

Research Note

**The influence of culture dates, genotype and size and type of shoot apices on *in vitro* shoot proliferation of *Vitis vinifera* cvs Thompson Seedless, Ribier and Black Seedless**

CLAUDIA BOTTI, L. GARAY and G. REGINATO

**Key words:** *Vitis vinifera*, tissue culture, micropropagation, genotype effect, culture date effect.

The genotype is one of the most crucial factors in the *in vitro* proliferation response and has been distinctly shown in some *Vitis* species (RAJASEKARAN and MULLINS 1981; CHEE and POOL 1983). The physiological condition of the donor plant has also shown a marked influence on shoot proliferation rates for several species. However, in grapevines, only CHEE and POOL (1985) mention this fact and FANIZZA *et al.* (1984) found no seasonal differences when culturing grapevines apices *in vitro*. With respect to the explant position in the plant (axillary *vs.* apical buds), the results are contradicting (YU and MEREDITH 1986; NOVAK and JUVOVA 1983; HWANG and KIM 1990; SUDARSONO and GOLDY 1991) probably due to its relation with the phenol content existing in the tissue (FANIZZA *et al.* 1984). The explant's size and physiological age are important factors in meristem selections for success in its survival and proliferation. However, no previous references were found in the literature concerning these aspects. The objective of this study was to evaluate the effect of the time (date) of culture as well as size and type of apex (apical or axillary) on the proliferation rate of shoots in three commercially important grapevine cultivars.

**Materials and methods:** Apices from apical and axillary buds (0.5 and 1.5 mm) of the cvs Ribier, Thompson Seedless and Black Seedless were cultured. The following media and concentrations of growth regulators were used: a) Initiation phase: MS 3/4 plus BAP 2.0 mg/l; b) Proliferation phase: MS plus BAP 2.0 mg/l; c) Elongation phase: MS plus BAP 0.5 mg/l plus GA 0.4 mg/l; d) Rooting phase: MS 3/4 plus IBA 0.5 mg/l. The culture was carried out in 6 different dates, every 15 d, starting on Sep. 27. The trial had a factorial design totalizing 72 treatments with 10 replications each. The cultures were kept at  $26 \pm 2$  °C, under 16 h of light with an intensity of  $30 \mu\text{E m}^{-2}\text{s}^{-1}$  PAR (photosynthetically active radiation) and were transferred to a fresh medium every 25 d. Shoots formed after each sub-culture were counted and the results were analyzed by ANOVA and Duncan test. For a commercial purpose, a potential amount of shoot/explant was calculated for each genotype after a one-year period of culture considering the best culture date.

**Results and discussion:** Due to the significant interaction found between genotype and culture date, the results were analyzed for each date and each genotype separately. Thompson Seedless is the cultivar with the highest potential value, 2,808,990 shoots/explant/year, considering the average number of shoots (AVS) obtained in the 4th date of culture after 3 sub-cultures (19.49) and a proliferation rate (PR) of 3.28. In the Ribier cv., considering its 3rd culture date (AVS = 10.44 and PR = 2.19), it could be possible to obtain 26,494 shoots/explant/year. For Black Seedless,

considering its 5th culture date (AVS = 5.05 and PR = 1.73), only 1,213 shoots/explant/year could be obtained. An additional culture carried out with Black Seedless in early February showed a 70 % survival and its PR reached 2.40 shoot/explant after the 1st sub-culture exceeding all 6 previous culture dates. Thus this cultivar could significantly increase its proliferation by being cultured in summer.

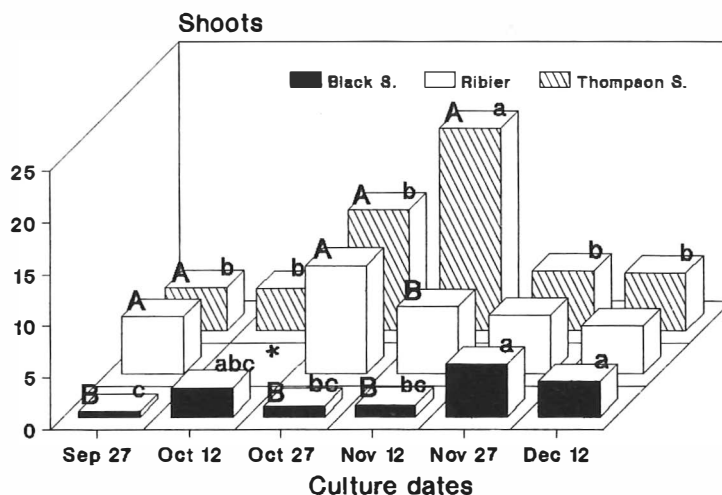


Figure: Mean number of shoots formed by *Vitis vinifera* apexes, cvs Black Seedless, Ribier and Thompson Seedless, after 3 sub-cultures and considering 6 culture dates. \* No results, due to loss of shoots by contamination. Capital letters show significant differences between cultivars for the same date of culture ( $p < 0.05$ ). Small letters show significant differences between culture dates for each cultivar ( $p < 0.05$ ).

- CHEE, R.; POOL, R. M.; 1983: *In vitro* vegetative propagation of *Vitis*. Application of previously defined culture conditions to a selection of genotypes. *Vitis* **22**, 363—374.
- — — —; 1985: *In vitro* propagation of *Vitis*. The effects of organic substances on shoot multiplication. *Vitis* **24**, 106—118.
- FANIZZA, G.; TANZARELLA, O. A.; CARROZZO, G.; GRECO, A.; 1984: Influence of *Vitis* source on *in vitro* shoot apex culture. *Ann. Appl. Biol.* **104**, 577—578.
- HWANG, J. H.; KIM, S. K.; 1990: The effects of plant growth regulators on *in vitro* growth of differentially chilled grape shoots. *J. Korean Soc. Hort. Sci.* **31**, 142—149.
- NOVAK, F. J.; JUVOVA, Z.; 1983: Clonal propagation of grapevine through *in vitro* axillary bud culture. *Sci. Hort.* **18**, 231—240.
- RAJASEKARAN, K.; MULLINS, M. G.; 1981: Organogenesis in internode explants of grapevines. *Vitis* **20**, 218—227.
- SUDARSONO; GOLDY, R. G.; 1991: Growth regulator and axillary bud position effects on *in vitro* establishment of *Vitis rotundifolia*. *HortScience* **26**, 304—307.
- YU, D. H.; MEREDITH, C. P.; 1986: The influence of explant origin on tissue browning and shoot production in shoot tip cultures of grapevine. *J. Amer. Soc. Hort. Sci.* **111**, 972—975.