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Research Note

In vitro grafting of grape with phylloxera resistant rootstock cultivars

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K e y w o r d s : micrografting, grape, scion, Phylloxera resistance rootstock.

Introduction: Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) feed on the sap of grape roots, causing damage and often death of vines (Makee *et al*. 2004). In case of phylloxera infestation current solutions include ripping out infested vines, fumigation of soils with methyl bromide, and replantation with tolerant rootstocks (Motosugi *et al*. 2002).

Micrografting has been successfully used in a wide range of horticultural plants including grape, as an effective method for the acquisition of clones which are free of soilborne pathogens, including viruses and virus-like diseases. This technique is also used to detect graft compatibility during early stages. Some important success has been reported for fruit trees, including citrus (Oiyama 1992), cherry (Ozzambak and Schmidt 1991), apple (Richardson *et al*. 1996) and grapes (Martino 1992).

The objective of the present study was to develop an efficient and robust micrografting system for the protection of grapes from phylloxera, using Millardet et de Grasset 01-14, Kober 5 BB, Couderc 3309 C and Rupestris du Lot rootstocks and, for mass production, favorable table grapes as scions.

Material and Methods: P l a n t m a t e r i a l a n d e x p l a n t p r e p a r a t i o n : Grape cultivars and rootstocks were sampled from the field at the National Horticultural Research Institute in Suwon, Republic of Korea, from late June until the first week of August in 1999 and 2003. The cultivars which were used to produce experimental scions included Kyoho, Campbell Early, Schuyler, and Tamnara. Millardet et de Grasset 101-14, Kober 5 BB, Couderc 3309 C and Rupestris du Lot were used as rootstocks. Shoot tips were sterilized with 70 % ethanol for 1 min; stirred for 12 min in 15 % sodium hypochloride, and rinsed 4 times in sterile water. The sterilized shoot tips were cut into pieces (30 mm in length), which were cultured (6-7 per jar).

R o o t s t o c k a n d s c i o n m u l t i p l i c a t i o n : For shoot multiplication we used MS basal medium (Murashige and Skoog 1962) supplemented with plant growth regulator (0.3-0.5 $mg \cdot l^{-1}$ benzyladenine, 3 % sucrose, and 0.8 % Gum Agar, Sigma). The pH of the media was adjusted with NaOH to 5.7 prior to autoclaving.

M i c r o g r a f t i n g m e d i u m : For micrografting we used half-strength MS medium which was free of growth regulators, but supplemented with 4 % sucrose and 0.8 % Gum Agar, Sigma). The pH of this medium was also adjusted to 5.7 prior to autoclaving (121 °C, 15 min).

M e t h o d o f g r a f t i n g : 3-week-old shoots were used as scions after the final subculture *in vitro*. Each scion consisted of a 5-10 mm long herbaceous stem with an axillary bud. Ten-week-old stems (length: 15-20 mm) were used for rootstocks. Cleft-grafting was applied as a micrografting method, thereafter the cultures were incubated in darkness at 27± 1°C for three d, then transferred to light (40 W cool-white fluorescent bulbs, average photosynthetic photon flux density: 100 $\mu mol \cdot s^{-1} \cdot m^{-2}$) in a growth room at 27 ± 1°C with a forced cooling fan system.

Results and Discussion: The success of micrografting primarily depends on the grafting technique. In this study cleft grafting proved to be the most successful and efficient method for *in vitro* micrografting (Figure A, B and C). Grafting success was determined by callus formation and rooting. Calluses tended to be formed at the junction of a graft union, arising from the living cells of both, scion and rootstock (Figure D, E, F, and G). Moore and Walker (1983) observed that adjacent callus masses tend to graft successfully in the absence of vascular differentiation. However, callus formation was determined by independent of grafting success, although callus growth constitutes a key factor in the development of the graft union; *e.g.* it physically joins the scion to the rootstock in the pear cactus (*Opuntia*) (Jeffree and Yeoman 1983).

Grafted shoots began to grow approximately 10 d after grafting. When the scion produced about 6 new leaves, the plants were transferred into small pots which contained an artificial soil mixture (vermiculite: peat moss = 5 : 1, v/v). The pots were enclosed in polyethylene bags and after 3-7 d the bags were gradually opened. After 4 weeks, the plants were transferred to large pots and moved to a greenhouse (Figure H and I).

After grafting cvs Campbell Early and Kyoho to different rootstocks we observed that, Kyoho exhibited a higher graft compatibility rate than Campbell Early (Table). When softwood graftings were compared with hardwood graftings, the former proved to be superior with regard to successful unions. In Korea, it has been reported that Kyoho, grafted to SO 4 is arable, as defined by evaluations of soluble solids, coloring, berry shattering and yield. Campbell Early grafted to each of the tested rootstocks had a significantly higher shoot vigor compared to non-grafted seedlings. The rootstock Couderc 3309 was successful in rooting, callus formation, and growth with all scion types (Table). Among the rootstocks or scions, we found that grafting compatibilities ranged between 11 % (Tam-nara/Rupestris du Lot) and 100 % (Schuyler/Couderc 3309).

In Korea, most grapevines were field-grafted with phylloxera-resistant rootstocks, although the production of rootstocks and scions in the field is an extremely time-con-

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Table

Effects of micrografting of cvs Kyoho, Campbell Early, Tamnara, and Schuyler to various rootstock cvs

Scions	Rootstocks	Scion length (cm)	Rate of rooting (%)	Rate of callus formation (%)	Rate of micrografting (%)
Kyoho	Millardet et de Grasset 101-14	9.56 a*)	0.80 a	0.63 a	19 b
	Couderc 3309	7.17 b	0.83 a	0.83 a	72 a
	Rupestris du Lot	12.60 a	0.69 ab	0.72 a	61 ab
	Kober 5 BB	5.26 b	0.27 b	0.44 a	27 ab
Campbell	Millardet et de Grasset 101-14	14.00 a	0.50 a	0.61 a	38 a
Early	Couderc 3309	11.36 a	0.66 a	0.66 a	66 a
	Rupestris du Lot	12.43 a	0.83 a	0.83 a	83 a
	Kober 5 BB	10.74 a	0.55 a	0.66 a	55 a
Tamnara	Millardet et de Grasset 101-14	7.64 b	0.75 a	0.83 a	75 a
	Couderc 3309	12.44 a	1.00 a	0.91 a	91 a
	Rupestris du Lot	4.70 b	0.11 b	0.00 b	11 b
	Kober 5 BB	5.03 b	0.22 b	0.55 ab	22 b
Schuyler	Millardet et de Grasset 101-14	11.50 a	0.83 a	0.83 a	50 b
	Couderc 3309	10.68 a	1.00 a	1.00 a	100 a
	Rupestris du Lot	9.03 a	0.55 a	0.66 a	22 c
	Kober 5 BB	7.56 a	0.61 a	0.72 a	38 bc

*) Mean separation in columns within scions by Duncan's multiple range test at $P \leq 0.05$. The mean values are from 10 replications (jars) with 3 subsamples (graftings) per jar.

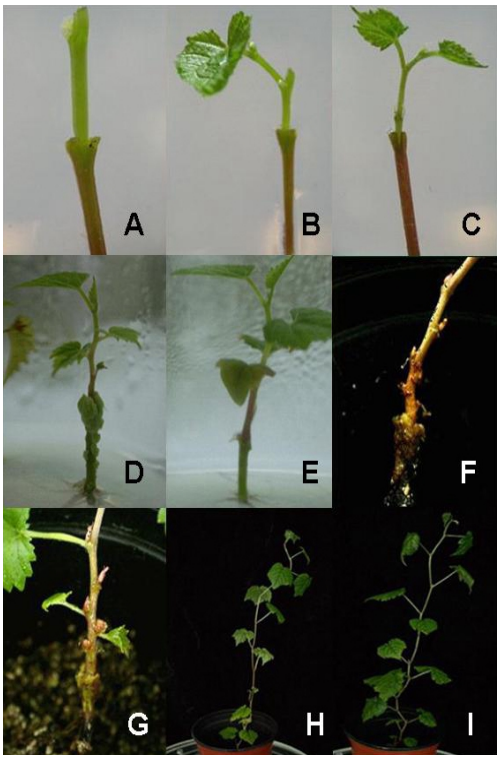

Figure: *In vitro* grafting of grape to Phylloxera resistant rootstocks (Schuyler/Couderc 3309). (**A**) no leaf, (**B**) one leaf, and (**C**) two leaves, (**D**) with callus, (**E**) no callus, (**F**) and (**G**) graftings after 6 weeks, (**H**) and (**I**) graftings after 8 weeks.

suming process. In this study, we propose mass production of phylloxera-tolerant grapevines *via* micrografting, involving *in vitro* culturing for the production of virus-free scions and rootstocks. Moreover, micro-grafting can also be successfully applied to predict more rapidly the grafting compatibility between newly developed scion and rootstock cultivars.

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