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BRIEF COMMUNICATION

Response of grape rootstocks to salinity: changes in root growth, polyamines and abscisic acid

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Abstract

Effects of salinity (0, 50, 100 and 250 mM NaCl) on growth, root:shoot dry mass ratio, osmotic potential (ψ_π), electrolyte leakage and contents of Na^+ and K^+, polyamines and abscisic acid (ABA) were studied in the grape rootstocks Dogridge, 1613, St. George and Salt Creek. In control rootstocks, the root length was highest in Dogridge and contents of K^+ and ABA in Salt Creek. Salinity treatments increased root Na^+ and decreased K^+ content and St. George exhibited highest Na^+ content and Na^+:K^+ ratio. The root:shoot dry mass ratio in all rootstocks increased upto 100 mM NaCl. With increasing NaCl concentration, putrescine, spermine and spermidine contents showed consistent increase and putrescine increase was highest in St. George and spermidine and spermine in the Dogridge and Salt Creek. Under salinity, the ABA content increased in all the rootstocks but more in Salt Creek and Dogridge than in St. George.

Additional key words: abscisic acid, NaCl-induced stress, putrescine, spermidine, spermine, *Vitis vinifera.*

Salinity is serious concern in grape production, owing to moderate sensitivity to saline environment. Among the strategies adopted for sustaining growth and productivity of vine cultivars under salinity, use of tolerant rootstocks is widely accepted (Singh *et al.* 1993). The salinity tolerance induced by rootstocks is attributed to root system restricting the movement and/or avoiding absorption and accumulation of toxic ions from the saline soils (Hepaksoy *et al.* 2006, Walker *et al.* 2002). The physiological mechanisms by which rootstock impart salinity tolerance are not well understood. Studies conducted earlier have revealed maintenance of better root growth (Neumann 1995) and high K^+:Na^+ ratio (Grattan and Grieve 1999) important attributes of tolerance to salinity. Abscisic acid (ABA) (Zhang *et al.* 2006, Viswanathan *et al.* 2005) and polyamines (Couee *et al.* 2004, Lakra *et al.* 2006) might be also important in stress responses. Thus in the present investigation, we studied the effects of NaCl treatments on ion contents, osmotic potential (ψ_π), electrolyte leakage, root growth, shoot and root biomass, and contents of polyamines and ABA in the grape rootstocks exhibiting differential salinity tolerance.

Stem cuttings of 4 grape (*Vitis vinifera* L.) rootstocks (1613, Dogridge, Salt Creek and St. George) obtained from mother plants maintained at the experimental farm of Indian Institute of Horticultural Research, Hessaraghatta, were kept for rooting in the sand mixed nursery beds. After 4 months, the well-developed healthy plants were transplanted to the pots filled with 20 kg mixture of red sandy loam soil, farmyard manure and sand (2:1:1, m/m). The water retention capacity of soil mixture assessed gravimetrically was 28.8 %. The plants were irrigated daily with uniform volume of water. After 2 months of transplantation, the plants were subjected to salinity treatments by irrigating daily with 1.5 dm^3 of 0, 50, 100 and 250 mM NaCl for 3 weeks. The 1.5 dm^3 of NaCl solution was found optimum for the soil saturation without seepage. During the experiment, the soil samples were periodically analyzed for salinity and the average soil salinities were 2.73, 4.64, 6.18 and 8.29 dS m^{-1} under control, 50, 100 and 250 mM NaCl, respectively. The

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Abbreviation: ABA - abscisic acid.

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experiment was designed under complete random design with 10 replications. During the experiment, the maximum and minimum temperatures ranged between 29.2 - 33.8 °C and 16.7 - 19.8 °C, respectively, and the average relative humidity at 13:00 was 46.2 %.

After 3 weeks of treatments, the plants were uprooted and the roots were gently washed in running tap water and dried by keeping between the pads of filter papers. Measurements were made on root length, and shoot and root dry mass. The sap from roots was extracted and osmotic potenial was measured by *Vapour Pressure Osmometer* (model *5520*, *Wescor*, Logan, UT, USA). Electrolyte leakage in the roots was measured according to the procedure of Campus and Pham-Thi (1997). The root Na^+ and K^+ contents were estimated after digestion with $H_2SO_4 + HNO_3 + HClO_4$ (1:7:20, v/v/v) employing flame photometer (*Photo Electric Instruments*, Jodhpour, India). For ABA analysis, roots were macerated in chilled 80 % methanol and filtered. The filtrate was evaporated in vacuum at 35 °C, the residue dissolved in water and pH was adjusted to 3.0. The aqueous acidic extract was partitioned twice against equal volumes of chilled diethylether. The ether phase was separated and dried over anhydrous sodium sulphate. The extract was kept over night at 4 °C, filtered and after evaporating ether, the residue was dissolved in 20 mM Tris buffer, pH 7.8. The ABA content was determined by ELISA (Weiler 1982) using laboratory raised polyclonal antibodies. Free polyamines content were estimated following the HPLC procedure of Flores and Galston (1982). Root sample (5.0 g) was extracted with 20 cm^3 of chilled 5 % (v/v) perchloric acid and the supernatant was benzoylated by adding 1.0 cm^3 of 2.0 M NaOH and 0.075 cm^3 of benzoyl chloride to 1.0 cm^3 of supernatant. After adding 2.0 cm^3 of saturated NaCl, the benzoylated polyamines were extracted with chilled diethyl ether. The ether phase separated was dried under nitrogen. The residue was suspended in 2.0 cm^3 methanol and passed through 0.45 µm membrane filter paper (*Millipore*, Massachusetts, USA). The methanolic solution after drying over nitrogen at 40 °C was dissolved in 0.1 cm^3 of methanol for HPLC (model *LC-10A*, *Shimadzu*, Kyoto, Japan) employing *µBondapak* C_{18} column and UV/visible detector (model *SPD-10A*, *Shimadzu*) adjusted to 282 nm. An isocratic solvent system of methanol (62 %, v/v) containing 1 % acetic acid at 1.0 cm^3 flow rate was used to resolve

Table 1. Na^+ and K^+ contents, $Na:K^+$ ratio, osmotic potential, electrolyte leakage, root length and root:shoot dry mass ratio in the roots of grape rootstocks under salinity (data represent means ± SE, *n* = 4)

Parameter	Rootstock	Control	50 mM	100 mM	250 mM
Na^+ [nmol g^{-1}(d.m.)]	Dogridge	4.49 ± 0.47	4.62 ± 0.57	4.94 ± 0.35	13.75 ± 1.31
	1613	5.10 ± 0.93	6.64 ± 0.91	9.64 ± 0.75	19.64 ± 2.04
	Salt Creek	4.52 ± 0.65	4.06 ± 0.66	5.21 ± 0.94	9.49 ± 1.36
	St. George	7.35 ± 1.08	11.76 ± 1.27	16.33 ± 1.44	23.45 ± 3.09
K^+ [nmol g^{-1}(d.m.)]	Dogridge	43.75 ± 3.65	32.18 ± 2.85	25.62 ± 3.98	40.38 ± 5.11
	1613	49.23 ± 4.09	33.94 ± 3.12	37.77 ± 2.66	31.73 ± 2.84
	Salt Creek	59.37 ± 3.15	28.79 ± 1.95	25.28 ± 4.09	35.20 ± 3.47
	St. George	39.80 ± 4.95	37.36 ± 3.65	38.61 ± 3.07	29.17 ± 3.27
Na:K ratio	Dogridge	0.10 ± 0.004	0.14 ± 0.016	0.19 ± 0.015	0.34 ± 0.011
	1613	0.10 ± 0.002	0.20 ± 0.015	0.25 ± 0.018	0.62 ± 0.034
	Salt Creek	0.08 ± 0.002	0.14 ± 0.08	0.21 ± 0.024	0.27 ± 0.013
	St. George	0.18 ± 0.010	0.31 ± 0.037	0.42 ± 0.031	0.80 ± 0.071
Osmotic potential [-MPa]	Dogridge	2.71 ± 0.81	2.76 ± 0.92	3.11 ± 1.08	3.56 ± 0.65
	1613	3.38 ± 0.69	3.51 ± 0.23	3.73 ± 0.19	4.58 ± 0.38
	Salt Creek	3.71 ± 0.52	3.20 ± 0.92	3.82 ± 0.75	4.00 ± 0.44
	St. George	3.07 ± 0.59	4.18 ± 0.17	4.60 ± 0.38	5.07 ± 0.31
Electrolyte leakage [µS mg^{-1}(d.m.)]	Dogridge	0.72 ± 0.008	0.65 ± 0.012	0.67 ± 0.009	1.02 ± 0.007
	1613	1.26 ± 0.018	0.98 ± 0.023	1.90 ± 0.085	3.16 ± 0.042
	Salt Creek	0.99 ± 0.015	0.83 ± 0.022	1.04 ± 0.074	2.00 ± 0.055
	St. George	1.58 ± 0.087	1.80 ± 0.108	2.60 ± 0.031	3.79 ± 0.049
Root length [m]	Dogridge	1.31 ± 0.119	1.62 ± 0.121	1.87 ± 0.168	1.10 ± 0.091
	1613	0.92 ± 0.061	1.09 ± 0.097	0.77 ± 0.051	0.65 ± 0.024
	Salt Creek	1.15 ± 0.087	1.44 ± 0.107	1.48 ± 0.119	0.85 ± 0.041
	St. George	0.86 ± 0.044	0.95 ± 0.076	0.69 ± 0.053	0.51 ± 0.034
Root:shoot dry mass ratio	Dogridge	0.59 ± 0.004	1.10 ± 0.023	1.43 ± 0.014	1.29 ± 0.031
	1613	0.58 ± 0.023	0.93 ± 0.009	1.07 ± 0.052	0.93 ± 0.032
	Salt Creek	0.63 ± 0.044	1.00 ± 0.027	1.20 ± 0.017	1.08 ± 0.065
	St. George	0.74 ± 0.037	0.79 ± 0.041	0.83 ± 0.023	0.83 ± 0.017

Table 2. Polyamines and abscisic acid contents in the roots of grape rootstocks under salinity (data represent mean ± SE, *n* = 4).

Parameter	Rootstock	Control	50 mM	100 mM	250 mM
Putrescine [μmol g^{-1}(d.m.)]	Dogridge	1.606 ± 0.051	1.464 ± 0.024	1.647 ± 0.068	2.035 ± 0.037
	1613	1.648 ± 0.062	1.614 ± 0.087	3.118 ± 0.411	5.043 ± 0.071
	Salt Creek	2.067 ± 0.122	1.752 ± 0.077	2.508 ± 0.308	2.905 ± 0.092
	St. George	3.074 ± 0.153	2.575 ± 0.097	6.049 ± 0.854	8.600 ± 0.731
Spermidine [μmol g^{-1}(d.m.)]	Dogridge	1.197 ± 0.201	1.376 ± 0.101	1.850 ± 0.109	3.658 ± 0.231
	1613	1.359 ± 0.074	1.220 ± 0.153	1.862 ± 0.075	2.929 ± 0.098
	Salt Creek	1.404 ± 0.116	1.198 ± 0.066	1.986 ± 0.047	3.240 ± 0.125
	St. George	1.223 ± 0.063	1.576 ± 0.084	2.386 ± 0.101	2.681 ± 0.117
Spermine [μmol g^{-1}(d.m.)]	Dogridge	2.151 ± 0.362	1.938 ± 0.063	2.421 ± 0.087	5.968 ± 0.367
	1613	2.140 ± 0.185	1.831 ± 0.114	2.856 ± 0.311	4.697 ± 0.854
	Salt Creek	1.958 ± 0.163	1.539 ± 0.138	2.587 ± 0.201	5.530 ± 0.338
	St. George	2.065 ± 0.184	2.185 ± 0.231	3.152 ± 0.174	4.819 ± 0.311
Abscisic acid [ng g^{-1}(d.m.)]	Dog ridge	539.7 ± 35.1	592.3 ± 31.0	944.0 ± 52.3	1576.0 ± 46.8
	1613	581.7 ± 24.8	508.0 ± 29.2	706.3 ± 41.8	1348.3 ± 60.3
	Salt Creek	686.5 ± 33.6	695.2 ± 41.7	1038.7 ± 65.2	1845.0 ± 49.4
	St. George	461.2 ± 18.9	498.9 ± 37.2	716.2 ± 22.7	1129.2 ± 58.4

benzoylated polyamines. The quantification of polyamines (putrescine, spermidine and spermine) was performed using these as external standards (*Sigma*, St. Louis, USA).

In the untreated rootstocks, Na^+ content was highest in St. George and K^+ content in the Salt Creek. The Na^+:K^+ ratio ranging between 0.08 - 0.18 in the untreated plants was highest in St. George (Table 1). NaCl treatments increased Na^+ and declined K^+ contents; the response being dependent upon rootstocks and NaCl concentration. High Na^+ and low K^+ contents were recorded in the St. George under different NaCl concentrations. Under all salinity levels, Salt Creek revealed low Na^+ and 1613 and Dogridge high K^+ contents. The Na^+:K^+ ratio consistently increase with increasing concentrations of NaCl, but Salt Creek and Dogridge recorded lower values than other cultivars. The salinity induced changes in root Na^+ and K^+ contents leading to increase in Na^+:K^+ ratio has been documented in the salt-sensitive crops like barley and maize (Flowers and Hajibagheri 2001, Kholova *et al.* 2009). The genotypic variability in root Na^+ and K^+ contents of grape vine under salinity has been reported by Deshmukh (2003) and Storey *et al.* (2003). The electrolyte leakage, an indicator of the damage of membrane integrity, differed significantly in the control and NaCl-treated rootstock plants, and Dogridge and Salt Creek recorded lower values than other cultivars (Table 1). The electrolyte leakage was high in St. George under 50 mM NaCl and in 1613 under 100 and 250 mM NaCl. Distinct variations (-2.71 to -3.71 MPa) were observed in the root ψ_π among the NaCl-untreated rootstocks. Increasing salinity further decreased ψ_π (Table 1). Under salinity, Dogridge and Salt Creek showed less declined ψ_π than St. George. All these results revealed that Dogridge and Salt Creek are relatively tolerant, whereas St George is susceptible.

Root length in the NaCl-untreated rootstocks showed distinct variations, with Dogridge revealing highest and St. George lowest values (Table 1). In the Dogridge and Salt Creek, the root length increased up to 100 mM NaCl (Table 1). In the other two rootstocks, the root length increase was evident only under 50 mM NaCl. The higher NaCl concentrations declined root length. There are reports that salinity stress reduces root extension growth and also influences negatively the root hair length and density in many plant species (*e.g.* Peterson and Farquhar 1996). According to Munns *et al.* (1995), there are two components of salinity that are related to salinity-induced changes in plants; one linked to osmotic effect operating under low salinity, and the other to toxic effects due to excessive salt accumulation or imbalance in nutrient under high salinity. The increase in root length observed under low or moderate salinity levels in the rootstocks could possibly be the outcome of the first component, and may be of adaptive significance through osmotic adjustment. The root:shoot dry mass ratio, showing increasing trends upto 100 mM, declined under 250 mM NaCl (Table 1). This ratio, in general, was higher in the salinity treated rootstocks than in the control ones and Dogridge exhibited relatively high values. The high root:shoot dry mass ratio depicts greater vulnerability of shoots than roots to salinity, and could possibly be the result of reallocation of photosynthates to roots under salinity. Previous studies in cotton (Meloni *et al.* 2001) as well as in soybean (Kant *et al.* 1994) also reported increase in root:shoot ratio by salinity.

Under 50 mM NaCl, the polyamine contents were not much altered but increased under 100 and 250 mM NaCl (Table 2). In the tolerant rootstocks (Dogridge and Salt Creek), spermidine and spermine contents showed greater increase than in other cultivars. In contrast, the susceptible rootstock (St. George) experienced greatest changes in putrescine content under 100 and 250 mM

NaCl. These changes appeared as the outcome of possible sensitization of polyamine biosynthesis pathway by NaCl leading to increased availability of precursors ornithine/arginine and/or induction in the activities of respective enzymes. The direct relationship of spermidine and spermine with root:shoot dry mass ratio and inverse relationship with Na^+:K^+ and electrolyte leakage under high NaCl concentrations may suggest involvement of spermidine and spermine in the salinity tolerance in grape rootstocks. The contribution of spermine and spermidine to salinity tolerance has also been reported earlier in rice (Chattopadhyay *et al.* 2002). Zao *et al.* (2007) stated that polyamines act as protector for plants against salinity by improving K^+/Na^+ homeostasis through restricting Na^+ influx into roots and thus preventing loss K^+ from cell.

In the NaCl-untreated rootstocks, the ABA content ranging between 461.2 - 686.5 ng g^{-1}(d.m.) was highest in Salt Creek (Table 2). Following NaCl stress, the ABA content increased (1.3 - 192.0 %) in all rootstocks; the increase being highest in the tolerant rootstocks (Table 2). Positive correlation between ABA and spermidine and spermine contents, and negative correlation between ABA and Na^+:K^+ ratio was observed under higher NaCl concentrations. The ABA increase under salinity has been reported in many plant species (*e.g.* Cramer and Quarrie 2002), and is stated to play a role in plant adaptation to salinity by influencing stomatal and hydraulic conductance (Freundl *et al.* 2000) and by improving growth through changes in cell wall extension (Cramer *et al.* 1998). Karmoker and Van Steveninck (1979) and Behl and Jeschke (1981) reported inhibition of Na^+ transport by ABA. There are also reports that ABA up-regulates polyamine biosynthesis in the plants subjected to salinity (Liu *et al.* 2005).

In conclusion, grape rootstocks exhibited considerable variations in the salinity tolerance as evident from changes in the Na^+ and K^+ contents and their ratios, root growth, root:shoot dry mass ratio, osmotic potential, electrolyte leakage and polyamine and ABA contents. The tolerant rootstocks accumulating higher ABA and spermine and spermidine contents under salinity, exhibited low Na:K ratio and electrolyte leakage, and high growth and root:shoot dry mass ratio.

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