Vitis **54** (Special Issue), 257–258 (2015)

Research Note

Micropropagation and *in vitro* germplasm conservation of Georgian wild grapevines

D. Maghradze[1)], R. Ocete[2)], J. L. García[3)] and M. Cantos[3)]

[1)] Institute of Horticulture, Viticulture and Oenology. Georgian Agricultural University, Tbilisi, Georgia
[2)] Applied Entomology Laboratory, Faculty of Biology, Sevilla University, Spain
[3)] Institute of Natural Resources and Agrobiology of Sevilla, (IRNAS, CSIC), Sevilla, Spain

Key words: tissue culture; South Caucasus; plant development.

Introduction: Wild grapevine *Vitis vinifera* ssp *sylvestris* Gmel., considered the wild ancestor of the cultivated grapevine *Vitis vinifera* ssp *sativa* D.C., is a typical representative of the Georgian flora (Maghradze *et al*. 2012). Fifty populations of wild grapevine have been described in this zone of the south of Caucasus. Population size varies from 1 to 20 plants, with an average of 3.8 plants per site (Chkhartishvili *et al*. 2005, Maghradze *et al*. 2006). It grows mainly in river gorges, the common habitat of wild grapevine. The lack of human selection has resulted in a highly conserved genetic diversity, so it can play an important role as plant genetic resource for further improvement of grapevine cultivars.

In vitro vegetative propagation of grapevine plants (micropropagation) has been used successfully by different authors (Cantos *et al*. 1993, Nicholson *et al*. 2012). These authors reported the development of a high number of plants starting with a few initial explants, in a relatively small space. *In vitro* growth is often strong due to rejuvenation, disease free, optimal nutrition balance and independence of the seasonal period. This technique is usually used for a suitable material conservation.

The objective of this research was testing the response of five Georgian wild grapevine accessions to micropropagation and comparing the resulting plants with other commercial and wild grapevines.

Material and Methods: Cuttings from five wild grapevine plants from two Georgian populations, Bagichala 10 (G10) and Tedotsminda 01 (G1), 06 (G6), 17 (G17) and 21 (G21) located in Aragvi river basin at Dusheti district and in Liakhvi river basin at Gori district correspondently, Kartli Province, East Georgia were taken, base-dipped in a solution with 2.5 % sucrose and 0.6 % of the fungicide benomyl and placed in a growth chamber with 23 ± 2 °C, 111 µmol·m^{-2}·s^{-1} light intensity and 16 h photoperiod. From these cuttings, sprouting shoots with 3-4 buds, 1.5 cm long were chosen and disinfected by the following steps: (a) immersion in 70 % ethanol for 1 min; (b) immersion in 12 % sodium hypochlorite (10 % active chlorine) with some drops of Tween-20, for 12 min; and (c) rinsing with sterilized water (three times, 5 min. each time).

After disinfection, uninodal explants (0.3-0.5 cm long) with one bud, were placed separately in sterile test tubes (25 x 150 mm) with 8 mL of culture medium (Sarmiento *et al*. 1992), modified with 2.5 % sucrose, 0.072 mg·L^{-1} of BAP, 0.024 mg·L^{-1} of NAA and 0.6 % agar, pH 5.7. Each tube was covered with a plastic cap, sealed with parafilm and placed in a growth chamber at 23 ± 2 °C, 30 µmol·m^{-2} s^{-1} and 16 h photoperiod. Explants from rootstock varieties 'Ramsey', '110-Richter' (110R), '161-49 Couderc' (161-49), '41B' and 'CH' (from a saline semi-arid zone in Arica-Chile); from *Vitis vinifera* varieties 'Superior Seedless' (SS), 'Malvasia' and 'Pedro Ximénez' (PX), and from the Andalusian wild grapevines 'Serag', '14/Córdoba/3' (CO3); '14/Rute/1' (CO9); '14/Montoro/4' (CO8); '14/Montoro/3' (CO7) and '23/Guarromán/2' (J3) (Ocete *et al.* 2007), were obtained from the *in vitro* germplasm bank of IRNAS-CSIC. Similar uninodal explants (Table) from these accessions were cultured in the same micropropagation conditions described above. After 60 d of *in vitro* culture the number of surviving plants, plant size (length, bud number per shoot and axillary shoot number) and root development (percentage of rooting and root number per plant) were determined.

For acclimatization, 10 rooted plants from each Georgian wild grapevine obtained in the micropropagation process were adapted to *ex vitro* conditions according to Cantos *et al.* (1998).

Statistical analysis was carried out using IBM SPSS Statistics v. 22. Data were analysed using ANOVA. Tukey test was applied for identification of important contrasts. Differences in percentages cases were compared using z test.

Results and Discussion: The average survival of all tested accessions was 78.5 % (Table). All the Georgian accessions, except G6 with a survival of 61 %, were above this value particularly G10 (100 %) and G17 (97.4 %). The three Georgian wild grapevines with higher survival ability were statistically higher than J3, 41B, PX and G6. The average stem length was 2.1 cm. This value is smaller ($p \leq 0.05$) than the length recorded for the Georgian accessions G10 (2.9 cm) and G17 (2.8 cm). These values are much lower than for 110R (4.5 cm) and CH (7.1 cm). The average number of buds per shoot for investigated accessions was 3.5 and the shoot number was 0.5, but the maximum values of these traits were detected in the plants belonging to CH (11.4 and 1.9 respectively), much higher than in other accessions ($p \leq 0.05$). The response of Georgian wild grapevines G10 and G17 was rather high, the average number of buds per shoot was 4.5 and the shoot number was 0.5 for the first sample and 5.4 and 1.0 for the second one which was significantly different in comparison with the same characteristics for the plants belonging to CH.

Explants of all accessions developed root system in the same micropropagation medium without any modification of phytoregulators. This behavior of grapevine explants with this medium is well known (Troncoso *et al.*, 1990; Troncoso *et al.*, 1999). The average rooting percentage in our experiment was 70.2 %, less than that reported by Troncoso *et al*. (1999) (89.7 %). Georgian wild grapevines G10, G17 and G21 presented very high percentages of rooted plants, 87.5, 94.7 and 89.7 % respectively. G1 explants re-

Correspondence to: Dr. M. Cantos Barragán, Dept. Biotecnología Vegetal, Spanish National Research Council (IRNAS-CSIC). Seville, Av. Reina Mercedes, 10, 41012 Sevilla, Spain. E-mail: cantos@irnase.csic.es

Table

Comparison among the average values of parameters obtained in the micropropagation of Georgian wild grapevine accessions (in grey colour) and the other considered accessions

Accession	Number of explants	Survival (%)	Stem length (cm)	Buds number per shoots	Shoots number	Rooting (%)	Roots number
G10	8	100 e*	2.9 c	4.5 def	0.5 abcd	87.5 f	1.6 a
G17	29	97.4 e	2.8 c	5.3 ef	1.00 cd	94.7 f	2.6 ab
Ramsey	53	96.3 e	1.3 abc	2.3 abcd	0.1 a	94.3 ef	2.9 ab
G21	57	93.2 de	1.7 abc	3.6 bcdef	0.4 abc	89.7 def	2.3 ab
G1	33	91.4 bcde	1.8 abc	3.0 abcde	0.1 ab	82.9 bcdef	2.2 ab
110R	56	90.3 bcde	4.5 d	5.9 f	1.0 cd	75.8 bcdef	2.7 ab
Serag	77	89.4 bcde	1.9 abc	4.5 def	0.7 bcd	77.4 bcdef	3.5 ab
CH	39	88.4 bcde	7.1 e	11.4 g	1.9 e	74.4 bcdef	2.6 ab
SS	122	83.6 bcde	0.3 a	0.8 a	0.1 a	82.8 cdef	5.7 c
CO3	36	78.6 bcde	1.3 abc	3.1 abcde	0.5 abcd	69.0 abcdef	2.4 ab
CO9	44	77.8 bcde	0.9 ab	1.4 ab	0.2 ab	71.1 bcdef	3.7 abc
161-49	24	77.8 abcde	1.5 abc	2.9 abcde	0.1 ab	59.3 abcdef	2.9 ab
Malvasía	67	74.7 bcde	1.4 abc	3.2 abcde	0.3 ab	51.9 ab	2.5 ab
CO8	42	68.8 abcd	1.3 abc	1.6 abc	0.1 ab	51.6 ab	2.9 ab
CO7	43	64.9 abcd	2.3 bc	3.4 bcdef	0.4 abc	64.9 abcd	3.6 abc
J3	42	61.8 a	1.1 ab	2.2 abcd	0.4 ab	56.4 ab	2.6 ab
G6	28	61 ab	1.4 abc	2.4 abcd	0.2 ab	56.1 abce	2.0 a
PX	30	56.9 ab	2.2 bc	4.2 cdef	1.07 d	56.9 ab	4.4 bc
41B	37	40 a	1.3 abc	1.5 ab	0.2 ab	36.7 a	3.7 abc
Average		78.5	2.1	3.5	0.5	70.2	2.9

* In each column, means followed by the same letter are not statistically different ($p = 0.05$).

duced this percentage to 82.9 and the lowest rooting was observed for G6, only 56.1 % with the trend described for the aerial part. The average number of roots was 2.99 for the plants of all 19 accessions. Plants of the five Georgian wild grapevines presented a lower number of roots, in a range between 2.6 (G17) and 1.6 (G10). These values were particularly low ($p \leq 0.05$) when compared to plants of Superior Seedless, 5.8.

Bud number per shoot was significantly correlated ($p < 0.0001$) with stem length ($r = 0.96$, $p < 0.0001$) for all accessions, including Georgian wild grapes. Other relationships detected were between shoot number and stem length ($r = 0.88$, $p < 0.0001$) and between shoot number and bud number per shoot ($r = 0.92$, $p < 0.0001$). There was also a significant correlation between survival and rooting ($r = 0.91$, $p < 0.0001$). On the other hand root number did not correlate with survival or rooting. These results demonstrated that the rooting was a more important parameter than root number for successful explant culture.

The Georgian plants showed in all cases a 100 % adaptation from *in vitro* to *ex vitro* conditions, indicating the suitable quality of the root system obtained by micropropagation.

Conclusion: In conclusion, we observed a good response of the considered Georgian wild grapevines to micropropagation and adaptation, surpassing other wild grapevines and cultivars with great importance in viticulture. In consequence, *in vitro* culture can be an appropriate conservation system for this Georgian material.

The article is a joint publication of the COST Action FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding".

CANTOS, M.; CUERVAS, J.; ZÁRATE, R.; TRONCOSO, A.; 1998: Embryo rescue and development of *Juniperus oxycedrus* subsp. *oxycedrus* and *macrocarpa*. Seed Sci. Tech. **26**, 193-198.

CHKHARTISHVILI, N.; MAGHRADZE, D.; GOGISHVILI, K.; TCHIPASHVILI, R.; 2005: CWR: Grapevine, small and minor fruits of Georgia (The Caucasus). 1st Int. Conf. Crop Wild Relative Conservation and Use. Agrigento, 14-17 September.

MAGHRADZE, D.; RUSTIONI, L.; TUROK, J.; SCIENZA, A.; FAILLA, O.; 2012: Caucasus and Northern Black Sea Region Ampelography. Vitis (Special issue).

MAGHRADZE, D.; MDINARADZE, I.; CHKHARTISHVILI, N.; GOGISHVILI, K.; CHIPASHVILI, R.; 2006: Inventory of wildly growing grapevines in Eastern Georgia. J. Vinodelie i Vinogradarsvo (Winemaking and Viticulture) **6**, 39 (in Russian).

NICHOLSON, K. L.; TARLYN, N.; ARMOUR, T.; 2012: Effect of phyllotactic position and cultural treatments toward successful direct shoot organogenesis in dwarf 'Pixie' grapevine (*Vitis vinifera* L.). Plant Cell Tiss. Org. Cult. **111**, 123-129.

OCETE, R.; CANTOS, M.; LÓPEZ, M. A.; GALLARDO, A.; PÉREZ, M. A.; TRONCOSO, A.; LARA, M.; FAILLA, O.; FARRAGUT, F. J.; LIÑÁN, J.; 2007: Caracterización y conservación del recurso fitogenético vid silvestre en Andalucía. FALCOR y Consejería de Medio Ambiente. Junta de Andalucía.

SARMIENTO, R.; VILLEGAS, A.; MAZUELOS, C.; GARCÍA, J. L.; TRONCOSO, A.; 1992: Influence of the nitrogen source and concentration on N fractions and free amino acid levels of grape vine explants. Plant Soil **144**, 255-258.

TRONCOSO, A.; VILLEGAS, A.; MAZUELOS, C.; CANTOS, M.; 1990: Growth and mineral composition of grape-vine rootstock cultured *in vitro* with different levels of ammonium nitrate. Plant Nutrit. Physiol. Applic. **41**, 653-654.

TRONCOSO, A.; MATTE, C.; CANTOS, M. LAVEE, S.; 1999: Evaluation of salt tolerance *in vitro*-grown grapevine rootstock varieties. Vitis **38**, 55-60.