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Studies on cold hardiness of grapevine roots

by

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Untersuchungen zur Frostresistenz von Rebwurzeln

Zusammenfassung: Bei 19 Rebsorten bzw. -arten, davon 16 Unterlagssorten, wurde die kritische Frosttemperatur bestimmt, bei der die Rebwurzeln abstarben. Zwischen den untersuchten Sorten wurden beträchtliche Unterschiede der Frostresistenz festgestellt, so daß die Selektion weiterer frostresistenter Unterlagsreben aussichtsreich erscheint.

Verschiedene Methoden zur Feststellung der Frostresistenz wurden verglichen. Als einfach und verläßlich erwies es sich, die Messung der elektrischen Leitfähigkeit des Wurzeldiffusats mit der Kulturmethode von Wurzelabschnitten zu kombinieren. Rasterelektronenmikroskopische Untersuchungen der Wurzelstruktur ergaben ebenfalls Korrelationen zur Frostresistenz, so daß aufgrund der strukturellen Befunde eine Prognose der voraussichtlichen Frostresistenz und eine Sichtung des Rebenmaterials vor der Durchführung weiterführender Kältetests möglich ist.

Key words: cold, resistance, frost damage, analysis, histology, root, variety of vine, rootstock, China.

Introduction

In some cold regions of China, such as in the northern part of the Liaoning Province, in the Jilin and Heilongjiang Provinces and the Inner Mongolian Autonomous Region, above-ground winter temperature usually goes down to −32 to −40 °C, and the low soil temperatures (Table 1) during winter often damage the roots of ungrafted *Vitis vinifera* grapevines seriously. In order to protect the roots from being killed, Beta (Carver × Concord) and *V. amurensis* are used as cold-resistant rootstocks since the 1960s, but the former is not hardy enough and not resistant to saline soil, whereas the latter is difficult to propagate by cuttings. Therefore, it was necessary to select better rootstocks.

Table 1

The minimum temperatures below ground level in the Shenyang region (°C)

Tiefstwerte der Bodentemperatur (°C) in der Region Shenyang

Year	Depth (cm)					
	10	20	40	50	60	80
1962—64 (Av.)	−14.3	−11.8	−8.3	−6.4	—	—
1982	−14.0	−11.5	−7.8	−6.3	−4.7	−2.1
1983	−14.8	−13.0	−7.9	−6.7	−5.5	−3.3

In this study, we determined root cold hardiness of 19 grape varieties and species. Several test methods were compared to choose the most suitable techniques. In order to find morphological indices for prediction of cold hardiness and for screening of grape materials tissue structure of roots was also investigated.

Materials and methods

Grapevine materials

3 fruiting and 16 rootstock varieties (or species) were used: *V. amurensis,* Beta (Carver × Concord), Beichun (Muscat Hamburg × *V. amurensis*), Gongniang no. 1 (Muscat Hamburg × *V. amurensis*), Gongniang no. 2 (*V. amurensis* × Muscat Hamburg), Heishan (Black Hamburg × *V. amurensis*), *V. riparia,* Kyoho (Campbell Early × Centennial), Campbell Early, Muscat Hamburg, SO 4 (*V. berlandieri* × *V. riparia,* hybrid of Teleki), Freedom (1613 no. 59 × Dogridge no. 5), Harmony (1613 no. 39 × Dogridge no. 5), Teleki 5 A (*V. berlandieri* × *V. riparia* no. 5), St. George (*V. rupestris*), *V. riparia* no. 580, *V. amurensis* × *V. riparia, V. amurensis* × *V. vulpina* (= *riparia*), *V. amurensis* × *V. labrusca.* 1-year-old test roots were collected from the field in November 1982 and 1983.

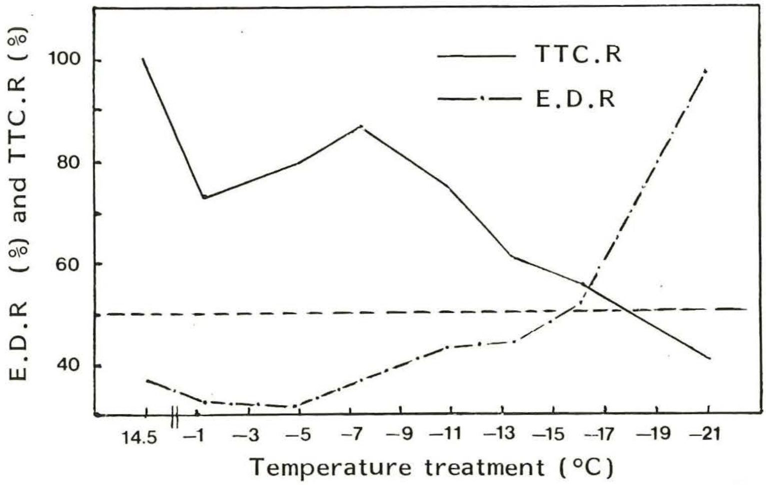


Fig. 1: Electrolyte diffusion rate (%) and TTC reduction rate (%) of *V. amurensis* after cold treatment.

Elektrische Leitfähigkeit des Diffusats (%) und TTC-Reduktion (%) bei *V. amurensis* nach Kältebehandlung.

Measurement of root cold hardiness

(i) Electrolytic conductance method and TTC (triphenyl tetrazolium chloride) reduction. — The measuring procedure and methods adopted in this experiment were basically routine (8, 12, 13). 1-year-old washed root pieces (about 2—3 mm in diameter) were cut to a length of 4 cm; samples for each treatment weighed 4 g. They were put into test tubes (3 × 20 cm) and exposed to different artificial temperatures for 4 h, then moved to 0 °C for 2 h. After that, they were put into a freezer under 3—6 °C for 16 h, and their cold hardiness was measured by the electrolytic conductance method with samples cut to 2—3 mm in length and weighing 1 g, as well as by the TTC reduction method using samples of 1—2 mm in length and weighing 0.1 g. Two replications were made for each treatment.

The results were calculated using the following formulas:
Electrolyte diffusion rate for treated material

$$\text{E.D.R} = \frac{\text{C 1 } (\mu\Omega/\text{cm})}{\text{C 2 } (\mu\Omega/\text{cm})} \times 100\ \%$$

TTC reduction rate for treated material

$$\text{TTC.R} = \frac{\text{O.D reading for treated material at 490 nm}}{\text{O.D reading for control at 490 nm}} \times 100\ \%$$

C 1 represents the electrolyte conductivity of treated material measured with an electro-conductometer Model DDS-11A, C 2 the electrolyte conductivity after boiling.

Optical density (O. D.) was measured with a spectrophotometer Model 721.

We adopted the 50 % threshold of TTC reduction rate and electrolyte diffusion rate as the physiological index of the critical chilling temperature for grape root (3, 6, 7).

(ii) Root section culture. — After the different low-temperature treatments, root sections were put into culture dishes and kept in a thermostat under 20—25 °C for about 2 weeks. The colour change of root cross-section surface, the appearance of callus or new roots were recorded. Roots were considered alive if callusing or new root growth occurred.

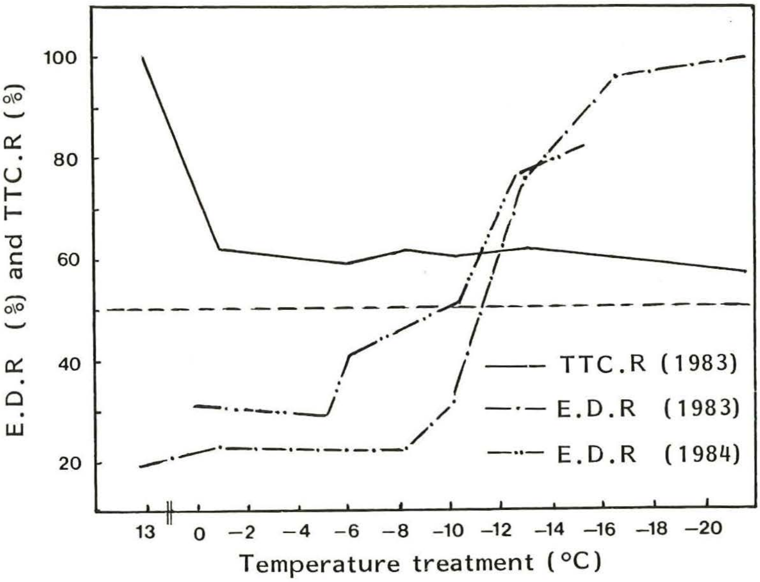


Fig. 2: Electrolyte diffusion rate (%) and TTC reduction rate (%) of Heishan after cold treatment.

Elektrische Leitfähigkeit des Diffusats (%) und TTC-Reduktion (%) bei der Sorte Heishan nach Kältebehandlung.

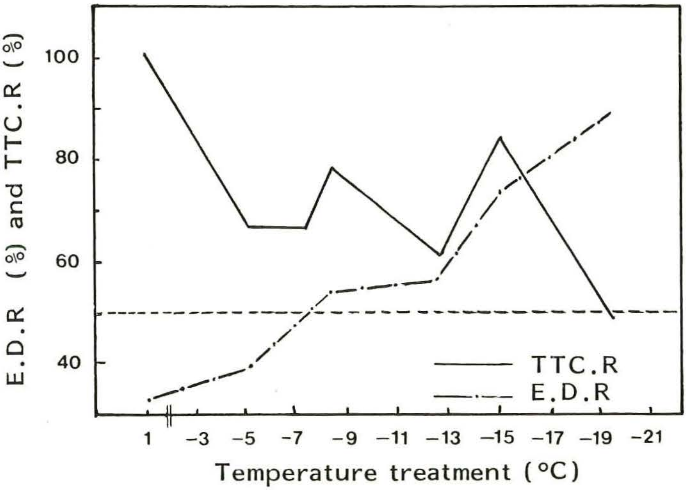


Fig. 3: Electrolyte diffusion rate (%) and TTC reduction rate (%) of Campbell Early after cold treatment.

Elektrische Leitfähigkeit des Diffusats (%) und TTC-Reduktion (%) bei der Sorte Campbell Early nach Kältebehandlung.

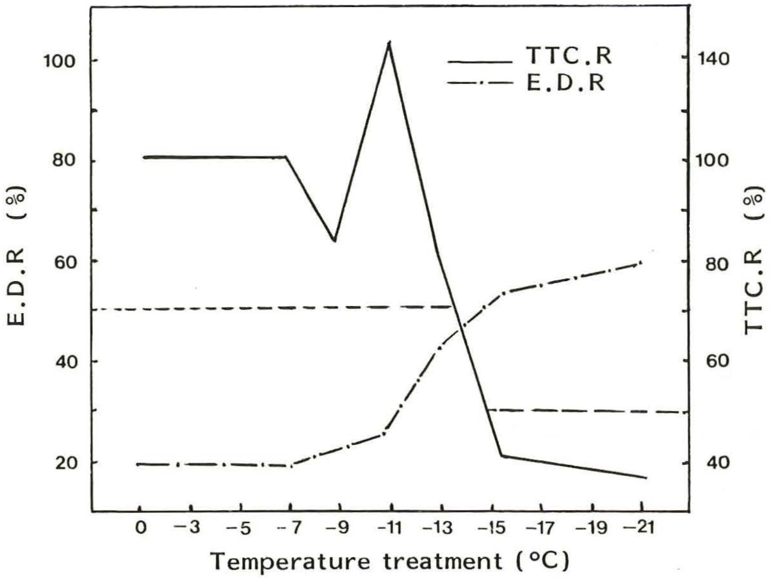


Fig. 4: Electrolyte diffusion rate (%) and TTC reduction rate (%) of *V. amurensis* × *V. riparia* after cold treatment.

Elektrische Leitfähigkeit des Diffusats (%) und TTC-Reduktion (%) bei der Kreuzung *V. amurensis* × *V. riparia* nach Kältebehandlung.

Evaluation of root structure

1-year-old roots, 2—3 mm in diameter, of 5 varieties (species), including *V. amurensis,* Beta, Beichun, Kyoho and Muscat Hamburg, were studied. After pretreatment as usual and cross sectioning, photographs of tissue structure were taken with a scanning electron microscope and the following characters were measured: Size of cortex and ray cells, size and density of the vessels, ratio of the bark area as per cent of the total area of root section.

Results and discussion

1. Measurement of critical chilling temperature for grape roots

In this part of the study, electrolytic conductance and TTC reduction methods were mainly adopted for measuring the critical winter temperatures of grape roots. At the same time, root section culture was used as a supplemental method to further verify the above-mentioned methods. The results of measurement for different varieties or species were as follows:

(i) *Vitis amurensis.* — E.D.R. and TTC.R measurements indicated critical chilling temperatures of −15.5 °C and −17.6 °C, respectively (Fig. 1). By using root section culture, we also found that root sections under −16 °C were browning seriously and could

Table 2

The critical chilling temperatures of grape roots (°C) measured by the electrolytic conductance method

Kritische Frosttemperaturen von Rebenwurzeln (°C) bei Anwendung der Leitfähigkeitsmethode

Species/cultivar	Winter 1983	Winter 1984	Average
Muscat Hamburg	—	− 5.2	− 5.2
Kyoho	− 6.7	− 6.8	− 6.7
Campbell Early	− 7.0	− 7.1	− 7.0
Harmony	—	− 8.0	− 8.0
Teleki 5 A	—	− 8.7	− 8.7
SO 4	—	− 9.0	− 9.0
Freedom	—	− 9.0	− 9.0
Gongniang # 1	− 9.2	− 8.9	− 9.1
Beichun	− 8.8	− 9.8	− 9.3
St. George	—	− 9.4	− 9.4
V. riparia # 580	—	− 9.8	− 9.8
Gongniang # 2	−10.0	−10.4	−10.2
Heishan	−10.1	−11.4	−10.8
V. amurensis × *V. labrusca*	—	−11.3	−11.3
V. riparia	—	−11.4	−11.4
Beta	−12.5	−12.7	−12.6
V. amurensis × *V. riparia*	—	−14.8	−14.8
V. amurensis × *V. vulpina*	—	−15.0	−15.0
V. amurensis	−15.5	—	−15.5

not recover after a 2 weeks' culture. Root sections under −13 °C were browning only slightly; the cambium was still white in colour and some new root growth occurred after culture. Thus, we consider the critical chilling temperature of *V. amurensis* roots to be about −16 °C. The result was basically consistent with HE's work (3) using the same methods and also FU's observation under field conditions (2).

(ii) Heishan. — The TTC reduction rate changed little between −1 °C and −21 °C (60 %) and the curve for TTC.R % was almost parallel to the temperature axis (Fig. 2). The results seemed unstable and unreliable. The critical chilling temperatures determined in 1983 and 1984 by the electrolytic conductance method were −10.1 °C and −11.4 °C, respectively. The result of root section culture was also consistent with the E.D.R. measurements.

(iii) Campbell Early. — As shown in Fig. 3, the TTC reduction rate did not follow a distinct pattern. The critical chilling temperature determined by electrolytic conductance method was −7.1 °C, which had also been confirmed by root section culture. Root sections under −5 °C treatment produced some new roots and callus after a 2 weeks' culture, and the root section under −7.3 °C treatment became brown and soft.

(iv) *V. amurensis* × *V. riparia*. — According to Fig. 4, the results gained by the two methods were very close to each other. The critical chilling temperatures were −14.8 °C and −15.0 °C, respectively; they were also coincident with those obtained by root section culture.

In addition to the 4 grapevines described above, we also tested the critical chilling temperatures of 15 other varieties.

As is seen from Table 2, there were (i) differences in cold hardiness among the 3 fruiting varieties. Muscat Hamburg was the most tender one, with a critical chilling temperature of −5.2 °C. These data could be used as an important guideline in formulating winter protection measures for self-rooted vines. The tender the variety, the wider and thicker the soil cover in winter should be.

(ii) There were great differences in cold hardiness among the 16 rootstocks tested. Harmony was most tender, with a critical temperature of −8 °C; *V. amurensis* with

Table 3

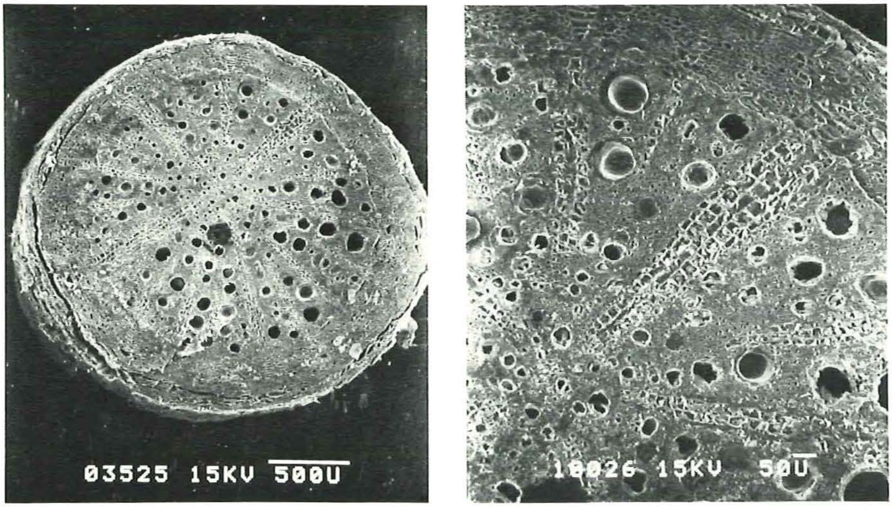
Characteristic of grape root structure for different varieties

Charakterisierung der Wurzelstruktur bei verschiedenen Rebsorten

Character	*V. amurensis*	Beta	Beichun	Kyoho	Muscat Hamburg
Cell size of cortex (L × W, μm)	15.5 × 6.5	23.0 × 16.7	28.9 × 18.6	38.2 × 25.9	39.1 × 25.8
Cell size of rays (L × W, μm)	28.4 × 21.1	33.3 × 23.8	41.1 × 21.1	45.4 × 24.7	49.3 × 26.9
Diameter of vessels (μm)	43.3	54.4	64.9	73.5	71.6
Density of vessels (no./mm^2)	29.6	37.2	39.0	43.2	44.9
Ratio of bark (% of total area)	40.0	49.3	51.8	57.2	63.1

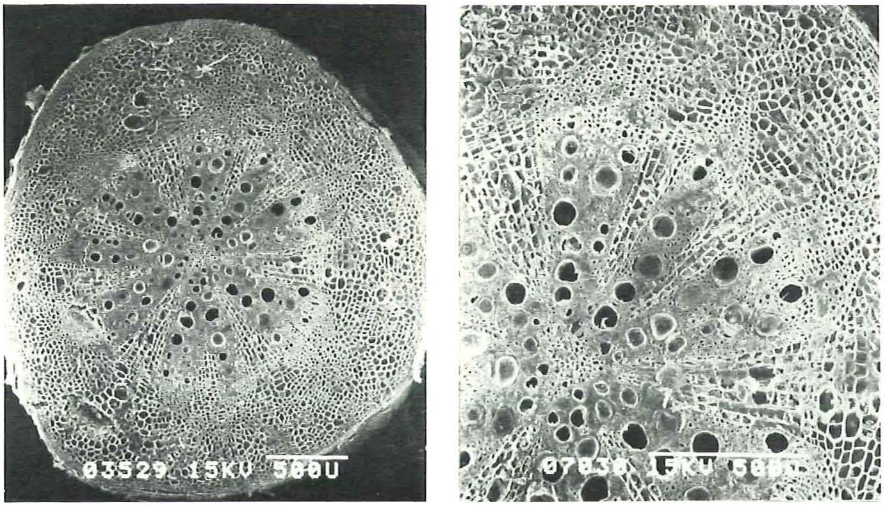
L = length, W = width.

A. *Vitis amurensis*



x 35 x 100

B. Muscat Hamburg

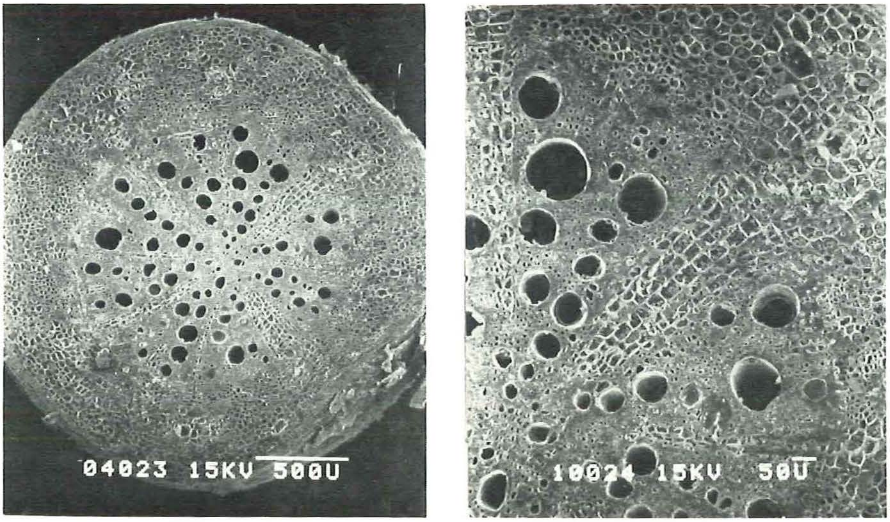


x 35 x 70

Fig. 5 A and B: Root structure of *Vitis amurensis* and a *V. vinifera* variety.

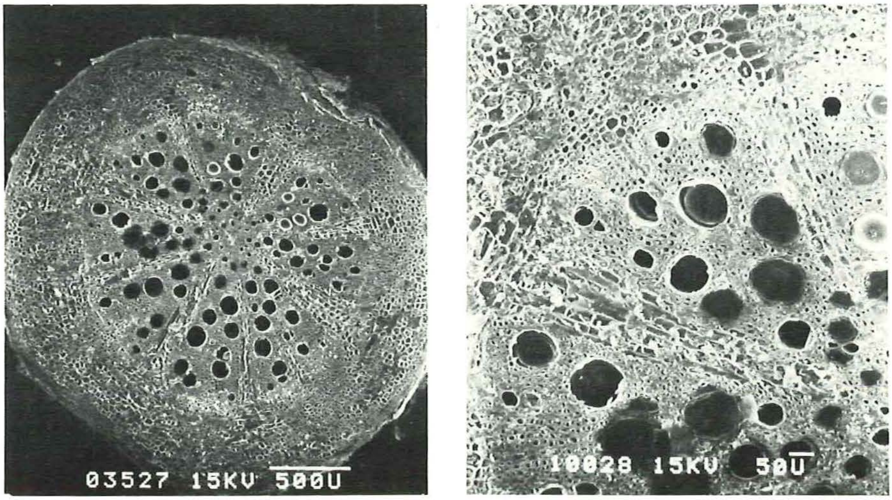
Wurzelstruktur von *Vitis amurensis* und einer *V.-vinifera*-Sorte.

C. Beta



x 40 x 100

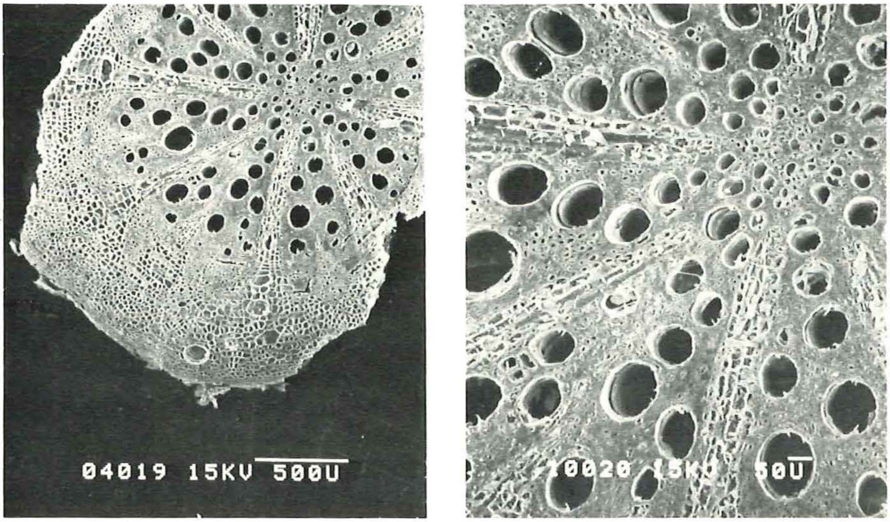
D. Beichun



x 35 x 100

Fig. 5 C—E: Root structure of three *Vitis* varieties.

Wurzelstruktur von drei *Vitis*-Sorten.


E. Kyoho

x 40

x 100

– 15.5 °C was most hardy. *V. riparia* and *V. amurensis* × *V. labrusca* were close to Beta in hardiness. *V. amurensis* × *V. riparia* was hardier than Beta and close to *V. amurensis*. These vines, rooting easier when propagated by cuttings and having multiple resistance, might become valuable rootstocks in cold regions, if further tests will be confirmatory.

According to the above-mentioned results, the electrolytic conductance method was easy to handle, fast, low in cost and most accurate in measuring cold hardiness of grape roots (1, 6). If root section culture is used as a supplementary method, reliability of the testing results can be further increased (3, 4, 11).

TTC reduction method was labour consuming and more expensive and its results were fluctuating and unstable (3). It seems that this method should not be adopted for determination of cold hardiness of grape roots. This conclusion is basically consistent with the work of STERGIOS and HOWELL (11).

2. Characteristics of grape root structure and its relation to cold hardiness

In 1982 and 1983, 5 grape varieties with different degree of cold hardiness were used as test materials. Root structure was investigated by means of scanning electron microscopy (see 'Materials and methods'). The results, presented in Table 3 and Fig. 5, showed the following trends:

(i) There existed great differences in the size of cortex cells and ray cells among different varieties. The hardiest grapevine, *V. amurensis*, possessed the smallest cells and the most compact tissues. The cultivated varieties Kyoho and Muscat Hamburg, which were of low hardiness, were larger in cell size and looser in tissue structure. It seems that cell size is closely related to cold hardiness. The smaller the cell size and the more compact the tissue, the greater the hardiness would be (7). We are of the opinion that smaller cell size and greater compactness of tissue may be used as a morphological index for forecasting cold hardiness in screening grape rootstocks.

(ii) Vessel size and density of *V. amurensis* were also the smallest among the 5 varieties tested, whereas those of Muscat Hamburg and Kyoho were the largest. It is presumed that when the vessels are larger and higher in density, the tissue would contain more free water. Some researchers reported that water content in the root is closely related with cold hardiness (5, 9). Therefore, the smaller the vessel size and the lower their density, the stronger the resistance to freezing would be.

(iii) There were some differences in the ratio of bark and xylem in root structure. The bark ratio of *V. amurensis* was lowest, while that of Muscat Hamburg was highest. The higher the cold hardiness of a variety, the lower the bark ratio will be. According to the opinion of most researchers, the bark of roots is most sensitive to winter temperatures and the xylem and pith are harder. This is because the bark contains mainly living cells, dormancy of the bark and cambium being weaker and some physiological activities still remaining in winter. Such tissues are more susceptible to low-temperature stress (5, 10). We found that the bark of roots was the first to become brown after low temperature treatment. Hence the ratio of bark and xylem can be used as another morphological index in forecasting cold hardiness for grape roots.

Summary

1. For 16 grapevine rootstocks, the critical chilling temperatures were determined. There were great differences in cold hardiness among these vines. The cold hardiness of several newly introduced rootstocks was higher than that of rootstock variety Beta and they showed even multiple resistance. Thus, after further testing experiments, they might become valuable rootstocks in cold regions.
2. There were some differences in cold hardiness among 3 fruiting varieties tested. This finding can be used as reference in formulating winter protection measures for own-rooted grapes.
3. Comparative examination of the methods for testing cold hardiness showed that the combination of electrolytic conductance method with root section culture was simple and reliable for measuring cold hardiness of grape roots, while TTC reduction method, because of its lack of regularity in test results, was not suitable for this purpose.
4. The characteristics of root structure have certain correlations with cold hardiness:
   - (i) Varieties with higher cold hardiness have smaller bark and ray cells than tender varieties and their tissue structure is more compact.
   - (ii) Vessel diameter is smaller and vessel density is lower in hardy varieties.
   - (iii) Varieties with higher cold hardiness have a lower percentage of bark and a higher rate of xylem in their roots.

Thus, these characteristics of root structure could be used as an additional index in forecasting and screening cold hardiness of grape roots.

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