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Resistance of autotetraploids of grapevine rootstock cultivars to phylloxera (*Daktulosphaira vitifoliae* Fitch)

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Summary

Resistance of colchicine-induced autotetraploids of four grapevine rootstock cultivars (Riparia Gloire de Montpellier, Rupestris St. George, Couderc 3309 and Kober 5BB) to phylloxera (*Daktulosphaira vitifoliae* Fitch) was evaluated using the aseptic co-culture technique of root segments and phylloxera in a petri dish, and compared with those of the original diploid and Cabernet Franc (*Vitis vinifera* L.). None or very few phylloxera nymphs grew to adults on the root of the rootstock cultivars and their autotetrapolids whereas 26.8 % of the eggs grew to adults on the roots of Cabernet Franc. Resistance of Riparia Gloire de Mont-pellier, Rupestris St. George, Couderc 3309 and their autotetraploids to phylloxera was also tested by planting in a phylloxera-infested vineyard and compared with that of Kyoho, a tetraploid table grape cultivar (*V. vinifera* x *V. labrusca* Bailey). The formation of galls on the root tips of tetraploid rootstock cultivars was not increased significantly as compared to that on their original diploid plants whereas galls were formed on 52.9% of Kyoho root tips examined. These results show that the autotetraploid rootstock cultivars used in this study had high resistance to phylloxera, thus they were not different from the original diploids.

K e y w o r d s : rootstock, tetraploid, phylloxera, resistance, aseptic co-culture.

Introduction

Table grape production with large-berried tetraploid grapevine cultivars such as Kyoho and Pione (*V. vinifera* x *V. labrusca* Bailey) has been greatly increased in the last three decades in Japan. Due to the high rainfall in Japan these cultivars grow vigorously, and often show berry shattering. In addition, the vigorous roots are likely to absorb nitrogen constantly in summer, which seems to be related to berry coloring and high acidity of berries of cv. Kyoho, while a reduced nutrient supply during maturation, especially nitrogen, promoted berry coloration and maturation (Okamoto *et al.* 1991). Therefore, we assumed that vigor control by appropriate low vigor rootstocks might improve berry quality.

These rootstocks may also induce early fruiting of cross-seedlings in breeding. Breeders need to cultivate many offspring vines on a limited field area, which may be enabled by using low vigor rootstocks.

The colchicine-induced autotetraploid grapevine rootstock cultivars are less vigorous than the original diploid (Motosugi *et al.* 1999). Although the cause of the vigor reduction is unknown, the growth of Kyoho grapevines grafted onto these autotetraploid rootstocks was also lower than that of vines grafted onto diploid rootstocks (Motosugi *et al*. 1999). Therefore, these tetraploid rootstock cultivars seem to have a potential as dwarf rootstock.

Autotetraploid rootstock cultivars have thicker and shorter roots than the original diploid and have a very coarse and compact root system (Motosugi *et al*. 1999). It is probable that these physiological and anatomical characters are associated with their adaptability to soil conditions since the roots of autotetraploid rootstock cultivars had a higher water content and reduced cold hardiness compared with the original diploids (Motosugi 2000).

Most *V. vinifera* and *V. vinifera* x *V. labrusca* grapevines are grafted due to their susceptibility to phylloxera (*Daktulosphaira vitifoliae* Fitch) (Howell 1987).

For continuous observation of the phylloxera feeding and the plant response Forneck *et al.* (1996) and Grzegorczyk and Walker (1997, 1998) developed an aseptic co-culture of *Vitis* species and grape phylloxera; this rapid and seasonally independent technique can be used in a laboratory to identify phylloxera resistance.

The objective of our study was to determine the phylloxera resistance of autotetraploid rootstock cultivars using the aseptic co-culture technique and to observe nodosity formation in root tips of vines planted in a phylloxera-infested vineyard.

Material and Methods

M i c r o p r o p a g a t i o n o f g r a p e v i n e : Riparia Gloire de Montpellier (*Vitis riparia* Michx), Rupestris St. George (*V. rupestris* Scheele), Couderc 3309 (*V. riparia* x *V. rupestris*), Kober 5 BB (*V. berlandieri* Planch. x *V. riparia*) and their colchicine-induced tetraploids (Riparia Gloire(4x), St. George(4x), 3309(4x), 5BB(4x)), and Cabernet Franc (*V. vinifera* L.) as control were micropropagated at 1-2-month intervals by using nodal microcuttings (*in vitro* cutting) as follows: 40 ml of the rooting medium consisting of MS mineral salts (Murashige and Skoog 1962) with nitrogen reduced to 33 %, MS vitamins, 30 $g \cdot l^{-1}$ sucrose, 0.5 µM naph-

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thalene acetic acid (NAA) and 0.8 % agar was put in a 200 ml glass bottle. After the medium was adjusted to pH 5.7, bottles were autoclaved. The shoots of the plants growing *in vitro* were cut into one-node segments without leaves, (length: 1-2 cm) and transferred into the rooting medium to develop shoot and root simultaneously. Culture glasses containing plants were kept in a growth chamber at 25±1 °C with a 16-h light period (fluorescent light, 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Bio-Lux A, NEC Lighting Ltd., Tokyo).

Phylloxera proliferation: Phylloxera eggs were collected from the roots of vines used in the field to test resistance to phylloxera (description later). The surface of the eggs was sterilized with 50 % ethanol for 7 min as described by GRZEGORCZYK and WALKER (1997), and rinsed with sterilized water three times. The eggs were then placed onto a sterilized filter paper in plastic petri dishes (diameter: 9 cm). The filter paper (Advantec Toyo No.1, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) was moistened with a liquid medium identical with that used for the nodal microcuttings. Several root tip segments (length: 2-3 cm) from Cabernet Franc (C. Franc) plants growing in 200 ml glass bottles were placed into the petri dish inoculated with phylloxera eggs. The petri dishes were sealed with Parafilm® M (American National Can, Greenwich, CT, USA) to maintain moisture and kept in the dark at 25±1 °C.

The nymphs hatched from the inoculated eggs within a few days after inoculation grew to adults within 3 weeks and then the adults laid many new eggs on the root tips of C. Franc one month after egg inoculation. The surface of the eggs collected from the C. Franc roots was sterile. These eggs were transferred to other C. Franc roots cultured *in vitro* in the same manner and proliferated.

In vitro test of grapevine resistance to phylloxera: Root tip segments (length: 2-3 cm) were dissected from the microcuttings of each diploid and tetraploid rootstock and the C. Franc grapevines when the roots had elongated to a length of 5-6 cm. Five root segments from each test cultivar and the tetraploids were placed on filter paper moistened with the liquid medium used in the nodal microcutting culture. Five root segments were placed in each petri dish with the root tips directed to the center. Ten eggs from the C. Franc roots described above were gently placed onto the center of the petri dish with a fine brush (Fig. 1). These petri dishes were sealed with Parafilm® M and kept in the dark at 25±1 °C.

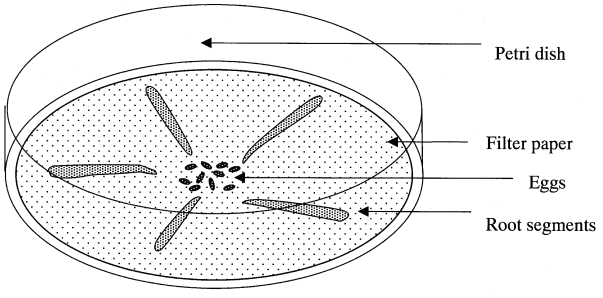

Fig. 1: The test for phylloxera resistance of roots of rootstock cultivars and their tetraploids in an aseptic co-culture (5 root segments and 10 phylloxera eggs were placed in each dish).

During a one-month culture, the numbers of living phylloxera including adults, nymphs, crawlers and eggs (population), and the mean date of the first oviposition (developmental time) were recorded periodically. The rate of survival of eggs to adults and the number of eggs laid per day in the first few days of oviposition (fecundity) were also counted.

The test was replicated with three dishes each for diploid and tetraploid Riparia Gloire, 3309, and St. George, twice in 4 dishes (total 8 dishes) for the diploid and tetraploid 5 BB. C. Franc was tested simultaneously as a control with 3-4 dishes in each test.

Field test of grapevine resistance to phylloxera: Micropropagated plantlets of Riparia Gloire, St. George, 3309, their colchicine-induced tetraploids and Kyoho, a tetraploid leading cultivar in Japan (control) were transplanted into a polyethylene pot filled with vermiculite and acclimatized.

After being raised in a greenhouse for a year, the vines were planted to a vineyard of the Department of Grape and Persimmon Research of the National Institute of Fruit Tree Science (NIFTS), Akitsu, Hiroshima, Japan, on April 28, 1999. The vineyard was infested with phylloxera because the own-rooted *V. labrusca* grapevines (selections from crossbreeding) had low vigor and many phylloxera-induced galls on the root tip. One plant each of the diploid and tetraploid rootstock and Kyoho grapevine were planted circularly 1 m apart from the trunk of an own-rooted selection grapevine. The planting design was a randomized complete block (4 blocks with own-rooted selection grapevines).

On September 29, roots of the vines were excavated and washed gently with water. Some of the roots were sampled and evaluated for gall formation by grape phylloxera on the root tips (nodosity) and phylloxera inhabitation on the galls. Percentages of gall formation were subjected to arcsine-square root transformation before statistical analyses. Data were subjected to analysis of variance (ANOVA) of a complete randomized block. Mean separation was accomplished by LSD at P=0.05.

Results and Discussion

In vitro test: Most of the phylloxera eggs hatched 1-3 d after inoculation. The first instar nymphs were observed probing on the root tip close to the meristemic zone. Root tips of C. Franc (control) began to swell only one day after feeding by the first instar nymphs (Fig. 2 A) and then bent abruptly at the site of feeding partially covering the insect in the crook. Within one-month co-culture, phylloxera nymphs grew to adults and then laid many eggs, and the nymphs of the second generation colonized around the adult on the root of C. Franc (Fig. 2 B). On the roots of C. Franc, 27 % of the inoculated phylloxera eggs grew to adults within about 20 d, and the population on the 30th day was about 6 times as large as the number of the eggs first inoculated (Tab. 1). On the other hand, on the root segments of the rootstock cultivars and their tetraploids, the first instar nymphs attempted to feed but the root tips did not swell

Table 1

Rate of survival of egg to adult, days to first oviposition, fecundity, and the population on roots of grape rootstock cultivars and their tetraploids, and Cabernet Franc in an aseptic co-culture (5 root segments and 10 phylloxera eggs)

Grapevines	Survival rate of egg to adult (%)	Days to first oviposition	Fecundity[a] eggs·adult^{-1}·d^{-1}	Population at day 30 (individuals per dish)
Cabernet Franc	26.8 ± 0.1[b]	19.6 ± 2.0	2.5 ± 0.4	57.5 ± 9.6
Riparia Gloire	0.0	-	-	0.0 -
Riparia Gloire(4x)	0.0	-	-	0.0 -
3309	3.3 ± 0.2	25.0[c] -	0.7[c]	4.3 ± 2.6
3309(4x)	0.0	-	-	0.0 -
St. George	0.0	-	-	3.7 ± 0.3
St. George(4x)	0.0	-	-	1.0 ± 0.6
5 BB	1.9 ± 0.1	19.0[c] -	0.3[c]	2.3 ± 0.8
5 BB(4x)	0.0	-	-	0.3 ± 0.1

[a]: Number of eggs laid per day during the first several days of egg laying.
[b]: Mean ± SE.
[c]: Only one phylloxera survived to adult stage.

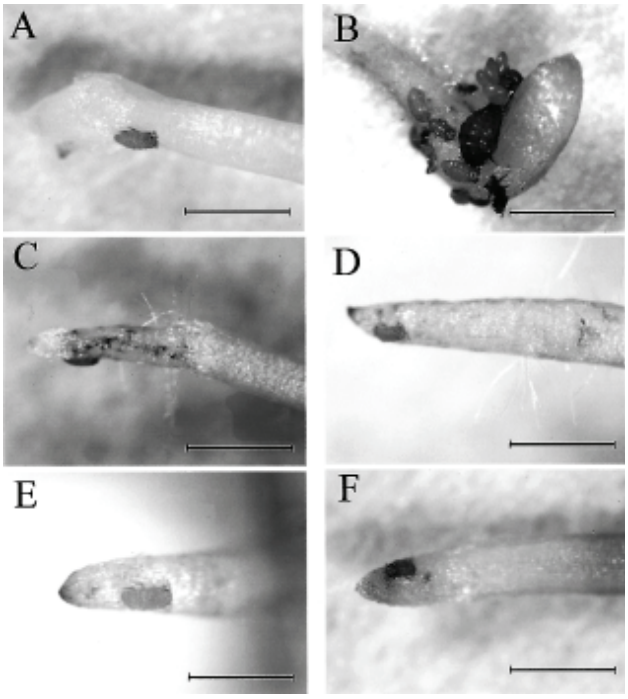

Fig. 2: Photographs of the aseptic co-culture of grapevine root tips and phylloxera. **A**: Root tip fed by a first instar nymph of Cabernet Franc began to swell within one day after feeding (on the 5th day of co-culture). **B**: A phylloxera colony on the nodosity formed on the root tip of Cabernet Franc root on day 28 of co-culture. **C**: Root tip fed by a first instar nymph of St. George on the 7th day. Brown spots around the feeding sites were developed. **D**: Root tip fed by a first instar nymph of St. George(4x) on the 7th day. **E**: Root tip fed by a first instar nymph of 5 BB(4x) on the 5th day. **F**: Root tip of 5 BB(4x) on day 30. One phylloxera nymph survived on the root tip but remained at the first instar stage. Scale bars = 1 mm.

(Fig. 2 C, D, and E) and developed only brown spots around the feeding sites (Fig. 2 C). Most of the first instar nymphs could not establish feeding sites on the root segments of the rootstock cultivars and their tetraploids and died. A few nymphs settled on those root tips but did not molt (Fig. 2 F), or grew slowly and remained immature during the 30 d of co-culture. On the roots of 3309 and 5 BB, in all dishes only one phylloxera nymph grew to an adult, and the adults laid significantly fewer eggs (lower fecundity) than adults on the roots of C. Franc (Tab. 1). On day 30 of co-culture, no insects (nymphs, adults and eggs) on the root tip segments of Riparia Gloire, Riparia Gloire(4x), and 3309(4x) survived, but a few insects (less than the number of eggs inoculated) were observed on the root tips of the other rootstocks and their tetraploids (Tab. 1).

Field test: Galls (nodosities) were formed in 52.9 % of the evaluated root tips in Kyoho vines, and numerous phylloxera individuals including adults, nymphs and eggs were found on the galls (Tab. 2). On the other hand, on the root tips of all the rootstock cultivars and their autotetraploids, only a few gall-like swelling tissues were observed and neither nymphs nor adults of phylloxera were found on the swelling tissues regardless of their ploidy.

These results show that the resistance to phylloxera of colchicine-induced autotetraploids of grapevine rootstock cultivars used in this study was high and not different from the original diploid rootstock cultivars in both aseptic co-culture and field tests. The fact that the tetraploid rootstock cultivars had shorter and thicker roots than the original diploid may not be related to phylloxera resistance. Phylloxera resistance of these rootstock cultivars is possibly based on physiological and biochemical mechanisms. Polyphenol oxidation has been considered to be one of the resistance mechanisms to aphids (Miles 1969). Roots of grape species such as *V. riparia* and *V. rupestris* developed a corky layer around phylloxera feeding sites, preventing development of tuberosity and survival of phylloxera (Boubals 1966). In this study, similar brown spots developed around the feeding site of the first instar nymphs on the root tip segments of the

Table 2

Percentage of gall formation and infestation of grape phylloxera on root tip samples of grape rootstock cultivars, their tetraploids and Kyoho grapevine planted in a vineyard infested with phylloxera

Grapevine	Gall formation (%)		Phylloxera infestation
Riparia Gloire	1.1	(0.060)[a]	-[b]
Riparia Gloire(4x)	2.8	(0.172)	-
3309	0.4	(0.103)	-
3309(4x)	2.9	(0.169)	-
St. George	0.8	(0.090)	-
St. George(4x)	1.0	(0.099)	-
Kyoho	52.9	(0.815)	+++
LSD(5 %)		(0.126)	

[a] arc-sine square root transformationed values.
[b] No phylloxera (-), infested with numerous phylloxera (+++).

resistant rootstocks and their autotetraploids. DE BENEDICTS and GRANETT (1992) mentioned that differences in phylloxera life-table parameters among rootstocks might explain the differences in resistance. In some rootstocks with *V. berlandieri* parentage survival of phylloxera was markedly lower suggesting that a toxin lethal to nymphs may be involved in the resistance mechanism. In some rootstocks with *V. rupestris* parentage survival was not affected but developmental time was longer and fecundity was suppressed suggesting a nutritional deficiency or inhibitor (GRANETT *et al.* 2001). Because the root tip segments became weak and brown gradually after the one-month co-culture of root segments with phylloxera in our study, the investigation of phylloxera life-table parameters could not be examined for more than one month. Therefore, the significant differences in life-table parameters were not observed between the diploid rootstocks and their autotetraploids as well as between the rootstock with *V. berlandieri* parentage 5BB and the rootstocks with *V. rupestris* parentage, St. George and 3309. Detail investigations should be conducted with larger roots that can be cultured for a longer time.

Some biotypes or populations of phylloxera different in the adaptability to grape rootstock cultivars were reported in North America (DE BENEDICTS and GRANETT 1993, FERGUSSON-KOLMES *et al.* 1993, OMER *et al.* 1999) and in Europe (SONG and GRANETT 1990, KOCSIS *et al.* 1999). KOCSIS *et al.* (1999) demonstrated that the phylloxera populations collected from the colony on the rootstock cultivars showed higher survival, developmental and reproductive capacity on the roots of the rootstock cultivars Teleki 5 C and SO 4, than the population collected from *V. vinifera* roots and were genetically remote from those collected from the population of *V. vinifera* by RAPD DNA analysis. However, phylloxera used in the present study was derived from a *V. labrusca* vineyard of the NIFTS, Akitsu. No information is available for the host preference of that phylloxera. Therefore, in further studies, the effect of the host preference should be elucidated to find a useful tetraploid of the rootstock cultivars.

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