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Cold Resistance in Root and Cane of Own-root 'Kyoho' Grapevines

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Root and cane of own-root 'Kyoho' grapevines were tested for winter cold resistance using EC (electrical conductivity) and TTC (triphenyl tetrazorium chloride) tests, as well as anatomical observations and survival tests. Vines were exposed to various subzero temperatures for various durations in two separate experimental trials, one in December, one in January. The EC and TTC reduction of small roots (ϕ1−2mm) were not affected after being exposed to temperatures of −2.5 or −3.0°C for 24 days, though those of large roots (ϕ3−5mm) were slightly affected. After exposure to −4°C for only 12 hours the EC markedly increased and TTC reduction decreased in both small and large roots. Cells of cortex and ray tissues in the root were ruptured. New root growth on root cuttings taken from the vine previously exposed to −4°C was severely inhibited. These results indicate that 'Kyoho' roots cannot resist temperatures of −4°C or lower even for a single night during winter. On the other hand, 'Kyoho' canes survived −15°C even for 24 days. However, exposure to −20°C or −30°C greatly affected the EC and TTC reduction in the cane and inhibited bud bursting entirely.

Key words : cold resistance, Kyoho grapevine, root, cane

Introduction

A new planting system of grapevines, which combined root-zone restriction with high density planting in raised beds, was developed by Imai *et al.*[4,5] and Okamoto *et al.*[8]. It enabled the production of normal crop levels of 'Kyoho', similar to those usually produced in mature vineyards, only one or two years after establishment of the vineyard[4,7]. However, soil temperature inside the raised bed changed more rapidly and widely than that in normal vineyards[12]. It is supposed that raised-bed soil may freeze more easily during winter in cold regions.

'Kyoho' is now cultivated worldwide even in regions with extremely cold winters such as Ninxia, northwest China, where the air temperature can drop to −15°C or lower[11]. It has been reported that grape canes and buds can survive temperatures of −13°C or lower[6], but roots have weaker cold resistance than the above-ground parts[9,14,15]. Ahmedullah and Kawakami[1] demonstrated that the lethal temperature for 'Concord' (*V. labrusca*) roots was near −5°C.

In order to develop root-zone restriction planting of 'Kyoho' grapes in cold regions, we investigated the effects of subzero temperatures on 'Kyoho' canes and roots as well as their lethal temperatures during winter.

Materials and Methods

Plant materials and exposure to subzero

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temperatures. Two-year-old cutting vines of 'Kyoho' grape grown in 4 liter soil pots were used. Twenty-seven vines were removed from the pot soil on December 10th, 1996, then pruned to retain a single cane with 4 nodes. The vines were divided into 3 groups and each of them was wrapped in polyethylene film together with wet sand-peat mixture (1:1, w/w). After being kept at 0°C for 24 hours, each group was transfered into a refrigerator controlled at −2.5, −5.0 or −7.5°C for 24 days. Canes of 10 cm length, cut off at pruning, were divided into 4 groups and wrapped in polyethylene film. They were exposed to −7.5, −15, −20 and −30°C for 24 days. Similar experiments were carried out from January 10th, 1997, by exposing vines to −3, −4 or −6°C. Another 20 vines were kept as controls in an outdoor vineyard until early March.

EC and TTC tests. In the first experiment, carried out during December, between 5 to 10g of roots were sampled from the vines 2, 4, 16, and 24 days after the beginning of exposure to each subzero temperature. In the 2nd experiment during January, root samples were taken 3, 6, 12, 24, and 48 hours after the beginning of exposure. After thawing at 4°C, sampled roots were separated into small roots (ϕ1-2mm) and large roots (ϕ3-5mm), then cut into 5mm long segments. They were used for TTC and EC tests by following the procedures described by Ahmedullah and Kawakami[1]. A 3g sample of roots was soaked in 60ml of distilled water and shaken for 15 hours at 25°C. Electrical conductivity of the diffusate was determined by an EC meter. For the TTC test, a 0.5g sample of roots was added to 3ml of 0.6% TTC in 0.05M Na_2HPO_4-KH_2PO_4 buffer (pH 7.4) and 0.055% (v/v) Tween 80, then incubated at 30°C for 15 hours. After rinsing the root samples with distilled water, formazan produced in the samples was extracted with 10ml of ethanol 3 times. The red color was measured by a spectrophotometer at 530nm. Excised canes, exposed to −7.5, −15, −20, and −30°C for 24 days, were sliced into 2mm thick segments after thawing. TTC and EC tests were carried out using the same methods as for the root samples.

Anatomical observation. Ten to 15 small roots sampled from the vines exposed to different subzero temperatures for 24 days were fixed with FAA solution, dehydrated with BuOH-xylene series, and embedded into paraffin blocks. Sixteen μm-thick cross sections of the root samples were stained with basic fuchsin and alcian blue. Microscopic observations were carried out to find damage in various root tissues such as cortex, ray, phloem, and xylem.

Survival tests of roots, canes and vines. Large roots exposed to each subzero temperature for 24 days were cut into 5cm segments and soaked with 50ppm of IBA solution for 12 hours. They were set in a sand bed as "root cuttings" and incubated in the dark at 28°C. New root growth was recorded 4 weeks later. Canes exposed to subzero temperatures for 24 days were also incubated in a sand bed as "single-bud cuttings". Bud bursting was recorded 4 weeks later. All vines subjected to subzero temperatures for 24 days were buried in an outdoor vineyard, then planted in 2 liter pots and moved into a heated greenhouse on March 5th. The temperature of the greenhouse was kept higher than 18°C. Growth of new shoots and roots were recorded weekly.

Results

1. Changes in EC and TTC reduction of roots

In the first experiment, the EC of small roots was not affected by exposure to −2.5°C, though that of large roots increased slightly after exposure for 4 and 16 days. Exposure to temperatures of −5 and −7.5°C increased the EC of both small and large roots remarkably for 2 days after the beginning of the treatment. TTC reduction of small roots exposed to −2.5°C was lower than that of the control roots when measured 2 days after exposure, though such difference was not

detected thereafter. However, in large roots TTC reduction decreased significantly after exposure to −2.5°C for 4 days or longer. Remarkable decreases in TTC reduction of both small and large roots were found by exposing them to −5 and −7.5°C for 4 days (Fig. 1). In the second experiment, carried out one month after the first experiment, exposure to −3°C did not affect either the EC or the TTC reduction of small roots, though TTC reduction was decreased in large roots after exposure to −3°C for 12 hours or longer. Exposure to −4°C increased EC of small and large roots gradually and decreased TTC reduction between 6 and 12 hours after the beginning of the treatment. Exposure to −6°C increased the EC values rapidly during the first 6 hours of exposure and decreased TTC reduction during the first 3 hours (Fig. 2).

2. Changes in EC and TTC reduction of excised canes

EC and TTC reduction of excised canes were not affected after exposure to −7.5°C for 24 days, though EC increased and TTC reduction decreased after exposure to −15°C to some extent (Fig. 3). Exposure to −20°C and −30°C caused remarkable effects on both EC and TTC reduction; it doubled EC and lowered TTC reduction to one third of the values of the control canes.

3. Anatomical damage in root tissues

Slight damage such as ruptures in the wall of several cortical cells was observed in small roots exposed to −2.5 or −3°C for 24 days. However, large lacunae, seemingly formed after a lot of cells were ruptured, were observed in both cortex and ray tissues of roots exposed to −4 or −5°C (Fig. 4). In small roots subjected to −6 or −7.5°C,

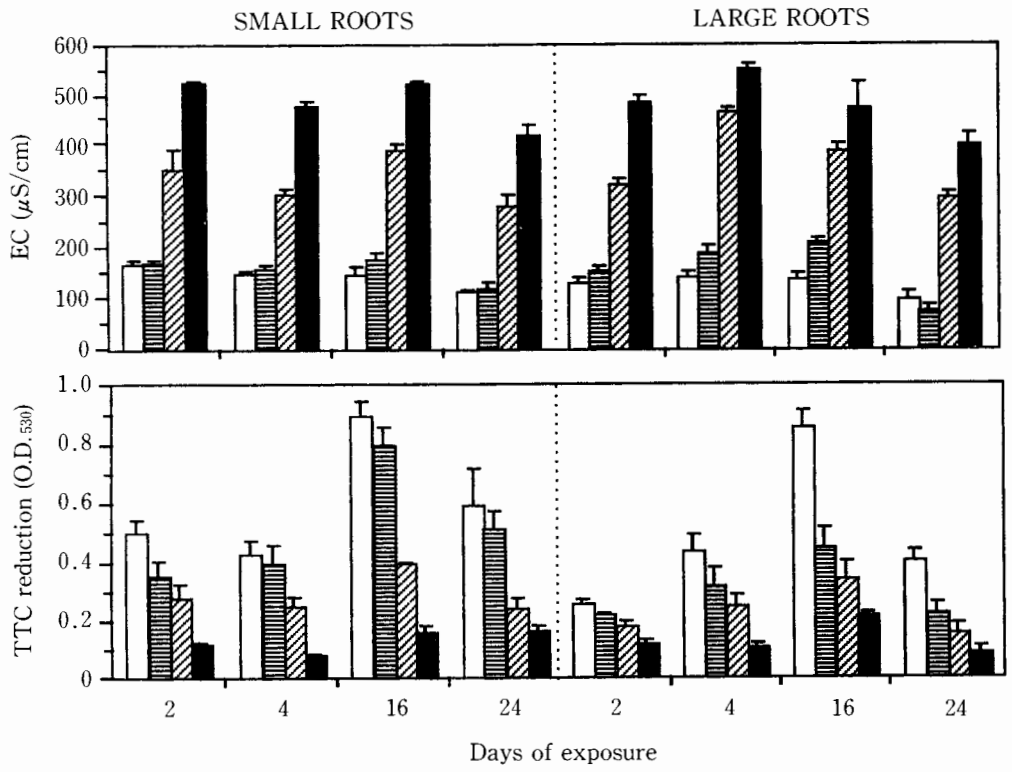

Fig. 1 Effect of exposure to subzero temperatures for various durations on the EC (upper) and reduction of TTC (lower) of small ($\phi < 2$mm) and large ($\phi > 3$mm) roots of Kyoho grapevines. Vines were exposed to −2.5°C (▤), −5°C (▨), and −7.5°C (■) from December 11th, 1996. Control vines (□) were kept outdoors. The EC (μS/cm) of diffusates of 3g of roots in 60ml of water was measured using an EC meter. TTC reduction was measured photometrically on 10ml of ethanolic extracts from 0.5g of roots and expressed as O.D. 530. Vertical bars show S.D.

large lacunae were also found in phloem tissue. Such severe damage in various root tissues was observed more frequently in lower temperature treatments.

4. New root growth on root cuttings and bud bursting from single-bud cuttings

Root cuttings taken from vines exposed to −3 °C for 12 hours developed new control-like roots during 4 weeks of incubation. However, new root growth on root cuttings from vines exposed to −4.0 °C or −6 °C was inhibited significantly. Such inhibitory effects of subzero temperatures became more obvious when vines were exposed for 24 days (Table 1). On the other hand, almost every bud on excised canes, exposed to −7.5 or −15 °C for 24 days, had burst when observed 4 weeks after incubating in a sand bed. However, few or no buds burst from the cane exposed to −20 or −30 °C (Data not shown).

5. Shoot and root growth on treated vines in the following spring

Vines exposed to −2.5 °C for 24 days normally developed new shoots and roots like control vines. However, on vines exposed to −5.0 °C

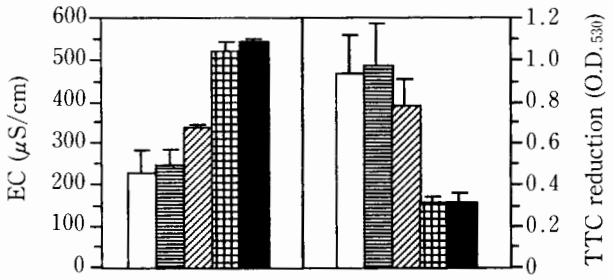

Fig. 3 Effect of exposure to subzero temperatures on the EC (left) and TTC reduction (right) of Kyoho canes. Excised canes were exposed to −7.5 °C (▤), −15 °C (▨), −20 (▦), and −30 °C (■) from December 11th for 24 days. Control canes (□) were taken from vines kept outdoors. Vertical bars show S.D.

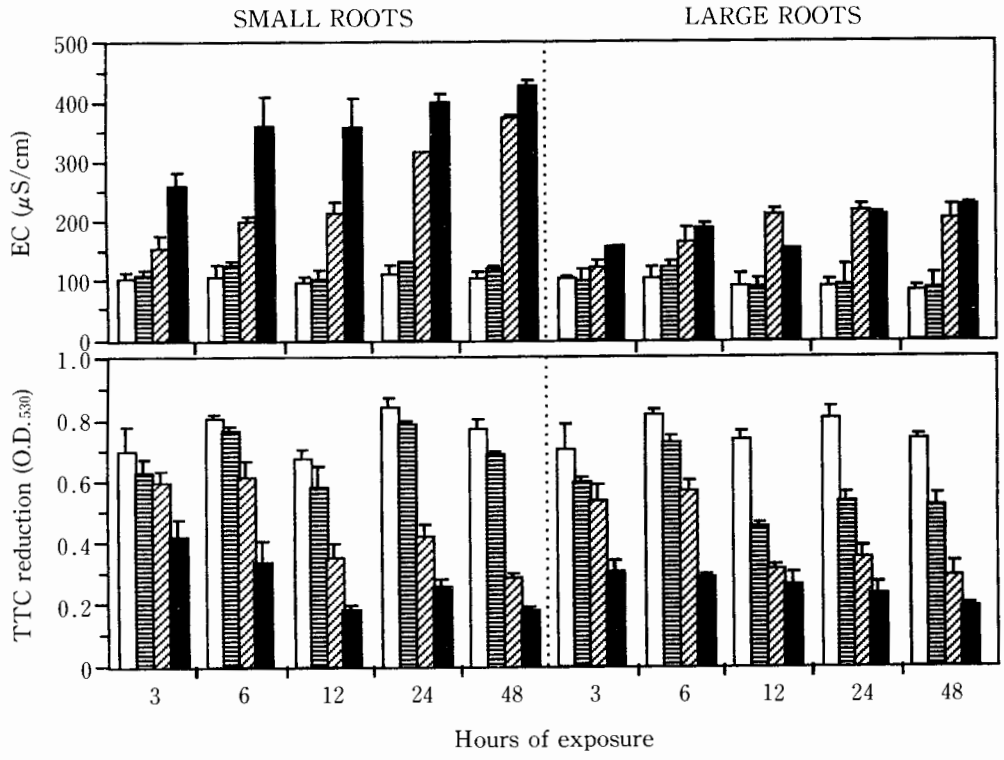

Fig. 2 Effect of exposure to subzero temperatures for various durations on the EC (upper) and reduction of TTC (lower) of small (ϕ<2mm) and large (ϕ>3mm) roots of Kyoho grapevines. Vine were exposed to −3.0 °C (▤), −4.0 °C (▨), and −6.0 °C (■) from January 10th. Control vines (□) were kept outdoors. Vertical bars show S.D.

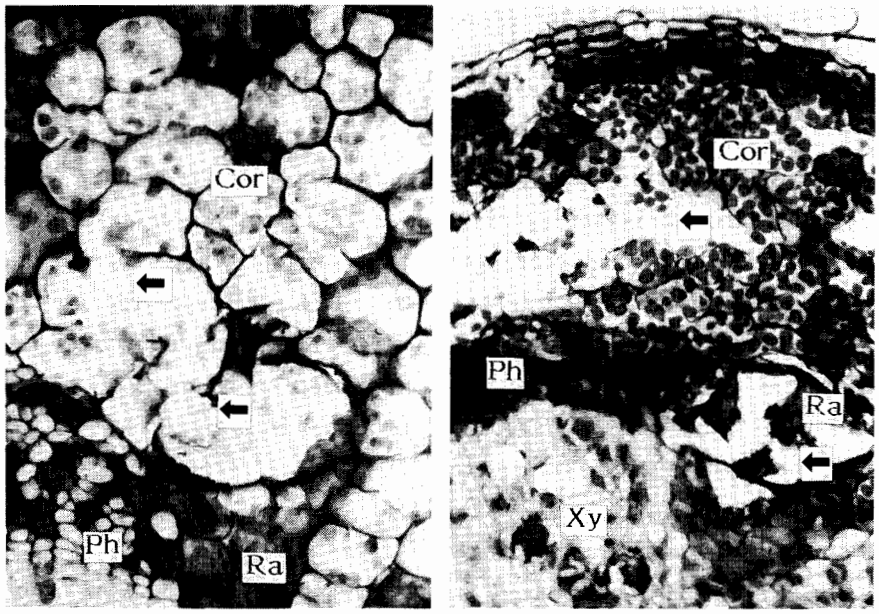

Fig. 4 Photomicrographs of the cross section of Kyoho roots exposed to subzero temperatures. Left:several cortical cells ruptured (←) after exposure to −2.5°C for 24 days (×200), right: large lacunae (←) can be observed in cortical and ray tissues exposed to −5°C for 24 days (×66). Cor,cortex; Ph, phloem; Xy, xylem; Ra, ray.

Table 1 New root growth from root cuttings taken from 'Kyoho' grapevines exposed to various subzero temperatures[a)]

Temp. (°C) treated	Duration of exposure		
	12h	24h	24d
No. of root cuttings with new roots[b)]			
−3.0	2.0ab[c)]	1.3a	1.7b
−4.0	1.3b	0.3b	0.0c
−6.0	0.0c	0.0c	0.0c
Cont.	2.3a	2.0a	2.7a
No. of new roots per root cutting[b)]			
−3.0	0.74a	0.54b	0.66b
−4.0	0.46b	0.06c	0.00c
−6.0	0.00c	0.00d	0.00c
Cont.	0.86a	1.00a	1.06a

[a)]Measured after 28 days of incubation in the dark at 28 °C. Control roots were taken from vines kept outdoors.
[b)]Averages of 3 replications using 5 root cuttings for each treatment.
[c)]Values without the same letters differ significantly (Duncan's multiple range test; $p<0.05$).

Table 2 Effect of exposure to various subzero temperatures for 24 days on new shoot and root growth of 'Kyoho' vines in the following spring[a)]

Temp. (°C) treated	No. of buds burst per vine	No. of shoots longer than 5cm per vine	Avg. shoot length (cm)	No. of new roots per vine
Exposure begun on December 11th, 1996				
−2.5	3.6a[b)]	2.3a	43.3a	326.7a
−5.0	2.1ab	1.0b	10.4b	11.7b
−7.5	2.3ab	0.3c	2.7c	0.0c
Exposure begun on January 10th, 1997				
−3.0	3.3a	1.7ab	41.2a	346.7a
−4.0	2.5ab	2.1a	17.9b	28.0b
−6.0	2.0b	1.1b	12.3b	13.0b
Cont.	3.4a	2.6a	58.8a	444.7a

[a)]Nine vines were used for each treatment. Each vine had a cane with 4 buds. Measured 4 weeks after bud bursting.
[b)]Values without same letters in each column differ significantly (Duncan's multiple range test; $p<0.05$).

shoot and root growth was inhibited significantly, though the number of buds burst per vine was similar to control vines. Vines treated with −7.5 °C developed only a few shoots and no roots (Table 2). In the experiment during January, shoot and root growth on vines exposed to −3.0 °C was not affected, but vines exposed to −4.0 or −6.0°C developed shorter shoots and significantly fewer new roots.

Discussion

The effectiveness of EC and TTC tests to

determine cold damage of plant tissues has been reported in various woody plants including grapes[1,3,9,10,14,15]. In our present experiments, close relationships were found between the values obtained from EC and TTC tests on large roots and the degree of vine growth occurring the following spring. The effect of cold temperatures on TTC reduction seemed to be more intense than on the EC as shown in the large roots exposed to −3°C in the second experiment. It could be noted that the TTC test is a more precise indicator for the cold resistance of grape roots than the EC test. Results of EC and TTC tests carried out during December showed that small roots of 'Kyoho' were not affected by −2.5 °C but affected by −5°C. In the second experiment in January, they survived −3°C but not −4 °C. Large roots, on the other hand, were affected after being exposed to −2.5°C or −3°C, which indicates that large roots may have weaker cold resistance than small roots. Wang et al.[13] reported that small roots of 'Kyoho' grapevines contained a higher level of sugars than large roots, which can result in the higher resistance against cell freezing owing to high turgid pressure of cell sap.

Cold damage of 'Kyoho' roots occurred during the first 3 to 6 hours of exposure to −4°C or lower. The rupture of cells in cortex and ray tissues would have resulted from cell freezing which might have occured after only a few hours. These results indicate that soil temperature around the root system of 'Kyoho' grapes should not drop to −4°C or lower even for a single night. Soil temperature at 10cm deep from the top of a raised bed drops to *ca* −4°C when the ambient air temperature drops to −10°C (Imai, 1999, unpublished data). Some modification of root restriction methods, such as heating the bed soil or burying it below ground, seems to be needed to develop this planting system in cold regions. The use of cold resistant rootstocks might be worth noting. Guo *et al.*[3] reported that roots of *V. amurensis* and its hybrids had higher cold resistance than those of *V. riparia* and other rootstock varieties.

Canes of 'Kyoho' grapevines survived −15°C, indicating that the cultivar 'Kyoho' is more cold resistant than *V. vinifera* cultivars[6]. However, in cold regions where the temperature can drop to −15°C or lower, the above-ground parts of 'Kyoho'vines should be covered with a thick sheet or buried below ground, as has been commonly practised in Ningxia[11].

References

1) Ahmedullah, M. and A. Kawakami : 1986. Evaluation of laboratory tests for determining the lethal temperature of *Vitis labruscana* BAILEY Concord roots exposed to subzero temperatures. Vitis **25** : 142-150.

2) Andrews, P.K., C.R. Sandidge and T.K. Toyama : 1994. Deep supercooling of dormant and deacclimating *Vitis* buds. Am. J. Enol. Vitic. **35** : 175-177.

3) Guo, X., W. Fu and G. Wang : 1987. Studies on cold hardiness of grapevine roots. Vitis **26** : 161-171.

4) Imai, S., G. Okamoto, and M. Endo : 1987. Effects of dense planting and root-system control on attaining greater early production and fruit stability of tetraploid grapes. Bull. Hiroshima Fruit Tree Expt. Sta. **12** : 1-9. (Japanese text with English abstract).

5) Imai, S., T. Fujiwara, S. Tanaka and G. Okamoto : 1991. Effect of soil moisture on vine growth and fruit production of Kyoho grapes growing on restricted rooting volume. Environ. Control in Biol. **29** : 133-140. (Japanese text with English abstract).

6) Mullin, M.G. , A. Bouquet and L.A. Williams : 1992. Biology of the grapevine. Cambridge University Press, Cambridge, pp. 176-177.

7) Ndung' u, C.K., G. Okamoto and K. Hirano : 1996. Use of water stress in forcing Kyoho grapevines to produce two crops per year. Am. J. Enol. Vitic. **47** : 157-162.

8) Okamoto, G., M. Noda, S. Imai and T. Fujiwara : 1991. Effect of levels of fertilizer application on vine growth and fruit development of 'Kyoho' grape. Sci. Rep. Fac. Agri. Okayama Univ. **78** : 27-33. (Japanese text with English abstract).

9) Pelett, H : 1971. Comparison of cold hardiness levels

of root and stem tissue. Can. J. Plant Sci. **51** : 193-195.
10) Steponkus, P.L. and F.O. Lamphear : 1967. Refinement of triphenyl tetrazolium chloride method for determination of cold injury. Plant Physiol. **42** : 1423-1426.
11) Wang, S., X. Liu and G. Okamoto : 1996. Improved berry set, ovule development and pollen tube penetration in 'Kyoho' grapevines in Ningxia. J. Japan. Soc. Hort. Sci. **65** : 27-32. (Japanese text with English abstract).
12) Wang, S., G. Okamoto and K. Hirano : 1997. Vine growth and fruit development of 'Pione' grapes planted in root-restricted buried and raised beds. J. Japan. Soc. Hort. Sci. **66** : 253-250. (Japanese text with English abstract).
13) Wang, S., G. Okamoto and K. Hirano : 1998. Effects of rooting-zone restriction on the changes in carbohydrates and nitrogenous compounds in 'Kyoho' grapevines during winter dormancy and early shoot growth. J. Japan. Soc. Hort. Sci. **67** : 577-582. (Japanese text with English abstract).
14) Wildung, D.K., C.J. Weister and H.M. Pellet : 1973. The temperature and moisture effects on hardening of apple roots. HortScience **8** : 53-55.
15) Wilner, J : 1955. Note on an electrolytic procedure for differentiating between frost injury of roots and shoots in woody plants. Can. J. Plant Sci. **39** : 512-513.

ブドウ'巨峰'自根樹の根と母枝の耐寒性

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冬季が寒冷な地域で'巨峰'の根域制限栽培を行うためには，根の耐寒性限界を知ることが必要である．そこで，'巨峰'の2年生挿し木個体を12月及び1月に種々の低温で処理し，根の EC 及び TTC テスト，組織形態の観察，根挿しによる発根テストなどを行った．その結果，−3.0℃で24日間処理しても直径1～2㎜の細根では EC，TTC 還元力とも変化がないが，直径3～5㎜の大根ではやや変化した．−4℃で12時間処理すると，細根，大根の EC と TTC 還元力が大きく変化し，根挿しの発根も抑制された．また，根の皮層と放射組織の細胞が破壊され，空洞が生じた．処理した個体の翌春の生長も著しく不良であった．したがって，自根の'巨峰'樹の根は−4℃以下の低温に1晩でも遭遇すると危険である．一方，母枝は−15℃で24日間処理しても EC や TTC 還元力の変化はなく，挿し木した後の発根も正常であった．しかし，−20℃処理では EC と TTC 還元力が大きく変化し，発芽も完全に抑制された．

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