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Full Length Research Paper

Response of some tomato cultivars to sodium chloride stress under *in vitro* culture condition

M. A. Aazami[1*], M. Torabi[2] and F. Shekari[3]

[1]Department of Horticulture, Faculty of Agriculture, University of Maragheh, Maragheh 55181-83111, Iran.
[2]Faculty of Agriculture Moghan, University of Mohaghegh Ardabili, Ardabil, Iran.
[3]Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh, Maragheh 55181-83111, Iran.

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The response of calli of six tomato cultivars (*Lycopersicon esculentum* Mill.) to salt stress was investigated under *in vitro* conditions. Callus relative growth rate (RGR), dry matter percentage (DM), osmotic potential and proline content were evaluated. Significant differences were found among cultivars regarding above traits. 'PS-10' had the highest RGR, while 'Roma' had the lowest amount of this trait under salt levels. Any increase in salinity levels in the media led to decrease of RGR and in contrast increased DM and osmotic potential in all treatments compared to control. In all cultivars, proline levels increased in response to salinity stress. High callus formation was correlated with low proline content. 'PS-10' and 'Imperial' had the highest callus formation and the lowest proline content. Significant differences were recorded in regeneration potential of cultivars under salt treatments. 'PS-10' possessed the highest and 'Roma' had the lowest regeneration rate. It is concluded that the more the salt tolerant genotype the more is the reduction in osmotic potential and proline content.

Key words: Tomato, callus, *in vitro*, sodium chloride stress.

INTRODUCTION

Plants respond to salinity stress through morphological, physiological and metabolic variations in their organs (Zhang et al., 2004; Amini and Ehsanpour, 2005). Salt stress is caused by various ions, mainly Na^+ and Cl^- which can be transported into and out of cells. Thus, salt stress has, in addition, the specific effect of ions present in the environment (Ben-Hayyim, 1987; Tewary et al., 2000; Zhao et al., 2009).

Tomato (*Lycopersicon esculentum* Mill.), one of the important and widespread vegetable crops in the world, is sensitive to moderate levels of salt in the soil. Tomato genotypes' response to salinity is genetic and species dependant and there is too much interest in screening and breeding for higher salt tolerance (Amini and Ehsanpour, 2005; Mohamed et al., 2007).

In vitro techniques make it possible to screen the required number of genotypes rapidly since *in vitro* plant cultures, even at different stages of development, may exhibit their capacity to withstand the stress (Tewary et al., 2000). In many species like tobacco, grape, rice, citrus and carrot salt tolerant lines have been isolated using *in vitro* techniques (Ben-Hayyim, 1987; Tewary et al., 2000; Vijayan et al., 2003).

The present report describes *in vitro* studies as an efficient method to study the effects of NaCl stress on callus tissue of six tomato genotypes.

MATERIALS AND METHODS

Seeds of six tomato cultivars ('Nora', 'PS-10', 'Peto', 'Roma', 'Imperial' and 'Pascal') were used in this study. The seeds were surface sterilized with 70% ethanol for 1 min followed by sodium hypochlorite (2%) for 10 min and thoroughly washed with sterile distilled water for three times. Then, seeds were incubated for germination in a ½ MS (Murashige and Skoog, 1962) medium under 16 h illuminations (70µ mol m^{-2} s^{-1}) and 28˚C. Thereafter, 10-14-days-old seedlings were used as explants. For callus induction, hypocotyl explants were placed in MS medium supplemented with 1 mgL^{-1} 2,4-D and 1 mgL^{-1} BA. For organogenesis, cotyledonary nodes were placed on shoot induction media (MS supplemented with 2 mgL^{-1}BA and 0.5 mgL^{-1} IAA). All the cultures were maintained at 25±1˚C under 16 h illuminations (70 µ mol m^{-2} s^{-1}). Salinity was simulated by the addition of NaCl at five concentrations

*Corresponding author. E-mail: Aazami58@gmail.com.

Table 1. Relative growth rate, dry matter percentage, osmotic potential and proline content in six tomato cultivars under NaCl stress.

	RGRgday^{-1}					DM%					O.P. MPa					Proline µmol/gFw				
NaCl (mM) / Cultivars	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100
Nora	0.050^{b}	0.045^{ab}	0.038^{bc}	0.029^{b}	0.023^{b}	5^{b}	6^{b}	7^{b}	8^{c}	9^{c}	-0.28^{b}	-0.51^{b}	-0.85^{b}	-1.0^{b}	-1.36^{b}	31^{b}	45^{ab}	106^{b}	273^{b}	377^{bc}
PS-10	0.059^{a}	0.048^{a}	0.042^{a}	0.036^{a}	0.030^{a}	8^{a}	9^{a}	10^{a}	13^{a}	15^{a}	-0.2^{d}	-0.38^{d}	-0.62^{d}	-0.91^{c}	-1.0^{d}	10^{d}	20^{d}	56^{d}	139^{cd}	241^{d}
Peto	0.054^{ab}	0.047^{a}	0.040^{ab}	0.030^{b}	0.022^{b}	6^{ab}	7^{ab}	8^{ab}	10^{b}	13^{ab}	-0.23^{c}	-0.48^{c}	-0.79^{bc}	-1.0^{b}	-1.16^{cd}	19^{cd}	22^{cd}	58^{cd}	196^{c}	291^{cd}
Roma	0.050^{b}	0.042^{b}	0.035^{c}	0.023^{c}	0.017^{c}	5^{b}	6^{b}	7^{b}	8^{c}	9^{c}	-0.33^{a}	-0.78^{a}	-1.21^{a}	-1.69^{a}	-1.83^{a}	40^{a}	48^{a}	174^{a}	310^{a}	466^{a}
Imperial	0.059^{a}	0.047^{a}	0.040^{ab}	0.035^{a}	0.024^{b}	8^{a}	9^{a}	10^{a}	11^{ab}	12^{b}	-0.21^{cd}	-0.41^{cd}	-0.73^{c}	-1.0^{b}	-1.31^{bc}	10^{d}	25^{c}	56^{d}	105^{d}	401^{b}
Pascal	0.051^{ab}	0.045^{ab}	0.039^{b}	0.030^{b}	0.022^{b}	5^{b}	6^{b}	7^{b}	8^{c}	10^{bc}	-0.26^{b}	-0.47^{c}	-0.72^{c}	-0.98^{bc}	-1.26^{c}	22^{c}	40^{d}	66^{c}	140^{cd}	333^{c}

RGR, relative growth rate (gday^{-1}); DM, dry matter percentage (%); O.P., osmotic potential (MPa) and proline content (µmol/gFw). In the columns, values followed by same letters are not significantly different according to LSD at p≥0.05.

(0, 25, 50, 75 and 100 mM) to the media. The cultures were monitored for six weeks to study their growth potential and regeneration capacity. After six weeks the samples were evaluated for their relative growth rate (RGR), dry matter percentage (DM) and osmotic potential (ψ_s).

Callus RGR= (FW_2-FW_1)/ number of days

Where FW_1: The primary fresh weight, FW_2: The fresh weight at the end of test period, Callus DM= (DW_2/FW_2) ×100, DW_2: The dry weight at the end of test period

Osmotic potential was determined with an osmometer (model: 030), using sap extracts from fresh calli tissues. Osmolarity was expressed as MPa using the formula

ψ_s= 0.00227k,

where k= osmolarity in mosmol kg^{-1} (Mohamed and Tawfik, 2006).

For proline determination, 10 ml of 3% (w/v) aqueous sulfosalicylic acid solution was added to 0.4 g of fresh weight of callus samples, homogenized and filtered through layers of filter paper (Whatman No.1), and then proline assay was conducted according to the method described by Bates et al. (1973).

The experiment was carried out as completely randomized design with four replications. Data were subjected to analysis of variance using MSTATC software and the means were separated using LSD at 5%. To confirm result the experiment was repeated twice.

RESULTS

The relative growth rate, dry matter percentage, osmotic potential and proline content of stressed and non-stressed callus cultures of six tomato cultivars were summarized in Table 1. The relative growth rate (RGR) decreased significantly cv. Roma obtained the lowest RGR. Meanwhile, cultivars 'Imperial', 'Nora', 'Peto' and 'Pascal' had the average amounts with the high salinity stress treatments. All the cultivars showed increased DM percentage. Among the cultivars 'PS-10', 'Peto' and 'Imperial' showed higher DM content compared to other cultivars. The sap extract of non-stressed calli showed the lowest osmotic potential values in all cultivars compared to the stressed ones. In (p≥0.01) with increasing NaCl concentration so that, cv. PS-10 showed the highest and all treatments the osmotic potential was lower in the case of cv. PS-10 than that of others. The NaCl treated calli showed higher levels of proline compared to control. The highest amount of proline was accumulated in calli of cultivars 'Roma' and 'Imperial' under 100 mM NaCl treatments. The proline accumulation was significantly different in control medium compared to medium supplemented with NaCl. The cultivar 'Imperial' showed the lowest proline content under 0-75 mM NaCl treatments. But in 100 mM NaCl treatment 'Imperial' positively responded to proline accumulation.

Result showed that shoot formation from cotyledonary nodal explants of tomato cultivars decreased with increasing of NaCl concentrations (Table 2). At all NaCl treatments, the number of shoots in cultivars 'PS-10', 'Peto' and 'Pascal' were more than that of other cultivars. In all the cultivars, the number of shoots in each explant was significantly decreased with NaCl level. However, in 100 mM NaCl treatment this parameter was more decreased in each explant.

DISCUSSION

The addition of NaCl to the culture media decreased the osmotic potential of the media inducing salinity stress that adversely affected the

Table 2. The effects of salinity stress on mean percentages of callus and shoot formation in six tomato cultivars.

	Callus formation (%)					Shoot formation (Shoot/explant)				
NaCl (mM) / Cultivars	0	25	50	75	100	0	25	50	75	100
Nora	95.34^{b}	63.76^{c}	51.93^{c}	33.19^{cd}	14.61^{c}	8.7^{c}	7.1^{c}	5.3^{bc}	2.8^{cd}	0.9^{cd}
PS-10	99.21^{a}	81.33^{a}	69.96^{a}	50.26^{a}	25.32^{a}	11.9^{a}	9.9^{a}	8.3^{a}	4.7^{a}	2.4^{a}
Peto	97.25^{ab}	72.53^{b}	64.79^{ab}	39.23^{bc}	19.08^{b}	10.1^{b}	8.7^{b}	6.8^{b}	3.4^{bc}	1.5^{b}
Roma	94.68^{b}	55.81^{d}	42.09^{d}	25.90^{d}	7.88^{d}	7.3^{d}	5.6^{d}	3.7^{d}	1.4^{d}	0.3^{d}
Imperial	99.32^{a}	83.12^{a}	64.18^{b}	41.57^{b}	15.63^{bc}	12.1^{a}	9.8^{a}	7.9^{ab}	3.8^{b}	1.0^{c}
Pascal	96.54^{ab}	63.11^{c}	52.20^{c}	37.25^{c}	16.41^{bc}	9.1b^{c}	7.3^{bc}	5.1^{c}	3.1^{c}	1.3^{bc}

In the columns, values followed by the same letters are not significantly different according to LSD at p≥0.05.

callus growth and *in vitro* regeneration capacity of tomato cultivars. Several authors reported the use of NaCl for *in vitro* salinity screening in different plants (Vijayan et al., 2003; Zhao et al., 2009). Callus growing in the presence of increasing NaCl concentrations increased their dry matter percentage and reduced RGR in all tomato cultivars (Ben-Hayyim, 1987). Heyser and Nabors (1979) and Binzel et al. (1985) reported that tobacco callus growing in the presence of NaCl had the higher dry weight percent compared to control (Zahang et al., 2004; Amini and Ehsanpour, 2006). In this study, 'PS-10' and 'Peto' cultivars showed low osmotic potential in all NaCl treatments and it seems that these cultivars are more salt tolerant than other cultivars studied. Yang et al. (1990) reported that osmotic adjustment in callus results from both Na^+ and Cl^- accumulation. Similar results have been reported for calli of wheat genotypes (Farrukh, 2002).

With increasing of NaCl, the proline content of all cultivars significantly increased. The rates of accumulation were different depending on cultivars and NaCl levels. The results are in agreement with Emilio et al. (1998) for *L. esculentum* and *Lecopersicon pennellii.* Marthinez et al. (1996) reported a positive relationship between proline accumulation and NaCl tolerance in potato (Mohamed et al., 2007; Aghaleh and Niknam, 2009).

The regeneration potential was decreased with increasing NaCl levels. A similar observation was found by Yusef et al. (1994), Cano et al. (1998) and Mercado et al. (2000) in tomato using tissue culture techniques for *in vitro* selection for salinity tolerance (Hamdy, 2002). Liu and Li (1991) found that callus was formed on 5% NaCl, but was less vigorous and the shoot-formation rate was decreased as compared with the control treatment.

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