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In vitro Propagation of Two Grapevine (*Vitis vinifera* L.*)* Cultivars under Conditions of Salt Stress

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Key words: Canonannon, Chenine Blanc, Hyperhydricity, Salt tolerance

Abstract

This study was aimed to investigate salt tolerance of two grapevine cultivars, 'Chenine Blanc' and 'Canonannon' through *in vitro* propagation on medium containing different concentrations of NaCl. Single-node shoots were cultured on MS with 1.0 mg/l BAP in combination with 0.1 mg/l IBA and containing 0.25, 0.50, 0.75, 1.00 or 1.50% NaCl. NaCl free medium was used as control. Shoots of both cultivars were cultured on the same MS containing 0.25, 0.50, 0.75 or 1.00% $CaCl_2$ to reduce hyperhydricity. The shoots were transferred to rooting medium followed by acclimatization in greenhouse. Number and length of shoots and roots, number of leaves and nodes, length of nodes, fresh and dry weight of roots and shoots decreased significantly in consistent trend as the concentration of NaCl increased. 'Canonannon' cultivar was found to be significantly more tolerant to NaCl than 'Chenine Blanc' in all parameters. The lowest percentage of hyperhydric shoots were obtained on medium containing 0.25% $CaCl_2$. Therefore, 'Canonannon' cultivar can be planted in relatively saline soils as it is more tolerant to salt than 'Chenine Blanc'.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest, extensively cultivated and economically important fruit crops in the world. It is cultivated under varied agro-ecological conditions, right from tropical to temperate and from cool-humid to hot-arid conditions. According to Aazami (2010), higher proportion of grapevine production is recorded in temperate regions of the world. However, some cultivars have cultivation potential under high temperatures of tropical and subtropical conditions. Grapevine is grown worldwide for a variety of purposes including fresh fruit, juice, jams, jellies, wine, raisins and other processed products (Ferreira et al. 2004). It is also a major horticultural crop

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with great applications in food and pharmaceutical industries. It is economically the most important fruit crop in the world. Regardless of its enormous uses, grapevine cultivation is affected by different biotic and abiotic stresses. Salinity is one of the major abiotic stresses affecting grapevine production. Agricultural productivity is severely affected by soil salinity. An excess amount of salt in the soil adversely affects plant growth, development, and crop productivity. It induces a wide range of perturbations at the cell and whole plant levels. The detrimental effects of salts result not only from a water deficit with relatively high solute concentrations in the soil but also from specific NaCl stresses. According to Jaleel et al. (2008), salinity affects plant growth in a variety of ways, reducing water uptake, causing toxic accumulation of sodium and chloride, and reducing nutrient availability. Salinity problem is considered as significant factor affecting grapevine production in many regions of the world including Ethiopia, as it reduces the value and productivity of the affected irrigated land (Gadalla 2009).

Investigating the effect of salt on plant growth on the field is time consuming, labor intensive, needs large space, and expensive. Several laboratory experiments were conducted to test the hypothesis that a vigorous plantlet surviving in a saline medium under *in vitro* condition would be tolerant to salinity in the field owing to a higher competitive ability and lower growth retardation **(**Ekanayake and Dodds 1993). In order to quickly and efficiently evaluate the salt tolerance of grapevine, salinity tests have been conducted on some grapevine varieties under *in vitro* conditions. Plant materials used in the salinity test were propagated under *in vitro* conditions using the axillary buds as explants (Hamrouni et al. 2008). Results showed that those varieties tolerant to salt under *in vitro* conditions were also found to be tolerant under field conditions indicating that *in vitro* testing of salinity can be used to identify salt tolerant varieties. Therefore, the objective of the present study is to investigate the effect of different concentrations of sodium chloride on *in vitro* shoot multiplication and rooting of two grapevine (*Vitis vinifera* L.) cultivars, Chenine Blanc and Canonannon.

Materials and Methods

About 1.0 to 2.0 cm shoots of grapevine varieties (Chenine Blanc and Canonannon) were excised from greenhouse growing plants and washed under running tap water followed by rinsing in 70% ethanol. The shoots were then surface sterilized in 20% sodium hypochlorite for seven minutes. The sterilized shoots were then cultured on MS shoot multiplication medium with 1.0 mg/l BAP in combination with 0.1 mg/l IBA and 30 g/l sucrose. The pH of the medium was adjusted to 5.8 and 7.0 g/l agar was added. The medium was then autoclaved at 121°C for 15 minutes and 50 ml was dispensed into each sterile Magenta GA-7 culture vessels. The cultures were maintained at 27 ± 2°C and light intensity of 40 µmol/m^2/s at 16 hrs photoperiod. Unless and otherwise indicated, all cultures were maintained at these culture conditions. The shoots were subcultured every four weeks.

In vitro multiplied shoots with single node from the third subculture were excised from proliferating shoot cultures and cultured on semi-solid MS with 1.0 mg/l BAP in combination with 0.1 mg/l IBA, 3% sucrose containing different concentrations of NaCl (0. 00, 0.25, 0.50, 0.75, 1.00, and 1.50%). Six shoots were cultured in each Magenta GA-7 culture vessels containing 50 ml medium in five replications. Number of shoots and buds/explant, shoot length, number of leaves/shoot, and fresh and dry weight were recorded after four weeks. Visible symptoms and dead shoots due to effect of salt on shoots were also recorded.

Single-node shoots from the third subculture were excised from proliferating shoot cultures and cultured on MS supplemented with 1.0 mg/l BAP in combination with 0.1 mg/l IBA, 3% sucrose containing different concentrations of calcium chloride (0, 0.25, 0.50, 0.75, and 1.00%). Six shoots were cultured in each Magenta GA-7 culture vessels containing 50 ml medium in five replications.

Semi-solid MS was supplemented with 2.0 mg/l IBA and 3% sucrose containing different conc. of NaCl (0, 0.25, .50, 0.75, 1.00 and 1.50 %). Six shoots were cultured in each culture vessels containing 50 ml medium in five replications. Number and length of roots/explant was recorded after four weeks. Roots of plantlets were washed under running tap water. The plantlets were transferred to glasshouse and planted in 12 cm diameter pot each containing cow-dung, sand and red soil in the ratio of 1 : 2 : 1, respectively. Plantlets were covered with light transparent polyethylene bags and watered daily for the first two weeks. Then, the bags were gradually removed and watered every other day. The number of survived plants was recorded after a month. Thirty plantlets of each cultivar were planted. Experiments were set up using a RBD. There were six shoots in each culture vessels containing 50 ml medium in five replications for each cultivar and the experiments were repeated. ANOVA and LSD were used to compute the percentage and mean number. The data were analyzed using SPSS version 16 statistical software. A difference at probability level of $p \leq 0.05$ was considered significant for all analyses.

Results and Discussion

The results showed that all the growth parameters of both cultivars of grapevine were significantly affected by different concentrations of NaCl in a consistent trend. With increasing the concentration of NaCl from 0.25 to 1.50%, the number and length of shoots in both cultivars decreased significantly. The reduction was more distinct in Chenine Blanc than Canonannon (Table 1 and Figs 1A-F). The mean number of new shoots/explant that was produced by Chenine Blanc and Canonannon was 5.200 ± 0.38 and 5.767 ± 0.38, respectively on NaCl free medium whereas this number decreased to 3.800 ± 0.23 and 4.600 ± 0.39 at 0.25% NaCl in Chenine Blanc and Canonannon, respectively. No shoots of Chenine Blanc survived at 1.0% NaCl whereas a mean number of 0.933 ± 0.18 new shoots were produced by Canonannon. No shoots were survived in

Table 1. Effect of different NaCl concentrations on mean shoot number, shoot length, leaf and node number/explant.

NaCl (%)	Chenine Blanc				Canonannon			
	Number of new shoots/ explant	Length of shoots/explant (cm)	Number of leaves/shoot	Number of nodes/ explant	Number of new shoots/ explant	Length of shoots/explant (cm)	Number of leaves/shoot	Number of nodes/explant
0.00	5.200 ± 0.38^{a}	3.55 ± 0.19^{a}	4.47 ± 0.21^{a}	3.77 ± 0.26^{a}	5.767 ± 0.38^{a}	4.83±0.22^{a}	6.67 ± 0.41^{a}	4.10 ± 0.27^{a}
0.25	3.800 ± 0.23^{b}	2.19 ± 0.12^{b}	3.07 ± 0.20^{b}	2.27 ± 0.23^{b}	4.600 ± 0.39^{b}	3.68±0.20^{b}	4.63 ± 0.38^{b}	3.50 ± 0.22^{b}
0.50	1.733 ± 0.19^{c}	1.44 ± 0.09^{c}	1.73 ± 0.16^{c}	1.57 ± 0.11^{c}	3.333 ± 0.27^{c}	2.99±0.14^{c}	2.93 ± 0.28^{c}	2.93 ± 0.21^{c}
0.75	1.067 ± 0.17^{d}	0.99 ± 0.17^{d}	0.87 ± 0.13^{d}	0.83 ± 0.11^{d}	1.667 ± 0.12^{d}	2.04±0.16^{d}	1.63 ± 0.24b^{c}	2.30 ± 0.15^{d}
1.00	0.000 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.933 ± 0.18^{e}	1.25±0.11^{e}	0.97 ± 0.27b^{c}	1.53 ± 0.17^{e}
1.50	0.000 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.000 ± 0.00^{e}	0.00±0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{f}

The data are given as mean ± SE. Means having the same superscript letter in a column were not significantly different at 5% probability level.

medium containing 1.5% NaCl in both cultivars. Similarly, with increasing the concentration of NaCl, the number of leaves and nodes/explant decreased significantly in both cultivars (Table 1). However, the rate of decrease was lower in Canonnanon than Chenine Blanc. The highest number of leaves/explant was 4.47 ± 0.21 in Chenine Blanc and 6.67 ± 0.41 in Canonnanon on NaCl free media and all the media containing NaCl exhibited lower number of leaves in both cultivars. Chenine Blanc produced 3.77 ± 0.26 nodes/explant whereas Canonnanon produced 4.10 ± 0.27 nodes/explant on NaCl free medium and all NaCl containing media produced lower number of nodes.

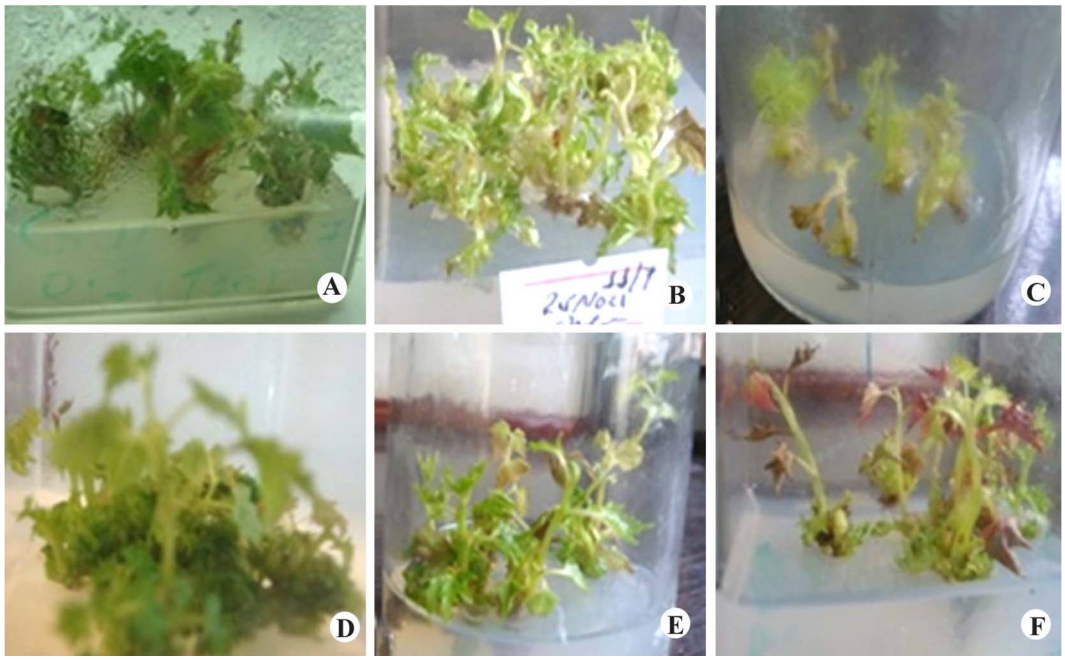

Fig. 1. Effect of NaCl on the development of grapevine shoots after four weeks of culture. A, B and C are the response of Chenine Blanc shoots cultured on medium containing 0.00 (control), 0.25, and 0.5% NaCl, respectively. D, E and F are the response of Canonan on shoots cultured on medium containing 0.00 (control), 0.25, and 0.5% NaCl, respectively.

The results showed that growth in shoot height of the grapevine of both cultivars was strongly affected by presence of NaCl in the medium although Canonannon was found to be more tolerant to NaCl than Chenine Blanc in all parameters. This is in agreement with the results of Berrichi et al. (2010) on jojoba plant and Gandonou et al. (2008) on seedlings of sugarcane. This effect of different concentrations of NaCl on shoot height was also observed in other parameters in similar trend. NaCl significantly decreased the number of leaves/shoot as compared to the control in both cultivars. The effect of different concentrations of NaCl on number of leaves/shoot was observed in this study as indicated by Rasmia and El-Banna (2011) on palm date plantlet, Freipica and

Levinsh (2010) on daggered coastal plant species and Shatnawi et al. (2010) on *Chrysanthemum morifolium*. The number of nodes also decreased as the salt concentration increased.

Different concentrations of NaCl significantly reduced shoot fresh and dry weight of both cultivars. Generally, Canonannon showed higher fresh and dry weight than Chenine Blanc, but on medium containing 0.5% NaCl, Cheine Blanc exhibited slightly higher fresh weight than Canonannon. Therefore, this indicates that Canonannon cultivar is more tolerant to salinity than Chenine Blanc (Table 2). The results of this study are in conformity with Sivritepe and Eris (1999) who studied the effect of NaCl on *in vitro* growing grapevine cultivars Cavus, Muskule and Sultani Cekirdeksiz. They reported that the degree of NaCl tolerance is different among these cultivars.

Table 2. Effect of different NaCl concentrations on mean fresh and dry weight of shoots and roots.

NaCl (%)	Chenine blanc				Canonannon			
	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)
0.00	247 ± 19^{a}	32 ± 2^{a}	242 ± 13^{a}	24 ± 2^{a}	261 ± 16^{a}	43 ± 3^{a}	299 ± 29^{a}	31 ± 3^{a}
0.25	128 ± 7^{b}	23 ± 6^{a}	168 ± 13^{b}	20 ± 2^{a}	129 ± 26^{b}	23 ± 4^{b}	241 ± 36^{a}	27 ± 5^{a}
0.50	82 ± 6^{b}	11 ± 1^{b}	65 ± 2^{c}	8 ± 1^{b}	71 ± 4^{b}	14 ± 1^{b}	57 ± 3^{b}	6 ± 1^{b}
0.75	54 ± 4^{b}	8 ± 1^{b}	-	-	61 ± 5^{b}	13 ± 1^{b}	-	-

The data are given as mean ± SE. Means having the same superscript letter in a column were not significantly different at 5% probability level.

Some shoot cultures of both cultivars of grapevine showed hyperhydricity (vitrification). However, it was more pronounced in Chenine Blanc cultivar than Canonannon. This problem was reduced by adding $CaCl_2$ to the medium. The least number of vitrified shoots of both cultivars were observed at 0.25% $CaCl_2$. Surprisingly, at 0.5% and higher concentrations of $CaCl_2$ (0.75 and 1.00%), the shoots of both cultivars exhibited more hyperhydricity as compared to the control. The control was MS without $CaCl_2$. Sharma and Ramamurthy (2000) reported that $CaCl_2$ reduced hyperhydricity and induced the elongation of shoots in *Eucalyptus tereticornis*. The concentration of agar was also increased from 0.7 to 0.8%. The results showed that percentage of explants forming shoots and the percentage of hyperhydric shoots decreased with increase in concentration of agar. Reduction of hyperhydric shoots in response to increased agar concentration was reported by several authors including Abdoli et al. (2007) who reported the effect of agar concentration on hyperhydricity of sunflower.

Shoots started root production within two weeks of culture on rooting medium. The percentage of shoots that produced roots and the number and length of roots varied

between cultivars and among treatments (Figs 2A-F). When the concentration of NaCl increased, both cultivars exhibited significant decrease in the number and length of roots in consistent trend (Table 3) and this is in agreement with the results of Mohamed et al. (2010) who worked on potato. This decrease in root number and length was more pronounced among treatments than between cultivars. No roots were produced on rooting medium containing 1.0 and 1.5% NaCl in both cultivars. Canonannon exhibited higher tolerance to salinity than Chenine Blanc as it produced more roots with better length. Alizadeh et al. (2010) reported similar results from their work on other cultivars of grapevine. Freipica and Levinsh (2010) also reported similar results on different daggered coastal plants.

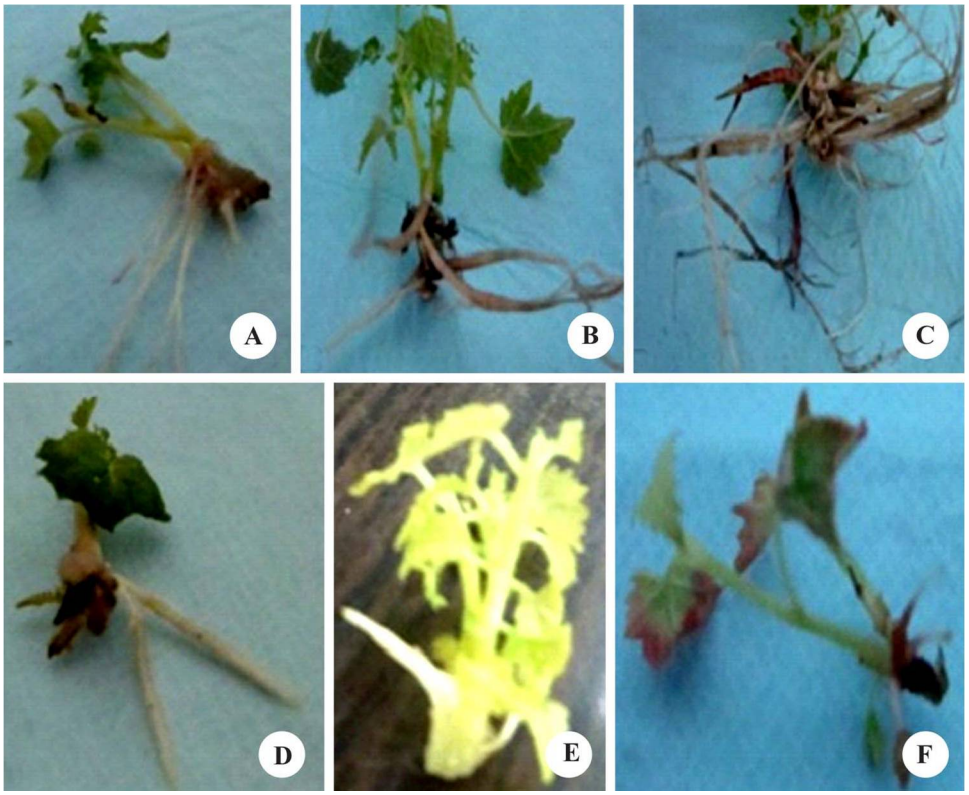

Fig. 2. Effect of different concentrations of NaCl on *in vitro* rooting of grapevine after 30 days of culture. A. Chenine Blanc on NaCl free medium, B. Canonannon on NaCl free medium, C. Chenine Blanc on medium containing 0.25% NaCl, D. Canonannon on medium containing 0.25% NaCl, E. Chenine Blanc on medium containing 0.50% NaCl and F. Canonannon on medium containing 0.50% NaCl.

With increasing concentration of NaCl, there was significant decrease of both the dry and fresh weight of root in both cultivars. The shoot and root fresh and dry weights were significantly different among different concentrations of NaCl and between the two

cultivars. The reduction was more pronounced in Chenine Blanc than Canonannon (Table 2). In the case of Chenine Blanc, the fresh weight was 242 ± 13 mg on NaCl free medium and this weight was reduced dramatically to 168 ± 13 mg on medium containing 0.25% NaCl. Similarly, the fresh weight of Canonannon decreased from 299 ± 29 mg on NaCl free medium to 241 ± 36 mg on medium containing 0.25% NaCl indicating that this cultivar is more tolerant to NaCl at this concentration. However, at 0.5% NaCl, the fresh weight was dramatically reduced to only 57 ± 3 mg. This is consistent with the results obtained from other grapevine cultivars (Alizadeh et al. 2010). The results of Khenifi et al. (2011) also support the results of the present study with regard to the effect of salinity on shoot fresh weight. Similarly, Shatnawi et al. (2010) reported with increasing concentration of NaCl in the medium, root dry weight of *Chrysanthemum morifolium* decreased. Among plantlets that were planted in glasshouse for acclimatization, 72% of Canonannon and 76% of Chenine Blanc survived (Figs. 3A-C). No aberrant plants were observed. Kinfe et al. (2017) reported that 92% of Chenine Blanc and 73.9% of Canonannon survived after acclimatization. This higher survival percentage as compared to the present study could be probably due to the difference in soil mixtures used.

Table 3. Effect of different NaCl concentrations on mean length and number of roots.

NaCl (%)	Chenine Blanc		Canonannon	
	Root length/ plantlet (cm)	Root number/ plantlet	Root length/ plantlet (cm)	Root number/plantlet
0.00	2.90 ± 0.17^{a}	5.30 ± 0.21^{a}	3.14 ± 0.14^{a}	6.57 ± 0.40^{a}
0.25	1.56 ± 0.18^{b}	3.53 ± 0.52^{bc}	1.88 ± 0.21^{b}	4.57 ± 0.62^{b}
0.50	0.86 ± 0.09^{c}	3.00 ± 0.44^{cb}	0.74 ± 0.13^{c}	3.10 ± 0.57^{c}
0.75	0.42 ± 0.11^{d}	1.93 ± 0.35^{d}	0.51 ± 0.06^{c}	1.13 ± 0.21^{d}
1.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
1.50	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}

The data are given as mean ± SE. Means having the same superscript letter in a column were not significantly different at 5% probability level.

Fig. 3. Grapevine plants in glasshouse during acclimatization. (A) Both cultivars after two weeks of acclimatization covered with polyethylene bags; (B) Chenine Blanc and (C) Canonannon after five weeks of acclimatization.

The results of the present study indicated that Canonannon cultivar is more tolerant to NaCl than Chenine Blanc in all parameters. In the areas where salt stress is a problem, Canonannon is preferred to Chenine Blanc. The present study contributes significantly to estimating the level of *in vitro* salt tolerance of these two cultivars.

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