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Screening for resistance to ripe rot caused by *Colletotrichum acutatum* in grape germplasm

M. Shiraishi[1)], M. Koide[2)], H. Itamura[2)], M. Yamada[1)], N. Mitani[1)], T. Ueno[1)], R. Nakaune[1)] and M. Nakano[1)]

[1)]Grape and Persimmon Research Station, National Institute of Fruit Tree Science, Higashi-Hiroshima, Japan
[2)]Department of Agriculture, Shimane University, Matsue, Japan

Summary

We screened 235 *Vitis* and six *Muscadinia* grapevine cultivars and selections conserved at the National Institute of Fruit Tree Science in Japan for resistance to grape ripe rot, caused by *Colletotrichum acutatum* Simmonds ex Simmonds. This fungus is insensitive to fungicides such as benomyl, diethofencarb, and iminoctadine-triacetate. We evaluated the disease resistance of nearly ripe berries from each cultivar and selection by artificial inoculation with *C. acutatum*. Analysis of variance of 20 cultivars and selections indicated that the genotype had a significant effect but that the year had no significant effect on the percentage of diseased berries. Genetic variance explained 85 % of total variance. Each cultivar or selection was classified into one of the following four classes based on its level of resistance to ripe rot: 50 highly resistant (≤ 20 % affected), 37 resistant (21-40 %), 48 susceptible (41-60 %), and 106 highly susceptible (≥ 61 %). Of the highly resistant cultivars and selections, we consider a diploid named 676-64 to be promising material for ripe rot resistant table grape breeding.

K e y w o r d s : breeding material, *Colletotrichum acutatum*, grape, muscadine, ripe rot resistant.

Introduction

Ripe rot is a devastating berry disease of field-grown grapevines (*Vitis* spp.) in Japan (Ozoe *et al.* 1972, Yamamoto *et al.* 1999, Fukaya 2001), Korea (Park *et al.* 1992), China (Wang *et al.* 2002) and the United States (Pearson and Goheen 1994). The berries are susceptible from small green size to the ripe stages, but they do not show symptoms until ripening. The diseased flesh of affected berries becomes reddish brown or rose-colored, and the surface is sunken (Winkler *et al.* 1974). Lesions enlarge in concentric zones until they cover the whole berry, which becomes a mass of sticky, salmon-colored conidia, which are spread to other parts of the vine by rain splash throughout the growing season. The infections result in severe financial losses when entire lots of berries are rejected.

Glomerella cingulata (Stoneman) Spaulding et Schrenk and *Colletotrichum acutatum* Simmonds ex Simmonds are the causal agents. They survive from one season to another on the vines as dormant mycelium in mummified berries and infected peduncles. Disease development is favored by warm (25-30 °C), wet weather. Conidia germinate, and penetrate the cuticle of green or ripening berries within 1 week under favorable conditions. Recent reports of site-specific incidences of *C. acutatum* resistant to benomyl (Fukaya *et al.* 1998, Sonoda and Pelosi 1988), diethofencarb (Ishii *et al.* 1998), and iminoctadine-triacetate (Fukaya 2002) indicate that fungicides are ineffective.

Because of the destructive nature of ripe rot, grape breeders are trying to enhance resistance in their breeding materials. Interspecific crosses have been made primarily between resistant American hybrids and susceptible European *V. vinifera* cultivars to increase ripe rot resistance in high-quality table grapes. The Grape and Persimmon Research Station (GPRS) of the National Institute of Fruit Tree Science (NIFTS) in Japan conserves more than 700 grapevine accessions, but little is known about the extent of ripe rot resistance in the collection. To provide this knowledge in an efficient manner, we developed a rapid screening assay for ripe rot resistance in grape (Shiraishi *et al.* 2006). The objectives of this study were (1) to screen the NIFTS grape germplasm collection for ripe rot resistance, (2) to determine levels of resistance to ripe rot among the germplasm, and (3) to select promising resistant grape breeding materials with good fruit quality.

Materials and Methods

We evaluated 235 *Vitis* and six *Muscadinia* grapevines held at GPRS for ripe rot resistance using one vine per cultivar or selection. We screened 110 accessions (18 cultivars and 92 selections) in 2004 and 111 (46 cultivars and 65 selections) in 2005. Tab. 1 shows the family number of each cultivar or selection with its parentage (bred at NIFTS since 1969). Eight cultivars and 12 selections were used for analysis of variance of the effects of genotype and year between 2004 and 2005 in a two-factorial test (no replications) of arc-sine transformed data. Fungicides were applied in both years to prevent latent infection with *C. acutatum* before berry sampling according to phenological stages defined by Eichhorn and Lorenz (1977): five to six leaves unfolded, famoxadone; inflorescence elongating, oxadixyl and chlorothalonil; beginning of flowering,

Correspondence to: Dr. M. Shiraishi, Fukuoka Agricultural Research Center, 1-129 Chikushino, Fukuoka 818-8549, Japan. Fax: +81-92-922-4916. E-mail: mikioshi@farc.pref.fukuoka.jp

Table 1

Cultivars or selections bred at the Grape and Persimmon Research Station (GPRS), National Institute of Fruit Tree Science (NIFTS) in Japan since 1969 with their parentage

Family No.	Parentage	Ploidy	Family No.	Parentage	Ploidy
72	Steuben × Muscat of Alexandria	2x	634	Alden × Rizamat	2x
73	Takasago × Campbell Early	2x	639	Italia × North Red	2x
78	Steuben × Seneca	2x	643	85-62 × Italia	2x
84	Steuben × Rosaki	2x	644	85-62 × Alphonse Lavallee	2x
85	Steuben × Rizamat	2x	645	Italia × Rizamat	2x
86	Seneca × Rizamat	2x	656	Yatomi Rosa × 617-14	2x
103	Katta Kurgan × Takasago	2x	658	119-14 × 622-21	2x
105	Neo Muscat × Cardinal	2x	659	119-14 × 619-2	2x
119	July Muscat × Neo Muscat	2x	660	72-129 × 86-29	2x
161	Campbell Early × Himrod	2x	661	72-129 × 617-14	2x
165	Sekirei × Emerald Seedless	2x	662	617-14 × 86-29	2x
168	Italia × A1706	2x	664	85-62 × 161-32	2x
224	Aki Queen × Aki Queen	4x	665	85-62 × 78-90	2x
301	Kyoho × Niabell	4x	666	85-62 × 86-29	2x
335	Benizuiho × Hakuho	4x	667	85-62 × 84-12	2x
347	Neo Muscat 4X × Centennial	4x	668	85-62 × 617-14	2x
350	Neo Muscat 4X × Hakuho	4x	669	105-37 × 622-21	2x
364	Aki Queen × Beniizu	4x	671	Shine Muscat × 634-81	2x
365	Beniizu × Aki Queen	4x	672	Shine Muscat × 119-12	2x
381	Honey Venus × Aki Queen	4x	674	Oriental Star × 119-12	2x
383	Honey Black × Centennial	4x	675	85-62 × 119-12	2x
384	Dark Ridge × Centennial	4x	676	85-62 × Shine Muscat	2x
389	Honey Venus × Shigyoku	4x	678	644-53 × Neo Muscat	2x
390	Fujiminori × Aki Queen	4x	682	633-3 × 86-29	2x
392	Honey Black × Ryuuhou	4x	683	Shine Muscat × 86-29	2x
397	350-19 × 335-26	4x	684	Oriental Star × 86-29	2x
399	335-26 × Sunny Rouge	4x	685	85-62 × Shine Muscat	2x
400	347-29 × 335-26	4x	686	85-62 × Oriental Star	2x
617	103-37 × Hiro Hamburg	2x	689	Oriental Star × 84-12	2x
619	105-54 × Alden	2x	806	350-19 × Aki Queen	4x
622	Italia × Alden	2x	808	347-29 × 384-25	4x
626	103-37 × 73-22	2x	811	Fujiminori × 364-30	4x
628	Katta kurgan × North Red	2x	813	Fujiminori × 335-26	4x
633	Ruby Okuyama × Madeleine Angevine	2x	817	Dark Ridge × 335-26	4x

famoxadone and iprodione; late flowering, metalaxyl and diethofencarb; fruit set, azoxystrobin; berries pea-sized, triflumizole; beginning of berry touch, famoxadone; beginning of berry ripening, copper sulfate.

Nearly ripe berries from each cultivar or selection were sampled on the basis of their palatability (sour to slightly sour) in 2004, and at 70 to 80 d after full bloom in 2005 together with the juice analysis of soluble solids content (SSC) by a hand refractometer (N1, ATAGO, Japan) and titratable acidity using an electrochemical method with an Acilyzer model 5G (Fujihira, Tokyo, Japan). In 2005, the period of berry sampling among the 111 accessions tested ranged from 60 to 90 d after full bloom, averaging 77 ± 7.9 d. The SSC ranged from 11.8 to 22.4 °Brix, averaging 16.7 ± 1.71 °Brix. The titratable acidity ranged from 0.50 to 1.20 % with average of 0.60 ± 0.15 %, coinciding with the optimum time for beginning commercial harvest.

Procedures for sample preparation and artificial inoculation are described in our previous report (Shiraishi *et al.* 2006). Two to three grape clusters per cultivar or selection were sampled, and the 25 to 30 berries were used for screening assay. Twenty berries were randomly selected and washed in tap water to remove any fungicide stuck to the skin. They were then surface-sterilized in 70 % ethanol for 30 s and rinsed once with sterile water. After being dried by kimtowel (Crecia, Tokyo, Japan), 10 berries were wrapped in a polyethylene bag (0.03 × 260 × 380 mm). Conidial suspensions were prepared from the stored *C. acutatum* isolates (CAB03) by flooding the culture plate with 5 ml of sterile water. The suspension was filtered through two layers of cheesecloth, and adjusted to a concentration of 1×10^5 spores·ml^{-1} using a hemacytometer (SUNLEAD GLASS, Tokyo, Japan). The berries were inoculated three times without surfactant (ca. 3.0×10^5 spores·ml^{-1} per 10 berries) with a hand sprayer. The inoculation was replicated twice per cultivar or selection. Inoculated berries were incubated at 25 ºC in the dark for 2 weeks. Diseased berries were evaluated as follows: The first sign of infection

usually appeared as small, faintly visible ripe rot colonies 3 to 4 d after inoculation; the disease was well developed after 11 to 14 d, and susceptible berries were normally covered with mycelia and spores by this time. The level of resistance was defined on a scale of percentage diseased berries per 10 berries according to Shiraishi *et al*. (2006): highly resistant (HR, ≤ 20 %; Figure, A), resistant (R, 21-40 %), susceptible (S, 41-60 %), and highly susceptible (HS, ≥61%; Figure, B).

Figure: Grape ripe rot resistance was rated on a scale of the percentage of diseased berries per 10 berries. **A**: Highly resistant 'Suiho' (15 %). **B**: Highly susceptible 'Pione' (100 %).

Results and Discussion

Interannual variation: Tab. 2 shows the percentages of diseased berries in 2004 and 2005 among 8 cultivars and 12 selections. Some cultivars and selections showed high resistance (HR, ≤ 20 %), among them were '668-108', '672-6', 'Muscat Bailey A', 'Steuben', '662-88', 'Suiho', 'Kyoho', 'Campbell Early'. The highly susceptible plants (HS, ≥ 61%) were 'Portland', 'North Red' and 'Neo Muscat'. A two-factorial test analyzed the effects of genotype and year in 20 cultivars and selections (Tab. 3). Analysis of variance indicated that the genotype had a significant effect but that the year had no significant effect on the percentage of diseased berries ($p < 0.01$). From the expectation of mean squares in Tab. 3, we estimated the variance components of genotype (σ^2_g), year (σ^2_y), and residual (σ^2) as 479.8, 10.1, 75.7, respectively. Given that the total variance is $\sigma^2_g + \sigma^2_y + \sigma^2$, the proportions of these components are 84.8 % (σ^2_g), 1.8 % (σ^2_y), and 13.4 % (σ^2), respectively. These results indicate that genotypic variance is large, so the data of 2004 and 2005 can be integrated and discussed in terms of a relatively small environmental influence.

Table 2

Percentages of diseased berries by rapid screening assay in grape cultivars and selections in 2004 and 2005

Cultivars or selections	2004 (%)	2005 (%)	Mean (%)[Z]
668-108	0	0	0a
672-6	5	0	3ab
84-12	10	0	5ab
Muscat Bailey A	10	0	5ab
Steuben	5	5	5ab
73-22 (Akitsu No. 7)	10	5	8ab
626-84	5	10	8ab
662-88	0	20	10ab
662-93	20	0	10ab
Suiho	15	15	15bc
668-50	5	25	15bc
668-61	20	10	15bc
660-127	25	10	18bc
Kyoho	25	10	18bc
Campbell Early	30	10	20bc
662-68	30	10	20bc
335-26 (Akitsu No. 20)	35	30	33bc
Portland	80	75	78d
North Red	90	90	90de
Neo Muscat	100	100	100e

[Z] Mean separation within columns by LSD test ($p<0.05$) of arcsine transformed data.

Table 3

Analysis of variance of the percentage of diseased berries in 2004 and 2005 in grape cultivars and selections

Source of variation	df	MS	F-value[Z]	Expectation of mean squares (MS)
Genotype	19	1035.5	13.68**	$\sigma^2 + 2\sigma_g^2$
Year	1	277.2	3.66[NS]	$\sigma^2 + 20\sigma_y^2$
Residual	19	75.7		σ^2

[Z] NS, not significant; **, significant at $p < 0.01$ based on arcsine-transformed data of the percentage of diseased berries.

Screening of grape germplasm: There was a wide range in the mean ratings for ripe rot resistance among the cultivars and selections in both years combined (Tab. 4). These were tentatively classified as 50 HR, 37 R, 48 S, and 106 HS. HR and R grapevines are considered to be ripe rot resistant because few or no symptoms on the berries are observed in GPRS experimental fields under regular fungicide spraying.

Notable HR cultivars were introduced from North America: 'Alden' (0 %), 'Buffalo' (5 %), 'Pierce' (15 %), 'Seneca' (10 %), 'Steuben' (8 %), 'Urbana' (0 %), 'Van Buren' (5 %), and 'Wayne' (0 %) and Japan: 'Houman' (0 %), 'Kyoho' (18 %), 'Muscat Bailey A' (5 %), 'Shine Muscat' (15 %), 'Suiho' (15 %), 'Tano Red' (10 %), and 'Yoho' (10 %). Some HR cultivars such as 'Kyoho', 'Campbell Early', and 'Muscat Bailey A' are widely grown as leading cultivars in Japan. *Vitis vinifera* cultivars were generally susceptible (S or HS) with the five exceptions: 'Hakunan' (5 %, HR), 'Katta Kurgan' (5 %, HR), 'Morgen Schoen' (25 %, R), 'Rizamat' (35 %, R) and 'Unicorn' (40 %, R). 'Hakunan' and 'Morgen Schoen' are *V. vinifera* intraspecific hybrids between 'Katta Kurgan' and 'Kaiji' (HS). Most muscadine cultivars were susceptible, except for 'Nesbit' (10 %, HR) and 'Fry' (30 %, R). Carlos was highly susceptible, as reported by Daykin and Milholland (1984). 'Nesbit' and 'Fry' could be used to improve muscadine cultivars because ripe rot is a serious problem on muscadine grape

Table 4

Classification of ripe rot resistance based on the rapid screening assay of 241 grape germplasms

Level	Germplasm (cultivar or selection)[z]
Highly resistant (HR) (≤ 20 %)	Alden, Buffalo, Campbell Early, Hakunan (V), Houman, Katta Kurgan (V), Kyoho, Muscat Bailey A, Nesbit (R), Pierce, Seneca, Shine Muscat, Steuben, Suiho, Tano Red, Urbana, Van Buren, Wayne, Yoho, 72-129, 73-22, 84-12, 384-32, 397-75, 399-1, 626-84, 639-59, 656-29, 658-28, 660-127, 661-61, 662-3, 662-42, 662-68, 662-78, 662-88, 662-93, 662-96, 665-15, 666-24, 667-53, 668-12, 668-31, 668-50, 668-58, 668-61, 668-108, 671-51, 672-6, 676-64
Resistant (R) (21 % - 40 %)	Concord, Delaware, Fry (R), Honey Black, Kuroshio, Morgen Schoen (V), Ontario, Rizamat (V), Takasago, Unicorn (V), 161-32, 335-26, 383-26, 384-25, 399-4, 628-73, 634-70, 643-67, 658-26, 658-67, 660-23, 660-35, 661-23, 662-56, 662-99, 662-154, 664-7, 664-16, 667-9, 668-49, 668-50, 668-53, 668-64, 668-66, 671-112, 682-68, 813-18
Susceptible (S) (41 % - 60%)	Aki Seedless, Hakuho, Himrod, Nagano Purple, Niagara, North Black, Oriental Star, Ruby Okuyama (V), Summit (R), Triumph (R), Wallace (R), 86-29, 119-12 (V), 224-22, 224-56, 347-29 (V), 364-30, 365-2, 365-18, 383-35, 392-43, 633-3 (V), 634-12, 634-21, 634-72, 645-67, 658-9, 658-46, 660-125, 662-58, 662-66, 662-163, 664-6, 666-65, 668-8, 668-22, 668-39, 669-15, 671-124, 672-11, 675-103, 676-51, 678-3, 684-11, 685-38, 685-4, 686-3, 817-231
Highly susceptible (HS) (≥ 61 %)	Aki Queen, Azumashizuku, Benizuiho, Black Hamburg (V), Cabernet Sauvignon (V), Carlos (R), Dark Ridge, Flame Muscat (V), Fuefuki, Fujiminori, Golden Muscat, Gorby, Gros Colman (V), Hiro Hamburg (V), Honey Seedless, Honey Venus, Italia (V), Kai Noir, Kaiji (V), Koshu (V), Merlot (V), Muscat of Alexandria (V), Muscat of Alexandria 4X (V), Neo Muscat (V), North Red, Pione, Pizzutello Bianco (V), Portland, Rosaki (V), Rosario Bianco (V), Shinanosmile, Sunny Rouge, Tamayutaka, Yamanashi No.38 (V), Yamanashi No.42, Yamanashi No. 44 (V), 85-62, 165-12, 168-39, 301-1, 350-19, 380-4, 381-14, 384-26, 384-60, 389-1, 390-76, 390-84, 400-57, 617-14, 643-25, 645-39, 658-21, 658-34, 658-95, 659-17, 659-23, 659-25, 659-30, 659-45, 660-136, 661-110, 661-113, 662-7, 662-10, 662-16, 662-39, 662-46, 662-98, 662-164, 666-17, 666-29, 667-51, 668-6, 668-23, 668-35, 668-56, 668-60, 668-68, 668-111, 668-115, 668-124, 668-131, 668-133, 669-5, 669-17, 671-17, 671-108, 671-120, 671-122, 674-14, 675-125, 675-147, 675-153, 676-57, 676-75, 683-3, 684-23, 685-2, 686-53, 806-3, 808-3, 811-13, 813-24, 817-18, 817-587

[z] All genotypes are derived from *Vitis labruscana* × *V. vinifera* except for *V. vinifera* (V) and *Muscadinia rotundifolia* (R).

cultivation, particularly in the warm, humid areas of the southeastern United States (PEARSON and GOHEEN 1994).

The original source of resistance to grape fungal diseases was probably North American germplasm derived from *V. labruscana* × *V. vinifera*, so-called " American hybrids" . Table grape breeders in North America and Japan have long used these hybrids intensively for breeding disease resistant, releasing many resistant cultivars. However, we found several cultivars and selections susceptible to ripe rot in American hybrids (e.g. 'Niagara' 45 %, 'Himrod' 50 %, 'Portland' 78 %, 'Gorby' 100 %, and 'Pione' 100 %). No pattern of ripe rot resistance correlating with geographical origin or species parentage was evident. Interspecific crosses between American hybrids and *V. vinifera* (lines 661, HR × HS; 676, HS × HR; 662, HS × S; 668, HS × HS) have shown that resistance to ripe rot possibly resulting from polygenic inheritance is quantitatively expressed in seedling populations. Further genetic analysis at the GPRS is under way to confirm this hypothesis.

Promising resistant breeding materials: In the GPRS breeding program, *V. vinifera* cultivars have been recurrently crossed with American hybrids to develop cultivars with excellent fruit quality. However, there has been a corresponding decrease in disease resistance among them (numbered selections in the lowest row in Tab. 4). Of the HR and R grapevines, '72-129' (Akitsu No. 21), '73-22' (Akitsu No. 7), '84-12', '397-75', '626-84', '662-96', '671-51', '676-64', and 'Shine Muscat' are promising breeding materials in terms of ripe rot resistance by the outcome of screening grape germplasms. In particular, '676-64', a diploid selection, is highly resistant (10 % diseased berries after artificial inoculation), and considered to be a superior breeding material on account of its crisp flesh and large berries (11-15 g, av. 12 g). Artificial inoculation provides an effective means to evaluate resistance to grape ripe rot caused by *C. acutatum*. We found significant variation in resistance among the GPRS grape germplasm collection, and identified highly resistant

breeding materials. Information from this study provides a basis for selection and improvement of table grapes with disease resistance.

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