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# Micrografting Technology in Grapevine (*Vitis vinifera* L.)

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## ABSTRACT

Micropropagated plantlets of *Vitis amurensis* Rupr. were used as rootstock and 'Jing Xiu' (*V. vinifera* L.) as scion. The effect of different graft methods *in vitro* on the survival rate for grafting and acclimatization of transplants was studied in this experiment. Results showed that the survival rate for grafted and acclimatized transplants by different graft methods differed greatly. Among eight micrografting methods, micropropagated *V. amurensis* Rupr. plantlets cultured for 30-40 d with or without roots and without leaves were selected as rootstock. Stem tips of 'Jing Xiu' *in vitro* for 20-30 d were selected as scion material. The survival rate for grafting was comparatively high, 90% for shoot tip/stem with roots and no leaves and 85% for shoot tip/stem without roots or leaves. Rootless and leafless stem segments used as rootstock, or stem tips and stem segments selected as scions demonstrated survival rates of acclimatized transplants higher than other graft methods used, 75% and 80%, respectively. The survival rate for transplants was 65% for shoot tip/stem with roots and no leaves, but it was lower than that of the two combinations of rootless rootstocks. This indicated that the physiological state of roots greatly affects the survival rate for transplanted, grafted plantlets. In order to increase grafted grapevine production and guarantee a higher survival rate, the perfect method for grape micrografting is proposed as: (1) plantlets with roots and without leaves as the rootstock, and a stem tip as the scion or (2) a stem segment without roots and leaves as the rootstock, and a stem tip as the scion.

**Keywords:** graft method, micrograft, *Vitis amurensis* Rupr.
**Abbreviations: IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **MS**, Murashige and Skoog (1962) medium

## INTRODUCTION

World production of grapes exceeded that of any other fruit until 1989. Now, grape production ranks second, only to orange (He 1999). It is popular because of its high nutrient and vitamin content and it can be eaten fresh or dried or processed to produce candy, wine, juice, and flavorings. The economic importance of grape products has stimulated a large volume of research which is concerned with both the basic cytogenetic and taxonomic problems encountered by breeders and by the demand for cultivars to adapt to varied climates and use (Luo 2004).

Micropropagation techniques are being applied to address the propagation needs and to bring about rapid improvement in plants and they has many advantages for the clonal propagation of fruit trees (Stushnoff and Fear 1985). However, poor *in vitro* response from mature trees is attributed to a lack of juvenility in older tissues and rejuvenation is sought as a measure to increase this (Pierik 1990). Micrografting as a means to rejuvenate adult tissues has been attempted in many woody perennials, which is performed under aseptic and high relative humidity conditions (Burger 1985; Hartmann *et al*. 1997; reviewed in Dobránszki and Jambor-Benczúr 2006). Alfaro and Murashige (1987) observed an improvement in rooting ability of microshoots in avocado by micrografting. The *in vitro* response of sequoia was found to improve by micrografting buds originating from old trees on to juvenating mature trees in *Picea* species (Ponsonby and Mantell 1993), *Hevea brasiliensis* (Perrin *et al*. 1994). In addition, *in vitro* micrografting may provide several advantages such as the elimination of viruses, year-round plant production, enhance compatibility studies and correlative relations between rootstocks and scions, make specific genotypic combinations to increase plant productivity, and extend ecological limits of a particular plant species or cultivar to tolerate edaphic conditions (Richardson *et al*. 1996; Hartmann *et al*. 1997; reviewed in Dobránszki and Jambor-Benczúr 2006). Ma *et al*. (1999) selected micrografing to detect micropropagated plantlets with known fan leafy virus, and the detection rate was 79.5%, with symptoms showing about 8-12 weeks from grafting (Ma 1999). Pathirana and McKenzie (2005) also selected this method to detect leafroll viruses early.

This paper reports on the most appropriate and reliable micrografing techniques, and optimal grafting materials for grape. In addition, methods are reported for the successful rooting of micrografted plantlets and the acclimatization of transplants.

## MATERIALS AND METHODS

### Plant materials for micrografting

Var. 'Shuang You' variety is a new wild grape variety. Wild grapes (*Vitis amurensis* Rupr.) are important wild resources in China. They are the hardiest among grape species and are the better materials as hardy stock (Song 1996; Song 2000). 'Jing Xiu' belongs to fresh variety and it has good quality, big grain, hard flesh and earliness (Li Shaohua 2004). *V. amurensis* var. 'ShuangYou' was obtained from the Special Products Institute of the Chinese Academy of Agricultural Sciences.

Its dormant annual hardwood was scrubbed with suds and incubated in 2% sucrose solution. The solution was changed for a new one every one week and the branches was sprayed with water twice or three times daily. New buds grow after one month and these were used as explant materials.

The new buds, obtained by removing the expanded leaves, were put in a sterilized jar and were washed twice with sterile distilled water. Buds were surface sterilized by immersion in 75% ethanol (30 s) followed by agitation for 1 min in 0.1% $HgCl_2$. After

Received: 25 November, 2006. Accepted: 28 January, 2007.

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rinsing three or four times in sterile distilled water, the buds were inoculated into pre-sterilized screw cap bottles (200 ml capacity, 5.5 cm × 9.5 cm OD×H) with B5 medium (Gamborg 1968) containing 1.1 μM IAA, 0.058 M sucrose and 0.8% (w/v) agar after adjusting the pH to 5.8. All cultures were incubated at 25 ± 2°C with a 14 h photoperiod at 35-40 μ.mol.m$^{-2}$s$^{-1}$ photosynthetic active radiation (PAR) provided by cool white fluorescent lights (made in China). When seedlings reached the top of the bottle in the initiation culture stage, tube seedlings were cut into single buds and transferred to half strength MS (Murashige and Skoog 1962) medium with 2.4 μM IBA, 0.058 M sucrose and 0.8% (w/v) agar at pH 5.8 for the multiplication and rooting cultivation. The incubation conditions were the same as the initiation culture stage. Micropropagated plantlets were used as rootstock. Micropropagated 'Jing Xiu' plantlets were used as scions (Zhu 2005).

## Grafting method

Under aseptic conditions, shoots of uniform length (2.0 cm) and diameter (1.0 mm) were selected from *in vitro* cultures and used as rootstocks and scions in micrografting. The top of the rootstock was slit (0.6-0.8 cm deep) and the scion was cut at an angle of 45° from the base (**Fig. 1**). In all cases, the rootstocks and scions were 2.0 and 1.0 cm long, respectively. Their diameters were kept as uniform as possible (ranging between 0.5 and 1.0 mm). Finally, the joined part was ligated with aseptic aluminium foil (1.0 cm × 0.5 cm).

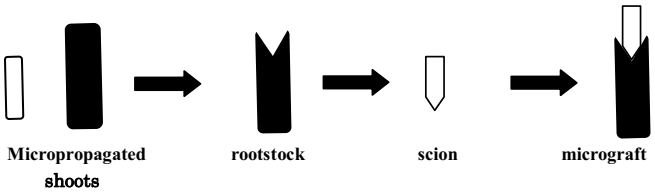


**Fig. 1** ***In vitro*** **micrografting of grape plantlets.**

## Culture conditions

Micrografted plantlets were cultured on half strength MS medium with 2.4 μM IBA, 0.058 M sucrose and 0.8% (w/v) agar at pH 5.8. All cultures were incubated at 25 ± 2°C in a 14 h photoperiod with 35-40 μ.mol.m$^{-2}$s$^{-1}$ PAR provided by cool white fluorescent lights (made in China).

## Acclimatization

Thirty plantlets for every micrografted method were removed from culture, the roots washed with running tap water. The plants were transferred to autoclaved river sand held in 50 cm × 50 cm styrofoam trays and covered with plastic for the first 2 weeks, to maintain high humidity. Plantlets were put into a culture room and watered every day for 2 weeks. After 15 days the plants grew the new roots and were transferred to soil outside the culture room.

## Data analysis

The formation of roots and rooting rate of micrografted plantlets were recorded on micrografts after 20 days, while graft survival rate and the survival of micrografted plantlets were recorded 30 days after micrografting and 30 days after acclimatization. Data was statistically analyzed by analysis of variance (ANOVA), and Tukey's test (α = 0.05%) was applied to separate the means using SPSS 11.5 software (SPSS, USA).

# RESULTS AND DISCUSSION

## The formation rate and rooting rate of different grafting methods

The formation of roots was observed 20 days after grafting and the rooting rates are displayed in **Table 1**. Successful root formation (formation rate >60%) was obtained in three methods in which the shoot tip was used as the scion,

**Table 1** Effect of different grafting methods on the formation rate of micrografted plantlets (n = 20).

| Scion/rootstock number | Formation rate (%) |
|---|---|
| 1 | 75.00 ab |
| 2 | 45.00 c |
| 3 | 90.00 a |
| 4 | 65.00 ab |
| 5 | 40.00 c |
| 6 | 30.00 c |
| 7 | 60.00 bc |
| 8 | 45.00 c |

Treatments: 1 = shoot tip/stem with roots and leaves; 2 = stem/rooted and leafed stem; 3 = shoot tip/rooted and leafless stem; 4 = stem/rooted and leafless stem; 5 = shoot tip/rootless and leafed stem; 6 = stem/rootless and leafed stem; 7 = shoot tip/rootless and leafless stem; 8 = stem/rootless and leafless stem.

resulting in a root formation rate of up to 90%. This indicates that micrografted plantlets germinated more rapidly when the shoot tip was used as the scion. A possible reason may be that the meristem of the shoot tip grows and splits faster than that of the stem. Stem segments can also be used as the scion in micropropagation when many micrografted plantlets are required.

Twenty days after grafting, the rooting rates of micrografting plantlets were determined (**Fig. 2**). All four methods resulted in a good rooting rate and there was no significant difference among them. The rooting rates were from 75% to 95%. This indicates that the rootstock absorbs water and nutrition well and has the ability to supply the necessary growth substances for scion development. For the four methods, the development rates ranged from 30% to 60% and were lower than rooting rates. The highest rate of development (60%) was displayed by the shoot tip/stem-without-roots-or-leaves method, which also had the highest rooting rate (up to 95%), so this method was used as the scion/rootstock and was determined to be the best among all eight methods.

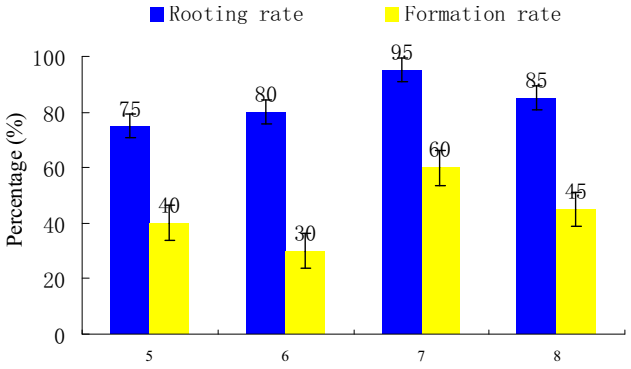


**Fig. 2 Effects of different grafting methods on the formation rates and rooting rates of micrografted plantlets.** Treatments: 1 = shoot tip/stem with roots and leaves; 2 = stem/rooted and leafed stem; 3 = shoot tip/rooted and leafless stem; 4 = stem/rooted and leafless stem; 5 = shoot tip/rootless and leafed stem; 6 = stem/rootless and leafed stem; 7 =shoot tip/rootless and leafless stem; 8 = stem/rootless and leafless stem.

During incubation, we observed that the cicatrized micrografted plantlets produced new leaves and sprouted normally and the plantlets grew well. Twenty days after grafting, the leaf size was comparable to that of the control micropropagated plantlets (**Fig. 3A**).

## Grafting survival rate

The grafting survival rates of different grafting methods are displayed in **Table 2**. There were distinct differences among different grafting methods. The survival rates were 90% and 85%, respectively for shoot tip/stem with roots and no leaves and shoot tip/stem without roots or leaves. These were the two best methods among the eight grafting methods.

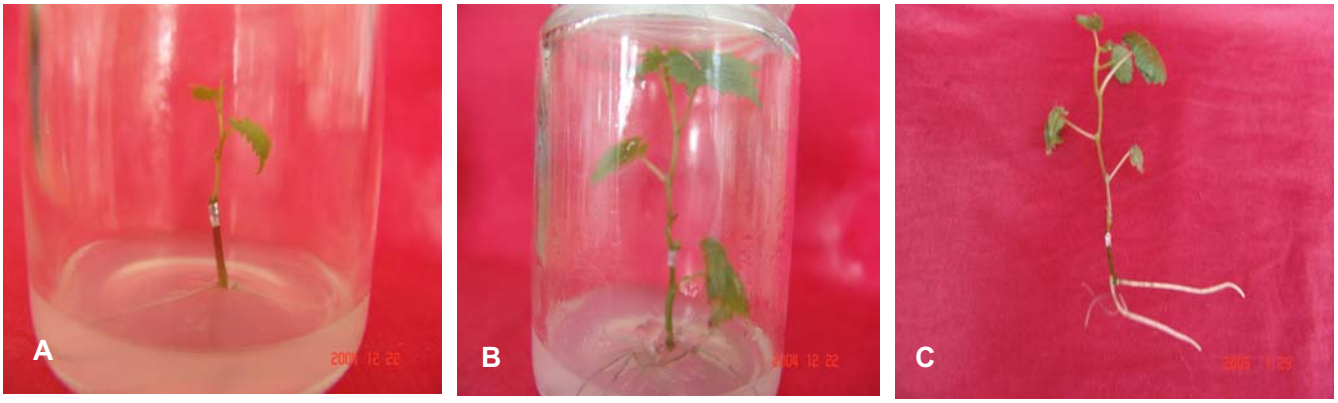


**Fig. 3** ***Vitis vinifera*** **'Jing Xiu' grafted plant** ***in vitro*****.** Growth of a micrografted grape plantlet (**A**) 20 days after grafting, (**B**) 30 days after grafting and (**C**) showing rooting of a 60-day-old micrografted grape plantlet 30 days after transplanting.

**Table 2** Effect of different grafting methods on the grafting survival rate (n = 20 grafts).

| Scion/rootstock number | Survival rate (%) |
|---|---|
| 1 | 75.00 abc |
| 2 | 50.00 c |
| 3 | 90.00 a |
| 4 | 70.00 abc |
| 5 | 55.00 c |
| 6 | 45.00 c |
| 7 | 85.00 ab |
| 8 | 60.00 bc |

Treatments: 1 = shoot tip/stem with roots and leaves; 2 = stem/rooted and leafed stem; 3 = shoot tip/rooted and leafless stem; 4 = stem/rooted and leafless stem; 5 = shoot tip/rootless and leafed stem; 6 = stem/rootless and leafed stem; 7 = shoot tip/rootless and leafless stem; 8 = stem/rootless and leafless stem.

The micrografted plantlets that survived with a rooted stem as rootstock developed earlier and grew better. Moreover, leaves were larger than on rootless plantlets whose stem was used as rootstock (data not shown). The reason for this may be that rootstocks with roots do not need to form roots after grafting since they can absorb nutrients directly from the medium.

Survival rates were higher when shoot tips were used as scion than when stem segments were used. Zhao *et al*. (1998) also found this phenomenon in their experiments. New leaves of micrografted plantlets sprouted earlier (7-8 d after grafting) than developing plantlets 10 days after graf-ting and sprouted, even if some plantlets did not germinate after 30 days.

Most survival rates were lower for methods in which the leafed stem was used as the rootstock except for shoot tip/stem with roots and leaves (**Fig. 4**). This result suggests that the micrografting with a leafless rootstock is better than with a rootstock containing a leaf(ves). The possible reason for this may be that more nutrition is allocated to the rootstock's leaf than is produced by photosynthesis.

In summary, a shoot tip/stem with roots and no leaves and shoot tip/stem without roots or leaves are the best methods among the eight micrografting methods. **Fig. 3B** shows the growth of a micrografted grape plantlet.

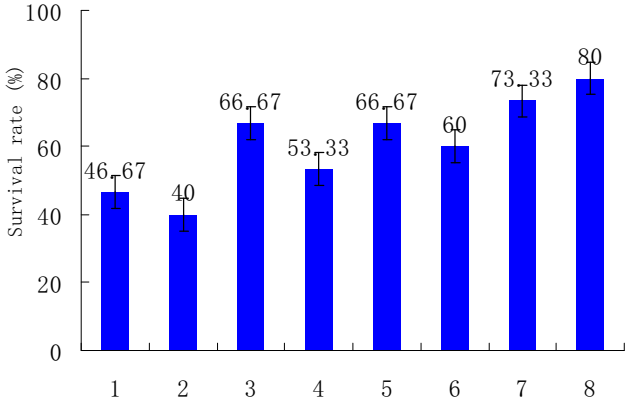


**Fig. 4 Effects of different grafting methods on the survival rates of micrografted plantlets.** Treatments: 1 = shoot tip/stem with roots and leaves; 2 = stem/rooted and leafed stem; 3 = shoot tip/rooted and leafless stem; 4 = stem/rooted and leafless stem; 5 = shoot tip/rootless and leafed stem; 6 = stem/rootless and leafed stem; 7 =shoot tip/rootless and leafless stem; 8 = stem/rootless and leafless stem.

## Acclimatization of micrografted plantlets

The acclimatization rates are shown in **Fig. 4**. There were distinct differences in the acclimatization rates among different grafting methods. Among the four methods, rootless stems were selected as rootstock expect for the shoot tip/rooted and leafless stem method. This suggests that the acclimatization rate of rootless micrografted plantlets is usually higher than that of rooted micrografted plantlets. The reason for this may be that rootstock's root ages over time. The ability to absorb water and nutrition is weaker and it will induce scion death because of water deficiency. **Fig. 3C** shows the rooting of a micrografted grape plantlet following transplant.

## CONCLUDING REMARKS

We have reported an effective *in vitro* micrografting method for grape. In this study, we found the appropriate method for micrografting grape and investigated the effect on the formation and rooting rates by different grafting methods, as well as grafting survival rate and acclimatization percentage of micrografted plantlets. These results are a significant contribution for production and research and for a greater understanding about micrografting-system-based morphogenesis.

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