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Rootstocks and wild grapevines responses to salinity

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Summary

A study of the effects induced by salt solutions applied on *in vitro* cultures and potted vines comparing five widely used rootstocks in Romania and six wild accessions from five populations of *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi was performed. For *in vitro* test, all genotypes were analyzed for toxicity symptoms appearance in media containing 17, 32 and 49 mM NaCl. The same accessions, as potted plants, were subjected to salt treatments with 51, 68 and 102 mM NaCl, and were evaluated in terms of growth reduction and toxicity symptoms emergence after one month of salt treatment. P, K and Na contents were detected in *in vitro* plantlets and potted plants. Results showed significant differences among genotypes and between the *in vitro* and potted treatments. The wild grapevine individuals, in comparison to the rootstocks, expressed a higher ability to adapt to the salt stress in both type of treatments. In comparison with the rootstocks, the wild grapevine individuals were characterized by a higher content of P and lower contents of K and Na, which could be directly correlated with their ability to uptake and accumulate a higher level of Na and Cl into their tissues.

K e y w o r d s : salt tolerance; *in vitro* culture; mineral nutrition.

Introduction

The osmotic stress induced by salinity is responsible for severe crop losses and affects plant growth. The grapevine, as a glycophyte plant, reacts to a high salinity in the soil by accumulation of NaCl within the vacuole, or by diminishing the NaCl entrance into cells, or by dilution of salt solutions after its entrance into the cells (Harborne 1993). Considered as moderately salt tolerant, grapevine plants performance is the result of the rootstock root system's ability to absorb nutrients from the soil and the scion's ability to translocate and accumulate these nutrients in vegetative structures. This is the reason of numerous studies of salinity tolerance performed with rootstocks (Dardeniz *et al.* 2006, Fort and Walker 2011) and grafted plants (Southey and Jooste 1991, Walker *et al.* 2010) aiming to assure yield grape quality on salt affected lands.

The first attempts to obtain grapevines with enhanced resistance to saline soil were performed under field conditions by using either sodium sulfate (Martynenko *et al.* 1973), or sodium chloride (Hassan and El Azayem 1990, Tesu *et al.* 1976) as selection agent. Different strategies were applied to select resistant or tolerant grapevine genotypes, or to improve this trait, but conventional methods have resulted in limited success. Differently, the biotechnology approaches proved to be more efficient to describe and highlight some physiological mechanisms related to saline stress. The variability of expression in tissue cultures has been effective in obtaining and selecting of salt-resistant somatic embryos (Lebrun *et al.* 1985) or regenerating plants with resistance to saline treatments (Skene and Barlass 1988) for different grapevine varieties, or rootstocks (Troncoso *et al*. 1999).

Beside physiological analyses, molecular methods aiming to understand the responses after progressive salt stress were applied. Tests with grapevine cultivars, rootstocks and wild grapevine (*Vitis vinifera L. ssp. sylvestris*) provided new information about osmotin gene expression levels, the early and late regulation of salt response genes, and revealed the possible use of molecular parameters to screen salt-tolerant genotypes (Agaoglu *et al.* 2004, Askri *et al.* 2011).

This study presents a comparative investigation for salinity tolerance under *in vitro* and pot conditions of five grapevine rootstocks widely used in Romania, and six wild plants collected from five different populations of *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi. The experiment was designed to establish a possible correlation between salt resistance and mineral content of plants after salt treatments applied in two different culture systems, *in vitro* culture of meristematic structures and potted plants, respectively.

Material and Methods

Potted plants belonging to five rootstocks ('SO4', 'Kober 5BB', '140Ru', 'Fercal' and 'Riparia Gloire'), and six different wild plants from five different populations of *Vitis vinifera* L. *ssp. sylvestris* (Gmelin) Hegi from Romania, identified along the Danube River (named Vs1, Vs10, Vs9DM, Vs3EB, Letea 1 and Letea 2), were used for this experiment.

The shoot tips were harvested from potted plants and the apexes were cultured on solidified Murashige and Skoog (1962) medium containing 1 mg·L^{-1} 6-benzyl-aminopurine, 0.5 mg·L^{-1} 3-indolylacetic acid and 20 g·L^{-1} saccharose, supplemented with 17 (V1), 34 (V2) and 43 (V3)

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mM NaCl. The medium without NaCl content was used as a control for the test. The viable and growing explants were selected at 30-d intervals and transferred to media with the same composition. All the cultures were maintained at 24 ± 1 °C under cool-white fluorescent light and 16-h photoperiod. The plant material obtained by multiplication on each medium was used for chemical analysis after 150 d of culture.

Potted plants with normal growth were sorted into four groups and watered every two days with solution of 51, 68, 102 mM NaCl, and also without NaCl (as control) for 30 d (Popescu *et al.* 2010). Phosphorus, potassium and sodium contents were determined on *in vitro* and potted plant material (without roots). For each treatment, in triplicate, samples were dried, ground and mineralized by dry ashing (500 °C); ash was treated with 5 mL of 2M HCl and the amount of elements were measured by flame photometer.

Data were analyzed by using analysis of variance, the mean values among treatments were compared by Duncan's test and the significance of the differences among genotypes were performed with *t* theoretical values (Fisher test) for three levels of significance (5 %, 1 % and 0.1 %).

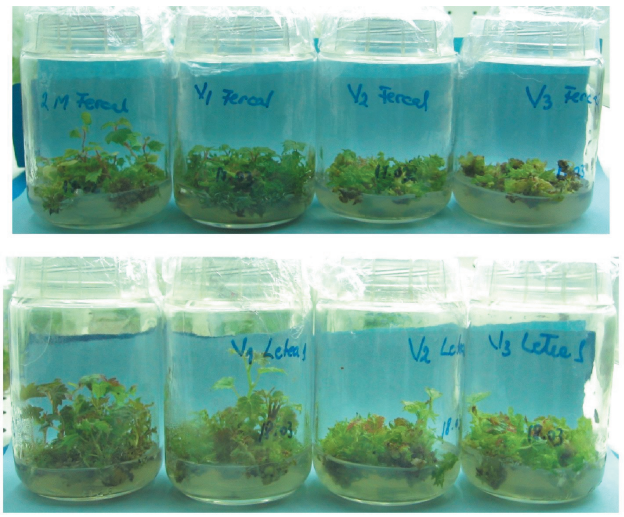

Fig. 1: The influence of NaCl on explants evolution and shoots development, after 5 passages on the same media composition. Example for rootstock 'Fercal 242' and wild plant Letea 1. From the left to the right: control (M), 17 (V1), 34 (V2) and 43 (V3) mM NaCl.

Fig. 2: Reduced rate of multiplication, the necrosis of the shoots and yellowing of the leaves as results of salt presence into the media composition. Example with wild plant *V. vinifera sylvestris* 10. From the left to the right: 43 (V3), 34 (V2) and 17 (V1) mM NaCl.

Results and Discussion

In vitro and potted plant development under saline stress: The first *in vitro* and potted plants response to the increasing salt concentration was the decrease in growth.

Apex explants on the control medium had a normal evolution and most genotypes produced shoots within 3 weeks of culture. The negative effects of salted media on apex differentiation were directly correlated with salt concentration (Fig. 1), and were significantly marked by a reduced rate of multiplication and sometimes by switching the normal development towards callus formation. This was the case of the explants from Kober 5BB, Vs1 and Vs10 genotypes, which were characterized by necrotic areas on the tips of shoots, yellow leaves, and callus formation at the base of shoots (Fig. 2). With these genotypes, the multiplication rate of transferred explants was the lowest, and the number of shoots decreased following the increasing NaCl concentration. In the media supplemented with 17 and 34 mM NaCl normal shoots were obtained, but shorter and with low ability of rooting, which occurred after a seven month-long period of culture. The decline of growth in plant material subjected to higher saline stress is associated with a decrease of photosynthetic capacity, as result of toxic accumulation of Cl ions in tissue structures (Qiu and Lu 2003). A longer period of culture is necessary for plant tissue to overcome this accumulation, to adapt to salty medium and to restore the growth/differentiation process.

With potted plants, the NaCl concentration had an indirect effect on absorption of other nutrients by the roots and their translocation through the plant body (Fozouni *et al*. 2012). Salt stress resulted in reduction of growth ability and on some specific physiological responses. Thus, it was noticed that the shoot elongation was inhibited and the lamina surface became withered, thinner and more affected by necroses with each salted water treatment. Necrotic symptoms of chloride toxicity appeared on the lamina of the lower leaves in all accessions, and became drastic in the end (Fig. 3).

The morphological aspects of *in vitro* cultures after 150 d on media supplemented with NaCl, and the behavior of potted plants watered with saline solutions, seemed to express the same degree of tolerance to salinity for the tested accessions. In our experiments, among rootstocks, 'Fercal' proved to be sensitive, 'SO4' and 'Kober 5BB' moderate tolerant, while '140Ru' and 'Riparia Gloire' were tolerant to saline solutions. Better results were obtained with *in vitro* shoots and potted wild grapevine plants, these being characterized by less affected areas on leaves. However, the tolerance to salt stress of the meristem structures (apexes) and whole plants was expressed at different level. With all genotypes, it was possible to maintain the capacity of multiplication and elongation of new shoots under

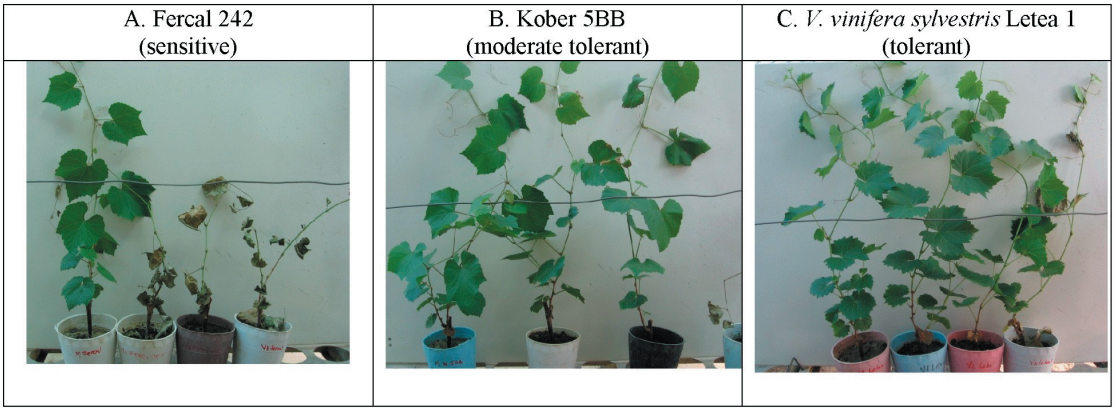

Fig. 3: The effects of different salt concentrations on plant development; potted plants after 15 treatments. From the left to the right: control (M), 51 mM (V1), 68 mM (V2) and 102 mM (V3) NaCl.

in vitro tests with saline solution up to 43 mM NaCl for a long period. With potted plants, the viability of plants was maintained for only 25-30 d after watering with salt solution in concentration up to 102 mM NaCl.

In vitro and pot salt stresses and mineral nutrients accumulation: Under saline conditions, greater amounts of ions are absorbed through the roots and are accumulated in plant tissues. A part of them are involved in physiological processes to maintain the tissue integrity, or to prevent the degenerative processes induced by the saline stress. The tolerance of plants to salty solutions was defined by Teakle and Tyerman (2010) as the ability of plants to restrict uptake of chloride from soil and subsequent transport to the shoot via xylem.

Among the main chemical elements which might be involved in nutritional imbalances and toxic accumulations in plants subjected to NaCl solution treatments in controlled conditions, phosphorus was found in higher quantities in potted plants. Its amount was increased progressively with salt concentrations from watery solutions, and the registered values were double in comparison with the *in vitro* plant material for almost all tested varieties (Table). Very significant higher content of P was found in 'Riparia Gloire' and Vs3EB accessions, and very significant lower content in 'SO4', 'Kober 5BB' and the two wild accessions from Letea forest. Phosphorus content in vegetative structures from *in vitro* cultures was similar for all tested genotypes, with no significant differences. We consider this to be the result of an efficient utilization of this element to maintain the integrity of tissue structures and proper functioning of cell membranes under saline stress.

The mineral content of Na and K in plant material revealed significant differences between rootstocks and the wild *V. vinifera* individuals, and very significant differences between *in vitro* and potted treatments (Table). When the plants are exposed to saline medium, the Na is either accumulated in their roots and excluded from leaves, or is accumulated in the shoots. With potted tests, our data proved a higher level of Na content in all rootstocks varieties, in comparison with wild accessions, ranging between 0.5 % ('Riparia Gloire') and 3.27 % ('140Ru' in V3). In the case of *in vitro* treatments, the level of Na accumulation was higher with *V. vinifera sylvestris* plants, in comparison with rootstocks varieties, ranging between 0.36 % (Vs1) and 2.91 % (Vs3EB). These results proved a different behavior of the two groups of plants: in the potted conditions, the tested rootstocks reacted as "Na includers", while in the *in vitro* tests the plants belonging to wild populations reacted as "Na excluders".

In vitro conditions favored absorption and retention of K and Na elements in vegetative structures, while potted treatments increased the absorption of P. The differences found between *in vitro* plant material and potted plants subjected to salt stress, and especially the reactions of different vegetal structures and cellular characteristics, indicate the involvement of different NaCl resistance mechanisms.

Potassium concentration into plant material during salt solution treatments is recognized to have a beneficial role in osmotic adjustment, and its accumulation in parallel with high Na uptake is considered to be a parameter for plant tolerance to NaCl (Poljakoff-Mayber and Lerner 1999). Our data with accumulation of K and Na in plant tissues under salt stress showed very significant higher contents for '140Ru' rootstock with potted plant tests, and significantly higher contents for 'Kober 5BB', 'Riparia Gloire' and four different accessions of *V. vinifera sylvestris* (Vs3EB, Vs9DM, Letea 1 and Letea 2) with *in vitro* plantlets. The higher capacity of '140Ru' to concentrate mineral elements involved in plant-defense response to saline stress was in agreement with similar experimental works (Walker and Clingeleffer 2009, Walker *et al* 2010).

According to Sathish *et al.* (1997), a high K/Na ratio is correlated with a high tolerance to salinity and was recommended as a selection criterion. With all tested genotypes this ratio decreased significantly under salt stress as compared to control, and the amplitude of reduction was higher for tissue culture tests (data not shown). The highest values of K/Na ratio and the highest decreases of this parameter in relation to the NaCl concentrations were found in 'Riparia Gloire' (from 2.7 to 0.3 with potted plant treatments and from 9.0 to 3.6 with the *in vitro* tests). In both culture systems, similar values were registered with three accessions of *V. vinifera sylvestris* (Vs1, Vs10 and Letea 2). In contrast, in 'SO4' and 'Fercal' rootstocks and two *sylvestris* accessions (Vs3EB and Vs9DM) the lowest level of K/Na ratio was found. Information on accumulation of

Table

Effect of saline treatments on nutrients tissue content

Treatment [mM NaCl]	Riparia Gloire	SO4	Kober 5BB	140Ru	Fercal	Vs1	Vs10	Vs3EB	Vs9DM	Letea1	Letea2
P (% dw) content in potted plants											
Control	0.55c[1]	0.31d	0.31c	0.38c	0.28c	0.45d	0.53a	0.58a	0.44c	0.29c	0.27c
V1[51]	0.55c	0.35c	0.39b	0.44b	0.31c	0.47c	0.48b	0.41c	0.40c	0.38b	0.34c
V2[68]	0.59b	0.39b	0.45a	0.54a	0.35b	0.49b	0.49b	0.49b	0.50b	0.37b	0.40b
V3[102]	0.66a	0.40a	0.46a	0.54a	0.41a	0.53a	0.49b	0.59a	0.53a	0.40a	0.46a
Mean[2] 0.44	0.58^{xxx}	0.36^{ooo}	0.40^{oo}	0.47^{x}	0.34^{ooo}	0.48^{x}	0.49^{x}	0.52^{xxx}	0.47^{ns}	0.36^{ooo}	0.37^{ooo}
P (% dw) content in i*n vitro* plantlets											
Control	0.26a	0.19c	0.22a	0.24a	0.20a	0.24a	0.31a	0.22b	0.22a	0.20b	0.25a
V1[17]	0.19c	0.20c	0.18b	0.22a	0.19b	0.20b	0.29b	0.22b	0.19c	0.25a	0.19b
V2[34]	0.17d	0.22b	0.19a	0.20b	0.20a	0.18c	0.28b	0.21b	0.20b	0.21b	0.18c
V3[43]	0.21b	0.29a	0.21a	0.23a	0.22a	0.16d	0.25c	0.25a	0.22a	0.18c	0.16d
Mean 0.21	0.21^{ns}	0.22^{ns}	0.20^{ns}	0.22^{ns}	0.20^{ns}	0.19^{ns}	0.28^{ns}	0.22^{ns}	0.20^{ns}	0.21^{ns}	0.19^{ns}
Na (% dw) content in potted plants											
Control	0.52d	0.58c	0.75d	0.93d	0.56c	0.30c	0.25d	0.22c	0.23d	0.35d	0.39d
V1[51]	0.92c	1.38b	0.98c	3.02c	1.28b	0.49b	0.44c	0.23c	0.44c	0.62c	0.67c
V2[68]	1.18b	1.71a	1.19b	3.19b	1.28b	0.49b	0.60b	0.31b	0.54b	0.81b	0.70b
V3[102]	1.93a	1.73a	1.42a	3.27a	2.29a	0.63a	0.67a	0.46a	0.81a	1.32a	0.90a
Mean 0.98	1.14^{ns}	1.35^{ns}	1.09^{ns}	2.60^{xxx}	1.35^{ns}	0.48^{ns}	0.49^{ns}	0.31^{ns}	0.51^{ns}	0.77^{ns}	0.67^{ns}
Na (% dw) content in i*n vitro* plantlets											
Control	0.43d	0.87c	0.41d	0.39d	0.49d	0.36d	0.40c	1.05d	0.50d	0.88d	0.42d
V1[17]	0.53c	1.15b	0.55c	0.58c	1.26c	0.82c	0.41c	1.58c	2.00c	1.66c	0.55c
V2[34]	0.75b	1.42a	0.75b	0.65b	1.54b	1.08b	0.48b	2.23b	2.13b	2.41b	0.89b
V3[43]	0.88a	1.43a	0.95a	0.70a	1.84a	1.17a	0.61a	2.91a	2.85a	2.80a	1.09a
Mean 1.11	0.65^{ns}	1.22^{ns}	0.67^{ns}	0.58^{ns}	1.28^{ns}	0.86^{ns}	0.48^{ns}	1.94^{x}	1.87^{x}	1.94^{x}	0.74^{ns}
K (% dw) content in potted plants											
Control	1.43a	1.01c	1.03d	1.14d	0.93c	0.82a	0.49a	0.51a	0.21b	0.83a	0.75a
V1[51]	1.03b	1.35a	1.23c	1.54c	1.47b	0.82a	0.39b	0.49a	0.12c	0.63c	0.65b
V2[68]	0.80c	1.24b	1.43b	1.76b	1.43b	0.76b	0.36b	0.47a	0.22b	0.66b	0.55c
V3[102]	0.60c	1.01c	1.67a	1.86a	1.63a	0.59c	0.28c	0.32b	0.55a	0.82a	0.41d
Mean 0.87	0.97^{ns}	1.15^{ns}	1.34^{xx}	1.58^{xxx}	1.37^{xx}	0.75^{ns}	0.38^{oo}	0.45^{ooo}	0.27^{oo}	0.74^{ns}	0.59^{ns}
K (% dw) content in i*n vitro* plantlets											
Control	3.88a	0.96d	4.17a	4.07a	2.75a	3.34a	3.43a	2.96a	3.32a	2.51b	4.05a
V1[17]	3.82a	1.20c	3.86b	4.18a	2.67b	2.94b	2.95b	2.42c	3.38a	3.10a	3.92b
V2[34]	3.75b	1.70b	3.66b	3.53b	2.62b	2.58c	2.76c	2.71b	2.62b	3.09a	3.87b
V3[43]	3.22c	1.82a	3.53c	2.99c	2.51c	2.27d	2.29d	2.28d	2.27c	2.37c	3.50c
Mean 3.00	3.67^{x}	1.42^{ooo}	3.81^{xx}	3.69^{x}	2.64^{ns}	2.78^{ns}	2.86^{ns}	2.59^{ns}	2.90^{ns}	2.77^{ns}	3.84^{xx}

[1] For each accession, nutrient and experimental conditions means followed by the same letter are not significantly different (Duncan test, P = 1 %). Each value represents the mean of three replicates.

[2] Comparison among genotypes: x, xx, xxx, o, oo, ooo: indicate significantly higher and lower to the mean value respectively (Fisher test at P = 5 %, 1 % and 0.1 %; ns: no significant).

potassium and/or sodium in shoots from potted plants, or *in vitro* propagated tissues, was useful to understand the correlation between osmotic adjustment, for which accumulation of Na and K is essential, and growth of plant under saline stress. This direct relationship was proven by the results of our experiment and suggested a higher salt tolerance expressed by the rootstocks '140Ru', 'Kober 5BB' and 'Riparia Gloire' and for three out of the six wild *V. vinifera sylvestris* accessions (Vs1, Vs10 and Letea 2).

Conclusions

The present study provided the first information on salt tolerance of some wild grapevine accessions collected along the Danube River, and suggests a possible test to select grapevine rootstocks and wild genotypes resistant to saline stress. The mineral content in all studied genotypes revealed that *in vitro* saline stress involved osmotic adjustment characterized by higher levels of K and Na uptake, and a lower accumulation of P, in comparison with potted tests.

The statistical analysis showed significant differences among the studied genotypes and very significant differences between their response to the *in vitro* and potted treatments.

In comparison with the analyzed rootstocks, the studied *Vitis vinifera sylvestris* individuals were generally characterized by lower contents of K and Na under saline stress, which could be directly correlated with their ability

to uptake and accumulate higher amounts of Na and Cl into their vegetative tissues. Among the investigated varieties, the rootstocks 'Riparia Gloire', '140Ru' and three of tested wild plants (Vs1, Vs10 and Letea 2) proved to be more tolerant to salinity. For salt-tolerance indicators were considered: good *in vitro* growth ability on media supplemented with NaCl, lower degree of leaves necrosis in potted plants watered with salty solutions, and a high K/Na ratio in comparison to control. Information on accumulation of potassium and/or sodium in shoots from potted plants, or *in vitro* propagated tissues, were useful to understand the correlation between the osmotic adjustment for which accumulation of Na and K are essential, and the growth of plant under saline stress.

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