	No. of plants regenerated	No. of plants fruited	Degree of seedlessness		
Score 0 × Score 0			Seedless	Seed traces	Seeded
Aki Seedless × Bronx Seedless	50	39	9 (23%)	24 (62%)	6 (15%)
× Himrod	18	9	2 (22%)	6 (67%)	1 (11%)
× Prima Seedless	31	19	4 (21%)	12 (63%)	3 (16%)
Akitsu No. 2 × Bronx Seedless	47	35	6 (17%)	20 (57%)	9 (26%)
× Himrod	47	40	12 (30%)	20 (50%)	8 (20%)
× Prima Seedless	82	59	15 (25%)	30 (51%)	14 (24%)
Mean	46	34	8 (24%)	19 (56%)	7 (21%)

genies showed in the following ratio of completely seedless : perceptible seed traces : seeded = 8 (24%): 19 (56%) : 7 (21%). On the percentage base, 'Akitsu No.2' x 'Himrod' gave the highest completely seedless seedlings (30%) and 'Akitsu No.2' x 'Bronx Seedless' the lowest (17%). It is noteworthy that seeded seedlings (11 to 26%) were always produced from crosses between the seedless accessions.

Discussion

Embryo culture is useful when there is poor embryo development or abortion after anthesis or pollination, thereby increasing potential grape germplasm combinations by intergeneric- (Goldy and Amborn, 1987; Goldy et al., 1988) and interspecific- or intraspecific-crosses (Cain et al., 1983; Spiegel-Roy et al., 1985; Gray et al., 1990; Ramming et al., 1990). The successful growth of ovule-cultured embryos depends on berry sampling time, genotypic combination, culture medium, and environment.

The weight of berries increased consistently as the fruit developed, but a corresponding ovule development did not always accompany berry growth. A similar trend was confirmed in berry development of seedless grapes (Spiegel-Roy et al., 1985; Wang and Horiuchi, 1990; Singh and Brar, 1992). The optimum berry sampling time in our study was found to be from 49 to 66 days after anthesis which corresponds to that reported by other researchers: 68 days (Emershad and Ramming, 1984); 43 or 69 days (Spiegel-Roy et al., 1985); 42 or 70 days (Emershad et al., 1989); 40 or 60 days (Gray et al., 1990); 52 or 66 days (Tsolova, 1990); 41 or 49 days (Gribaudo et al., 1993); and 60 or 70 days (Yamashita et al., 1995). Although the differences depend on the cultivar, these periods overlap the véraison stage of the berry development, ranging between 49 and 84 days (Winkler et al., 1974; Mulline et al., 1992; Coombe, 1992). Berry sampling at later stages, e.g. the 70th day or more, is not likely to be suitable for *in vitro* embryo rescue because the embryos enter dormancy progressively during véraison (Gray et al., 1990; Horiuchi et al., 1991). However, embryo germinability at earlier stages before the 40th day is low because of the immaturity of embryo per se (Cain et al., 1983; Horiuchi et al., 1991). Hence, embryo recovery was low at 20 and 30 days after anthesis. Gray et al. (1990) also found that culturing ovules 10 and 20 days after pollination led to poor embryo survival. Considering previous and our results, the optimum time for berry sampling for *in vitro* embryo rescue is 50 ± 10 days after anthesis.

Genotypic differences with respect to embryo/ovules development for the seedless females were significant, which agrees with previous studies (Spiegel-Roy et al., 1985; Goldy and Amborn, 1987; Emershad et al., 1989; Gray et al., 1990; Gribaudo et al., 1993). Cain et al. (1983) also indicated that the seedless pollen parent influences the frequency of viable embryos in crosses between seedless grapes. This is partly confirmed by our reciprocal cross tests. A significant variation in the recovery of viable embryos suggests that a degree of genetic compatibility exists among parental combinations. These findings should be helpful in determining how many ovule cultures (pollinations) are required to obtain the desired number of seedlings in a breeding program. Other factors affecting the behaviour of cultured embryos are as follows: media composition (Cain et al., 1983; Emershad et al., 1989; Gribaudo et al., 1993), interaction between genotype and medium (Gribaudo et al., 1993), and culture period and medium (Spiegel-Roy et al., 1985). With respect to embryo dormancy, it can be effectively broken by the application of exogenous cytokinins, especially BA (Gray et al., 1990). We observed that placement of dissected embryos on 5 μM zeatin MS medium stimulated rapid germination.

Polyembryony was infrequently encountered in the ovule culture. Durham et al. (1989) investigated the origin of polyembryos obtained during embryo rescue of progeny from crosses between seedless cultivars. They proposed that polyembryony originates through several mechanisms: i) fertilization of more than one egg cell in an embryo sac; ii) somatic embryogenesis from the zygote; and iii) embryogenic development from unfertilized gametic cells in addition to the zygote. Emershad et al. (1989) also showed that multiple embryo formations is significantly influenced by media,

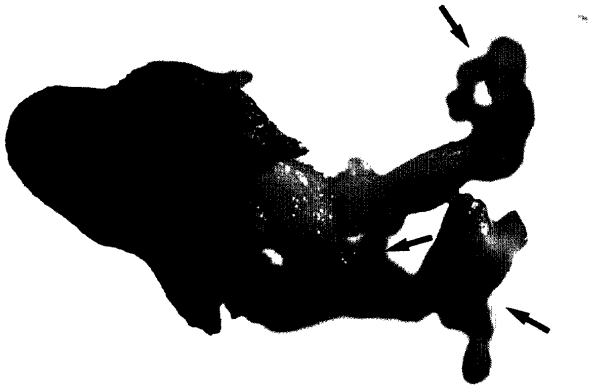

Fig. 2. Multiple embryos grown from ovules obtained from open-pollinated grape cultivar, 'Aki Seedless'. Arrows indicate embryos grown from hypocotyl-root axis of zygotic embryo.

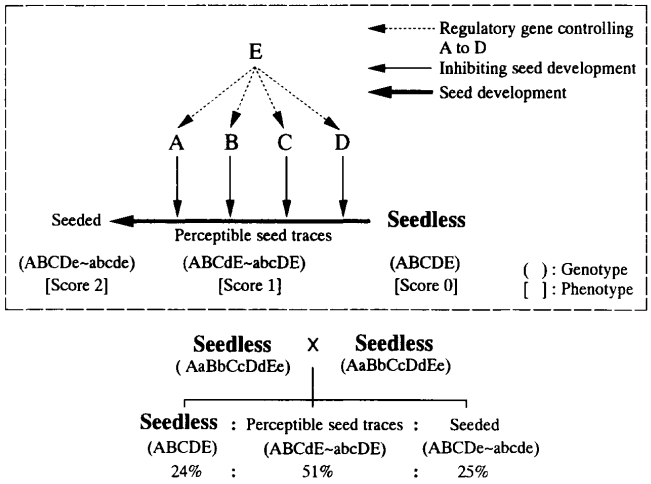

Fig. 3. Schematic illustration for the proposed genetic model of stenospermocarpic seedless grapes (Yamane, 1997). A, B, C, D: Dominant gene for complementary inhibiting seed development. E: Dominant regulatory gene controlling genes, A to D.

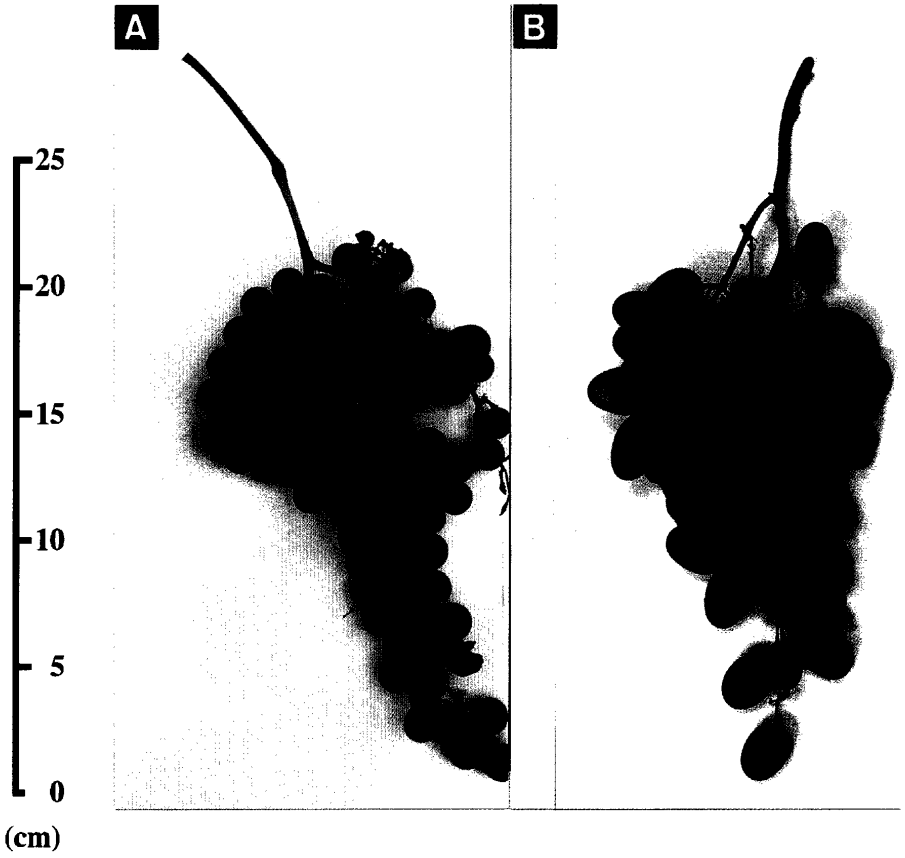

Fig. 4. Grapes of seedless-seedless crosses obtained via *in ovulo* embryo culture. A: Completely seedless with 2 to 3g in berry weight. B: Nearly completely seedless (negligible seed traces) with 5g in berry weight.

genotype, the interaction between media and genotype, and media and sampling time. Ramming (1990) concluded that the polyembryony is usually the result of somatic embryogenesis from the zygotic embryos and seems to be genotype-dependent. Thus, we assume that polyembryony arises somatically from zygotic embryos based on the position of multiple embryos attached to zygotic embryo.

Plant recovery rate through *in vitro* embro culture is generally low: 10.9 to 16.3% (Spiegel-Roy et al., 1985); 1.2 to 22.8% (Gray et al., 1987); 5.8 to 8.1% (Bouquet et al., 1989); 2.5 to 5.0% (Emershad et al., 1989); 0 to 7.5% (Tsolova, 1990); 0 to 14.8% (Gray et al., 1990). In our study, *in vitro* embro rescue resulted in 0.6 to 24.9% of cultured embryos developed into plants. This methodology requires careful laboratory techniques

and skilled labor to successfully rescue sufficient numbers of embryos for breeding purposes (Reisch and Pratt, 1996). Gray et al. (1990) speculated that embryo and plant development are independent events with different response stimuli during ovule culture.

Genetic basis of stenospermocarpic seedless grapes remains obscure due to the various degrees of seed development. The character of seedlessness has been reported to be controlled by a single or a few recessive genes (Stout, 1939; Weinberger and Harmon, 1964; Loomis and Weinberger, 1979; Spiegel-Roy et al., 1990; Bouquet and Danglot, 1996), although the possibility of dominant control has also been suggested (Stout, 1937). Roytchev (1998) proposed that seedlessness is a recessive trait but controlled also by dominant genes, entering into complex relationships in each specific hybrid combination. Crosses between completely seedless accessions revealed that the mean percentage of completely seedless: perceptible seed traces : seeded was segregated in a ratio of 24 : 56 : 21. When seedlessness is determined by recessive genes and the homozygous condition, no seeded phenotype should result from a cross between seedless parents. However, seeded seedlings were frequently observed here and by Gray et al. (1990). Based on a hypothesis by Yamane (1997)(Fig. 3), our segregation data may be explained by the following genetic model: inheritance of seedlessness is based on a complex system of four dominant genes, A to D behaving complementary in the direction of seed development inhibition, controlled by one dominant regulatory gene E. Thus, the probable phenotypic segregation in F_1 progenies from seedless x seedless crosses is expected as 24% (completely seedless): 51% (perceptible seed traces) : 25% (seeded), given that the seeded and seedless genotype are homozygous (aabbccddee) and heterozygous (AaBbCcDdEe), respectively. However, we could not undoubtedly support this model, because i) the plant recovery rate via *in ovulo* embryo culture was not sufficiently high as to make a genetic analysis, ii) it was not done to confirm the genetic origin to exclude the possibility of self-fertilization. The latter possibility is important when the number of recovered plants is extremly low. Gray et al. (1990) showed by isozyme analysis that 67 to 88% of the progeny are true hybrids between seedless grapes, and that about 12% of the progeny are not hybrids but most likely products of self-pollinations.

In our breeding work, *in vitro* embryo rescue has been performed since 1988, and the number of established plants in the vineyard totaled 634 in 1995. Of the seedless-seedless hybrids, the berry weight of completely seedless seedlings varies from 2 to 4 g (Fig. 4A), but in some genotypes, having relatively large berries (about 5 g with nearly unnoticeable seed traces), have been selected (Fig. 4B). Although the effect of specific parental combination is not negligible, conventional breeding methods of seeded x seedless crosses yielded 7 to 15% seedless progeny (Loomis and Weinberger, 1979; Spiegel-Roy et al., 1990). However, the proportion of seedless progeny was higher (av. 24%) in seedless x seedless crosses, indicating that smaller populations are required to obtain the same number of seedless seedlings than in seeded x seedless crosses. Hence, 'Akitsu No. 2' and 'F 4075' are promising parental stocks for the seedless-seedless hybridization. A completely seedless line, 'Akitsu No.2' bears large berries (5 to 6 g) with a high percentage embryo recovery. This accession also needs no emasculation because it is pollen sterile. Berries of 'F 4075' with noticeable seed traces range from 7 to 8 g and have a distinct muscat flavor. From a cross of 'F 4075' x 'CG 102295', 20 out of 57 plants which fruited, 7 (35%) were completely seedless (data not shown). To increase the efficiency of plant recovery via *in ovulo* embryo culture, research on the media composition, growth regulators, and culture period is ongoing.

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胚珠内胚培養による無核ブドウ交配間雑種

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摘 要

偽単為結果する無核ブドウの胚珠内胚培養において，果粒の採取時期および遺伝子型の相違が胚形成率に及ぼす影響について調査した．自然受粉を行った5品種では，果実発育の進行に伴って果粒重の増加が認められたのに対し，胚珠の長径は必ずしも増加せず，一定のままか，あるいは減少した．胚珠内胚培養による胚形成率は，満開後の経過日数に伴って有意に上昇し，特に40日目が顕著であった．培養した胚珠の中には多胚現象が認められることもあり，珠孔先端部において雑種胚の下胚軸から不定芽として発生することが多かった．自然受粉を行った5系統および6品種の満開期からベレゾーン期迄の経過日数では，49日から66日の変異が認められた．ベレゾーン期に採取した胚珠からの胚形成率には有意な系統間差異が認められ，変異幅は0%から24.7%であった．20組合せの無核ブドウ間の交配では，胚形成率において有意差が認められ，変異幅は2.7%から47.0%であった．試験管内での植物体形成率では，0.6%から24.9%の変異が認められた．胚珠内胚培養によって得られた無核ブドウ×無核ブドウの雑種第1代では，完全無核：種子痕跡をもつ：有核の出現頻度が24%：56%：21%であった．

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