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BRIEF COMMUNICATION

In vitro growth and leaf composition of grapevine cultivars as affected by sodium chloride

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Abstract

In vitro proliferated shoot culture of six grape genotypes (*Vitis vinifera* L.) were screened for tolerance to NaCl (0 to 200 mM). The cv. Perlette was found to tolerate 175 mM NaCl followed by cvs. Pusa Seedless and Beauty Seedless 150 mM NaCl. Na, K, Cl, Ca and Mg content increased upto 100 mM NaCl in most of the genotypes. Total sugar and proline content of stem tissue gradually increased under NaCl stress while leaf chlorophyll *a+b* content declined. Studies suggest that the *in vitro* screening procedure can be used for ranking the grape genotypes for salinity tolerance.

Additional key words : chlorophyll, minerals, proline, salinity tolerance, sugar content, *Vitis vinifera*.

Soil salinity arising due to irrigation water was the main factor associated with rapid decline in grape production in many traditional grapevine growing areas (Joolka *et al.* 1977, Khanduja *et al.* 1980 and Downton 1985). There exist a wide variability for soil tolerance among *Vitis vinifera* L. cultivars and *Vitis* spp. (Ehlig 1960, Alexander and Woodham 1968, Pandey and Divate 1976, Stevens and Harvey 1995). Zhang and Donnelly (1995) proposed *in vitro* procedure using stem segments as a quicker and reliable method for screening potato cultivars. Barlass and Skeene (1981) reported the effect of NaCl on *in vitro* growth of grape cultivars. Rains *et al.* (1980) proposed *in vitro* methods to select cell clones for salt stress. The present study was undertaken to screen *in vitro* some *V. vinifera* L. genotypes for their tolerance to NaCl, and according to effects of NaCl on morphological characters, mineral and biochemical compositions to select tolerant genotypes for growing in salt affected regions.

Six *Vitis vinifera* L. genotypes Cardinal (Car), Delight (Del), Beauty Seedless (BS), Pusa Seedless (PS), Perlette (Per) and Pearl of Csaba (PoC) were taken for the studies. Nodal segments from forced hardwood cuttings were established on Murashige and Skoog (1962) medium supplemented with sucrose 30 g dm^{-3} and solidified with agar (8.0 g dm^{-3}) and media pH adjusted to 5.8 before autoclaving. The cultivars were established and proliferated on the above medium containing 6-benzyl aminopurine (BAP) which was varied as found optimum for each of the cultivar. The proliferated cultures were subcultured (6-week) to procure sufficient number of explant, *i.e.*, double-node microcuttings, for the experiments. *In vitro* screening for salinity was carried out in 250 cm^3 Erlenmeyer flasks containing 100 cm^3 solidified shoot proliferation medium (MS with 2.0 μM BAP + 0.2 μM NAA) added with Analytical-Reagent (AR) grade NaCl (*Hi-Media*, Mumbai, India) at concentration 0 to 200 mM. The axillary buds sprouting on these microcuttings were taken for calculating the survival frequency. The proliferated cultures were further subcultured on elevated NaCl level upto 6 sub subculture (6-week) and the tissue mineral composition was estimated: Na and K contents were measured by flame

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photometer (*Systronics* 121, Ahmedabad India), Ca and Mg by Versene titration method and chloride by Buchlerchloridometer. Total sugar and protein contents for stem samples were measured according to Fales (1951) and Bates *et al.* (1973), respectively. Total chlorophyll content was estimated according to Lichtenthaler (1985). Dry mass was determined after drying at 65 °C to constant mass. The experiments were repeated thrice and the statistical analysis was done using randomised block design.

The six grape genotypes showed a marked difference in their *in vitro* NaCl tolerance (Table 1). Explant survival data suggested that cv. Perlette could tolerate NaCl upto 175 mM while, Pusa Seedless and Beauty Seedless survived at 150 mM. The cv. Cardinal was found most susceptible to NaCl as about only 14.5 % survival was recorded at 100 mM NaCl. The salinity tolerance of the grape genotypes can be summarized as Perlette > Pusa Seedless > Beauty Seedless > Delight > Pearl of Csaba > Cardinal.

Table 1. *In vitro* screening for survival [%] of grapevine genotypes on shoot proliferation medium supplemented with 0 to 200 mM NaCl. Means ± SE, $n = 45$ (MS medium + 2.0 μM BAP + 0.2 μM NAA; pH 5.8).

Genotype	0	25	50	75	100	125	150	175	200
Car	89.5±9.1	78.2±6.2	50.8±2.5	39.8±1.9	14.5±1.5	-	-	-	-
Del	95.4±8.8	81.8±9.1	42.5±5.1	32.2±2.5	21.9±2.4	16.5±2.4	-	-	-
BS	98.5±7.9	88.8±7.7	86.8±4.9	69.5±5.2	49.2±5.8	18.7±1.9	10.5±1.4	-	-
PS	96.8±5.1	79.7±8.1	68.7±4.4	65.7±3.5	44.7±4.3	25.8±4.3	12.8±2.1	-	-
Per	98.0±7.2	95.4±5.9	90.8±7.8	82.5±9.1	68.3±3.9	40.2±5.7	20.5±1.9	11.8±2.4	-
PoC	92.6±6.3	81.9±7.8	65.8±2.7	50.5±6.7	20.2±2.1	-	-	-	-

Table 2. Chloride, sodium, potassium, calcium and magnesium contents [mg g^{-1}(d.m.)] in petioles of grape genotypes grown *in vitro* on proliferation medium supplemented with NaCl (0 - 150 mM). Mean ± SE, $n = 12$

Ion	Genotype	0	25	50	75	100	125	150
Cl^-	Car	3.5±0.6	7.2±0.3	18.2±2.4	24.5±0.6	35.3±1.9	-	-
	PoC	3.2±0.7	5.6±0.6	7.9±0.3	16.8±0.3	21.9±0.6	-	-
	PS	4.4±0.1	6.3±0.7	9.7±0.3	13.2±0.4	15.5±0.3	16.3±0.9	18.5±1.0
	Per	3.9±0.3	8.2±0.5	10.2±0.7	14.7±0.8	19.2±1.4	21.3±2.1	24.2±2.5
Na^+	Car	6.8±1.0	9.7±1.1	12.4±1.4	11.3±0.7	10.7±0.4	-	-
	PoC	5.6±0.2	7.8±0.3	9.2±0.1	8.3±0.5	8.9±0.3	-	-
	PS	6.3±0.7	8.8±0.3	12.1±0.2	13.5±0.3	13.8±0.5	14.1±0.6	16.6±0.4
	Per	7.8±0.2	8.6±0.1	13.4±0.5	16.7±0.6	17.5±0.3	19.2±0.2	21.2±0.5
K^+	Car	7.9±0.4	9.8±0.3	12.2±0.5	14.5±0.2	15.3±0.1	-	-
	PoC	7.4±0.1	8.2±0.5	10.7±0.2	11.4±0.6	12.5±0.7	-	-
	PS	9.5±0.4	9.7±0.3	12.4±0.9	13.9±0.7	14.2±0.2	19.4±0.4	22.8±1.3
	Per	10.2±0.5	13.5±0.8	14.2±0.3	19.2±0.4	21.1±0.3	22.7±1.5	28.4±1.6
Ca^{2+}	Car	3.1±0.2	3.9±0.4	4.4±0.1	5.3±0.5	7.6±0.2	-	-
	PoC	3.8±0.5	4.4±0.1	4.8±0.6	6.5±0.3	7.9±0.3	-	-
	PS	4.9±0.3	6.3±0.3	7.2±0.7	8.3±0.3	9.5±0.2	12.2±0.7	11.2±0.1
	Per	5.1±0.4	8.9±0.5	12.1±0.2	12.8±0.5	13.2±0.2	14.4±0.8	13.8±0.4
Mg^{2+}	Car	4.7±0.2	5.4±0.3	6.9±0.1	8.7±0.5	9.1±0.3	-	-
	PoC	5.5±0.5	9.4±0.2	11.0±0.6	12.6±0.4	13.4±0.7	-	-
	PS	8.4±0.6	8.8±0.5	9.7±0.2	10.1±0.4	12.3±0.1	11.4±0.5	14.2±0.3
	Per	9.3±0.2	9.9±0.1	10.1±0.5	12.3±0.1	14.1±0.7	15.8±0.2	16.2±0.2

With the gradual increase in salinity there was a linear decline in the number of leaves per shoot and internodal length (data not presented). Fresh and dry mass increased at NaCl upto 50 mM and declined thereafter (data not presented). Symptoms of leaf damage, *i.e.* reduced size, marginal necrosis, was observed at 75 to 100 mM NaCl in

suceptible cultivars, while in tolerant cvs. at 150 to 175 mM NaCl. The results corroborates the findings of Barlass and Skene (1981) and the adverse affect of NaCl toxicity have been attributed to ionic imbalance, altered availability and uptake of other ions, decline in photosynthetic rate due to reduction in leaf area, lower stomatal density, accumulation of ions in leaf cell vacuoles, reduced carbon fixation (Prior *et al.* 1992).

The chloride content in leaf petiole increased with the increase of NaCl concentration (Table 2). In confirmation to the present study, Barlass and Skeene (1981) recorded higher Cl^- uptake in *in vitro* grown shoot apices of several grape genotypes and considered Cl^- toxicity as the major factor causing reduced growth in grapes. The trends noted in the present study corroborates the *in vivo* findings of Downton (1985), Alexander and Woodham (1968), Pandey and Divate (1976), Stevens and Harvey (1985) and Prior *et al.* (1992).

Table 3. Sugar [mg g^{-1}(d.m.)], proline [mg g^{-1}(d.m.)], and chlorophyll [µg g^{-1}(d.m.)] contents in leaf tissue of grapevine genotypes grown *in vitro* on shoot proliferation medium supplemented with 0 - 150 mM NaCl. Mean ± SE, $n = 12$.

	Genotype	0	25	50	75	100	125	150
Sugar	Car	175.0±13.4	179.0±11.2	194.0± 9.6	169.0± 7.2	170.0±13.2	-	-
	PoC	167.0± 6.3	154.0± 7.1	168.0± 3.5	134.0± 3.9	141.0± 4.8	-	-
	PS	215.0±18.1	255.0±16.4	312.0± 9.3	281.0± 7.9	269.0±14.1	265.0± 3.2	252.0± 8.2
	Per	240.0±21.9	248.0±19.6	269.0±31.1	265.0±11.9	268.0± 9.6	270.0±16.8	294.0±15.2
Proline	Car	3.9± 0.8	4.4± 0.3	5.1± 0.7	6.3± 0.8	6.4± 0.5	-	-
	PoC	6.4± 0.2	6.5± 0.3	7.2± 0.6	8.8± 0.5	8.9± 0.1	-	-
	PS	7.6± 0.3	7.5± 0.7	9.2± 0.2	10.2± 0.5	14.4± 0.8	18.9± 0.6	21.3± 1.2
	Per	5.9± 0.4	6.1± 0.2	6.8± 0.7	7.3± 0.1	10.4± 1.3	14.5± 2.1	17.8± 0.3
Chlorophyll	Car	24.5± 1.9	21.5± 1.2	20.8± 0.7	17.3± 0.2	14.9± 0.4	-	-
	PoC	48.9± 2.4	45.7± 1.9	43.6± 1.3	31.9± 1.4	23.7± 0.9	-	-
	PS	51.8± 2.9	48.4± 3.4	44.5± 2.1	42.7± 1.3	39.1± 2.2	33.9± 1.7	28.8± 1.1
	Per	63.4± 3.5	55.7± 2.4	49.6± 1.7	42.7± 2.9	34.3± 3.1	30.5± 1.8	25.3± 0.9

At 150 mM NaCl the sodium content was almost double in the tolerant cultivars, while in the susceptible culivars, Pearl of Csaba and Cardinal it rose upto 50 mM NaCl and declined thereafter. The potassium content in the leaves rose with increasing NaCl concentration and in general its content was higher than that of Na^-. Also Ca^{2+} and Mg^{2+} contents rose with the increase in NaCl concentration (Table 2). The result obtained in the *in vitro* condition agrees with the *in vivo* pot experiments of Joolka *et al.* (1977), Downton (1986) and Prior *et al.* (1992). The genotypic difference in the uptake of N and K exists and higher K^+/Na^+ ratio has been expressed in tolerant cvs. (Gorham *et al.* 1991). Nevertheless, tolerant cell lines have been shown to accumulate Na^+ and Cl^- (Watad *et al.* 1983, Binzel *et al.* 1989). The uptake of Ca^{2-} and Mg^{2+} showed similar pattern to those observed in salinized vines by Prior *et al.* (1992). High salinity due to indirect effect on uptake of other nutrients probably resulted in reduciton in growth and disturbance of several other physiological processes (Prior *et al.* 1992).

Total sugar content in shoot increased linearly under NaCl stress in the tolerant genotypes while in susceptible cvs. there was a decline after 50 mM NaCl. The findings corresponds to those of Binzel *et al.* (1989), who also observed that cells and tissues exposed to NaCl stress accumulate organic solutes thereby lowering the cellular osmotic potential and regaining the pressure potential. Similarly, increase in sugar content only in tolerant cvs. (Downton 1985) help them in osmotic adjustment (Reuveni *et al.* 1990). Proline accumulated with the increase in salinity in most of the grapevine cultivars. Increase in the proline content in the stressed tissue under *in vitro* condition has been observed by Dreier (1983) and Greenway and Munns (1980). Watad *et al.* (1983) supposed that this proline took part in osmotic adjustment. There was a marked reduction in chlorophyll content in leaf tissue with NaCl concentration. Marginal chlorosis was noted on the leaves of micro-cutting growing on high NaCl concentration, which later on resulted in necrosis. (Table 3). Similar findings were observed in chrysanthemum (Dalsou and Short 1987) and grapes (Walker *et al.* 1981) where net photosynthetic rate also declined in such conditions. Production of phenolics have been observed in grapevine cultivars, and also, *e.g.*, in salt-stressed legumes (Misra *et al.* 1996).

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