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BRIEF COMMUNICATION

Responses of two *Prunus* rootstocks to KCl induced salinity *in vitro*

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Abstract

The *in vitro* response of two *Prunus* rootstocks: GF 677 (*Prunus persica* × *Prunus amygdalus*), and Nemared (*Prunus persica*) to increasing concentrations of KCl of the culture medium was studied. Shoots were grown *in vitro* for 8 weeks on an Murashige and Skoog medium supplemented with 0, 5, 10, 15, 20, 40 or 80 mM KCl. By increasing KCl concentration from 0 to 40 mM, the number of shoots per explant was not significantly affected for both rootstocks. However, Nemared rootstock formed more shoots per explant than GF 677 under respective KCl concentrations of the medium. Inclusion of 80 mM KCl in the medium resulted in a reduction of growth of both rootstocks. Sodium, Fe, Mn, and Zn concentration in tissues of Nemared rootstock were significantly higher than the respective values of GF 677.

Additional key words: cell proliferation, ion toxicity, micropropagation.

Salinity is one of the most important environmental factors limiting plant growth. Salt stress can adversely affect plant survival, biomass and plant height. Upon exposure to salt stress, plants exhibit a wide range of responses at the molecular, cellular and whole plant level (Hasegawa *et al*. 2000). These include morphological and developmental changes (*e.g*. life cycle, inhibition of shoot growth and enhancement of root growth), adjustment in ion transport (uptake, extrusion and sequestration of ions, decrease in uptake of K^+, Ca^{2+} and Mg^{2+}) and metabolic changes (osmoregulation) (Xiong and Zhu 2002). Nutrient imbalances were observed in salt-stressed plants (Grattan and Grieve 1999). Rootstocks affect the nutritional status of the scion *in vitro* (Sotiropoulos *et al*. 2005) and proper choice of rootstocks can ameliorate the detrimental effects of salinity (Layne 1987). The objective of the present research was to study the effect of KCl induced salinity on growth and nutritional status of two *Prunus* rootstocks cultured *in vitro*.

The explants employed were shoots of two peach rootstocks: GF 677 (*Prunus persica* × *Prunus amygdalus*), and Nemared (*Prunus persica*). The shoots were about 25 mm in length preserved from previous *in vitro* cultures and maintained in the growth room. Each shoot was transferred and grown in a 15 × 100 mm glass test tube containing 5 cm^3 of the Murashige and Skoog (1962; MS) culture medium supplemented with 1 g m^{-3} benzyladenine (BA). Six KCl concentrations were used: 0, 5, 10, 15, 20, 40 and 80 mM. These KCl concentrations were added to the standard MS medium containing already 20.04 mM K^+ and 5.98 mM Cl^-. Therefore K^+ concentration (mM) of the treatments were as follows: 20.04, 25.04, 30.04, 35.04, 40.04, 60.04, and 100.04 whereas Cl^- concentrations (mM) were 5.98, 10.98, 15.98, 20.98, 25.98, 45.98, and 85.98. The pH of the media was adjusted to 5.8 before autoclaving at 121 °C for 15 min. The tubes were maintained in the growth room at 22 ± 1 °C and 16-h photoperiod per day (cool white fluorescent tubes, irradiance of 45 μmol m^{-2} s^{-1}, 400 - 700 nm). After 8 weeks in culture, the number of shoots, length of shoots, and fresh and dry mass of explants were measured. For determination of the mineral composition, leaves and stems from each plantlet were harvested and rinsed twice with distilled water. These organs were then dried at 68 °C for 48 h, ground to pass a 30-mesh screen, and dry ashed at 530 °C for 16 h. The

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Abbreviations: BA - benzyladenine; MS medium - Murashige and Skoog medium.
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residue was dissolved in 10 cm^3 of 6 M HCl and brought to a volume of 50 cm^3. These extracts were measured for K, Ca, Mg, Na, Mn, Fe, and Zn by atomic absorption spectrometry (*Perkin-Elmer* model *2380,* Wellesley, USA). Each treatment included fifteen replicates (tubes). The experiment was conducted and repeated twice, and the reported data are the means of the two experiments. The statistical design adopted was the randomized complete block. Differences between means were evaluated by using LSD test at $P \leq 0.05$.

By increasing KCl concentration of the culture medium from 0 to 40 mM, the number of shoots per explant was not significantly affected for both rootstocks (Table 1). However, the Nemared rootstock formed more shoots per explant than GF 677 under the same KCl concentrations, indicating a genotypic effect. Inclusion of 80 mM KCl in the medium resulted in the lowest number of shoots per explant of both rootstocks. Salinity can affect organogenesis and growth in *in vitro* cultures (Dimassi-Theriou 1998) and its effect is a function of concentration and type of salt added: NaCl at low concentrations exerts a significant effect on shoot proliferation of *Prunus cerasifera in vitro*. Furthermore, low concentrations of NaCl (10, 20 mM) stimulate shoot proliferation of kiwifruit (Sotiropoulos and Dimassi 2004).

Shoot length of GF 677 rootstock was not significantly affected when KCl concentration of the culture medium increased from 0 to 40 mM (Table 1). However, Nemared shoots grown on medium containing 15, 20 and 40 mM KCl were significantly lower in comparison to 5 and 10 mM. Fresh mass of GF 677 shoots grown on media containing 20 and 40 mM KCl were significantly higher in comparison to the rest treatments (Table 1). However, fresh mass of Nemared shoots was not significantly affected by KCl concentration of the medium. Nemared cultures had higher fresh mass than GF 677 when KCl concentration of the medium was 0, 5, 10, and 15 mM. Growth enhancement due to the presence of NaCl at certain concentrations has been reported for various *in vitro* cultures such as *Suaeda aegyptiaca* (Eshel 1985), and rice (*Oryza sativa* L.) callus (Lutts *et al*. 1999). The positive effect of NaCl at low concentrations on plant growth may be due to the increased osmolarity (Flowers and Läuchli 1983). Singh *et al*. (2000) reported that fresh and dry mass of *in vitro* cultures of various grapevine cultivars increased at NaCl concentration of the medium up to 50 mM. By increasing KCl concentration of the culture medium from 0 to 40 mM, dry mass of GF 677 cultures increased too (Table 1). On the contrary, dry mass of Nemared was not significantly affected by KCl concentration of the medium. Inclusion of 80 mM KCl in the medium resulted in the lowest length of shoots, lowest fresh and dry mass of both rootstocks. Plants are stressed in two ways in high salt environment: by the increase in osmotic potential of the medium as a result of high solute content, and by the toxic effects of high concentration of ions (Liu and Van Staden 2001).

Phosphorus concentration of shoots grown on media containing 40 and 80 mM KCl was significantly lower than the control for both rootstocks (Table 2). As KCl concentration in the culture medium increased, K concentration in tissues increased too. Potassium concentration of tissues of Nemared tended to be higher than those of GF 677 under the same KCl concentrations

Table 1. Effect of K^+ and Cl^- concentrations of the culture medium on the number of shoots per explant, shoot length, fresh mass, and dry mass of the of the *Prunus* rootstocks GF 677 and Nemared *in vitro*.

Rootstock	K^+ [mM]	Cl^- [mM]	Number of shoots [explant^{-1}]	Shoot length [mm]	Fresh mass [g]	Dry mass [g]
GF 677	20.04	5.98	1.93	10.55	2.16	0.19
	25.04	10.98	2.07	10.62	2.58	0.26
	30.04	15.98	2.07	10.94	2.69	0.29
	35.04	20.98	1.87	11.43	2.56	0.36
	40.04	25.98	2.47	11.02	3.60	0.39
	60.04	45.98	2.27	11.65	3.50	0.41
	100.04	85.98	0.86	7.21	1.62	0.11
Nemared	20.04	5.98	3.47	10.11	3.75	0.43
	25.04	10.98	3.00	10.67	3.46	0.38
	30.04	15.98	3.27	10.43	3.94	0.43
	35.04	20.98	3.67	9.06	4.21	0.43
	40.04	25.98	3.07	9.14	3.49	0.38
	60.04	45.98	3.00	9.00	3.99	0.42
	100.04	85.98	1.24	7.03	2.09	0.22
$LSD_{(0.05)}$			0.73	1.26	0.76	0.05

Table 2. Effect of K^+ and Cl^- concentrations of the culture medium on P, K, Ca, Mg, Na, Mn, Zn, and Fe concentrations of the *Prunus* rootstocks GF 677 and Nemared *in vitro*.

Rootstock	K^+ [mM]	Cl^- [mM]	P	K	Ca	Mg	Na	Fe	Mn	Zn
			[g kg^{-1}(d.m.)]					[mg kg^{-1}(d.m.)]		
GF 677	20.04	5.98	0.35	0.46	0.22	0.04	0.23	169	82	50
	25.04	10.98	0.33	0.72	0.19	0.04	0.20	194	109	70
	30.04	15.98	0.28	0.92	0.20	0.04	0.20	197	106	79
	35.04	20.98	0.23	1.11	0.21	0.04	0.20	203	112	81
	40.04	25.98	0.23	1.33	0.26	0.04	0.20	245	125	93
	60.04	45.98	0.19	1.77	0.24	0.06	0.18	260	112	94
	100.04	85.98	0.15	1.91	0.16	0.02	0.14	121	59	37
Nemared	20.04	5.98	0.27	0.72	0.25	0.07	0.36	279	177	127
	25.04	10.98	0.31	0.99	0.26	0.06	0.32	233	166	103
	30.04	15.98	0.25	1.66	0.31	0.07	0.34	272	171	116
	35.04	20.98	0.23	1.86	0.26	0.06	0.30	270	170	118
	40.04	25.98	0.24	1.67	0.25	0.05	0.28	224	139	96
	60.04	45.98	0.16	2.26	0.26	0.05	0.25	281	142	109
	100.04	85.98	0.13	2.41	0.18	0.04	0.19	132	109	77
$LSD_{(0.05)}$			0.05	0.22	0.06	0.02	0.04	19.1	12.9	11.7

of the media. Calcium and Mg concentrations of tissues of both rootstocks decreased at the highest KCl concentration compared to the control. Salinity may increase energy consumption, required for osmotic regulation and competition of transported ions. This may subsequently lead to a reduction of metabolically important ions such as K^+ and Ca^{2+} (Kwon *et al*. 1995). Sodium concentration of tissues of both rootstocks decreased as KCl concentration of the media increased. Sodium, Fe, Mn, and Zn concentration in tissues of Nemared rootstock were significantly higher than the respective values of GF 677. Iron and Zn concentrations of tissues of GF 677 increased as KCl concentration of the media increased from 0 to 40 mM, whereas it decreased at 80 mM. Sotiropoulos and Dimassi (2004) reported that Fe, Mn and Zn concentrations of kiwifruit shoots *in vitro* were not significantly affected in the presence of 0 - 80 mM NaCl. The same researchers also reported that by raising NaCl concentration from 10 to 80 mM, Na and Cl concentrations of kiwifruit cultures increased whereas K and Ca decreased. Nutrient imbalances may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant's internal requirement for that essential element (Grattan and Grieve 1999).

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