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Full Length Research Paper

In vitro response of promising tomato genotypes for tolerance to osmotic stress

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Drought is a major abiotic factor that limits plant growth and productivity. Tomato is an important vegetable crop and area under production is limited by irrigation water scarcity. Four cultivars of tomato were grown as callus cultures under conditions of water stress, which was induced by addition of polyethylene glycol (6000) in the medium. The presence of PEG in the medium decreased relative growth rate and increased dry matter content in all treatments compared with the control. In all cultivars, proline levels increased in response to water stress. Also, there was decreased shoot induction in all cultivars with increase PEG treatments. The number of shoot forming in PS-10 and Peto was higher then Roma and Nora cultivars. This result can be used for *in vitro* screening and manipulations of tomato cultivars for improvement of drought tolerance.

Key words: Callus, drought, polyethylene glycol, tomato.

INTRODUCTION

Tomato (*Solanum lycopersicon*) is one of the most important vegetable crops grown in word (Kulkarni and Deshpande, 2007). Most crop plants, including the cultivated tomato, are sensitive to abiotic stress, although differences between tomato cultivars have been reported (Rus-Alvarez and Guerrier, 1994; Cano et al., 1996). Drought is an environmental stress which is a major barrier to productivity of agricultural crops throughout the world (Noaman et al., 2004). Plants exposed to drought stress respond by various resistance mechanisms. These mechanisms range from whole-plant characteristics such as life-cycle timing (maturity), deep root systems, to cellular-level functions (osmoregulation) (Mohamed et al., 2000). A single trait alone may not ensure successful survival; however, it may enhance overall water stress resistance. It seems likely that the cellular-level compounds of drought-tolerance mechanisms are important and improvement at this level could have a positive impact on whole plant tolerance (Mohamed et al., 2000; Errabii et al., 2006). *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 (Gosal and Bajaj, 1984; Tewary et al., 2000). Polyethylene glycol (PEG) of high molecular weights have long been used to simulate drought stress in plants as non-penetrating osmotic agents lowering the water potential in a way similar to soil drying (Larher et al., 1993). Simulation of drought stress under *in vitro* conditions during the regeneration process constitutes a convenient way to study the effects of drought on the morphogenic responses (Sakthivelu et al., 2008). This study was aimed to investigate the changes in growth and osmotic potential of calli and regeneration of tomato cultivars as affected by PEG - induced water stress.

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Abbreviations: PEG, Polyethylene glycol; **2, 4 –D,** 2, 4 - dichlorophenoxyacetic acid; **MS**, Murashig and Skoog media; **RGR,** relative growth rate; **DM,** dry matter percentage; **ψ_s,** osmotic potential; **LSD,** least square difference.

MATERIALS AND METHODS

Tomato seeds of four cultivars (Nora, PS-10, Peto and Roma) were used in this study. The seeds were surface sterilized with 70% ethanol for 1 min and then with sodium hypochlorite (2%) for 10 min

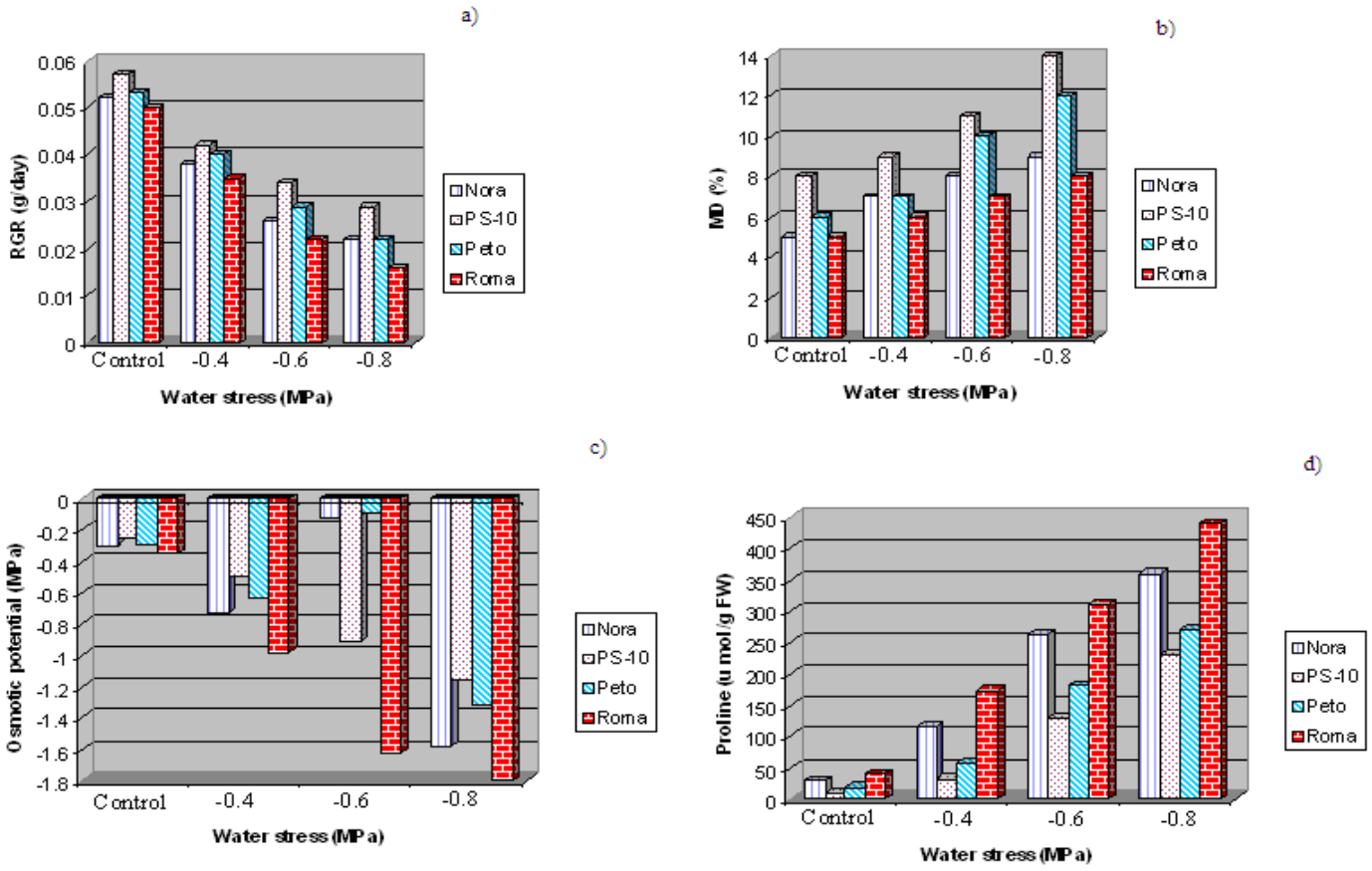

Figure 1. a) Relative growth rate; b) Dry matter percentage; c) Osmotic potential and d) proline content of four tomato cultivars callus cultures on PEG media.

and thoroughly washed with sterile distilled water for three times. Then, the seeds were kept for germination in a ½ MS (Murashig and Skoog, 1962) media and incubated under 16 h illuminations (70 µ mol m^{-2} s^{-1}) at 28°C. Then seedlings from 10 - 14-day-old were used as explants. For callus induction, hypocotyle explants were placed in MS medium supplemented with 1 mgl^{-1} 2, 4 -dichloro-phenoxyacetic acid (2, 4 -D) and 1 mgl^{-1} BA and for organogenesis cotyledonary nodes were placed on shoot induction media (MS media supplemented with 2 mgl^{-1} BA and 0.5 mgl^{-1} IIA). All the cultures were maintained at 25 ± 1°C under 16 h illumination (70 µ mol m^{-2} s^{-1}). Drought was simulated by the addition of polyethylene glycol (6000) at four concentrations (0, 200, 270 and 295 gL^{-1}) to the media. The cultures were kept for six weeks to study their growth potential and regeneration capacity. After six week the samples were analyzed for their relative growth rate (RGR), dry matter percentage (DM) nad osmotic potential (ψ_s).

Callus RGR = (FW_2 - FW_1)/ Number of days
FW_1: The fresh weight primary
FW_2: The fresh weight end of test period

Callus DM = (DW_2/FW_2) × 100
DW_2: The dry weight at end of test period.

Osmotic potential was determined with an osmometer (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 sulfo-salicylic acid solution was added to 0.4 g of fresh weight of callus samples and homogenized and filtered through layers of filter paper (Whatman No.1), then proline assay conducted according method of Bates et al. (1973).

The experiment was carried out in four replicates. Data were subjected to analysis of variance and the means were separated using LSD at 5%. To confirm result the experiment was repeated twice.

RESULTS

The relative growth, dry matter percentage, osmotic potential and proline content of stressed and non-stressed callus cultures of four tomato cultivars were summarized (Figure 1).

The relative growth rate (RGR) decreased significantly ($P \geq 0.01$) with increasing PEG concentrations (Figure 1a). In PEG treatments, cv. PS-10 showed the highest and cv. Roma had the lowest RGR; however, the cultivars Nora and Peto obtained amounts in-between. With high drought stress treatments, all the cultivars showed increased DM percentage. Among the cultivars, PS10 and Peto showed better DM content than two other

Table 1. The effects of water stress on mean percentages of callus formation and shoot formation in four tomato cultivar.

Cultivar	Water stress (MPa)							
	Callus formation (%)				Shoot formation (Shoot/explant)			
	0	- 0.4	- 0.6	- 0.8	0	- 0.4	- 0.6	- 0.8
Nora	95.67^{ab}	51.76^{c}	26.33b^{c}	7.93^{c}	8.5^{bc}	5^{bc}	2.5^{bc}	0.7^{c}
PS-10	98.33^{a}	71.43^{a}	41.07^{a}	17.89^{a}	11.6^{a}	8.3^{a}	4.3^{a}	1.9^{a}
Peto	96.78^{ab}	65.33^{b}	33.02^{b}	11.65^{b}	9.7^{b}	6.7^{b}	3.1^{b}	1.1^{b}
Roma	93.58^{b}	43.88^{d}	19.67^{c}	4.08^{d}	7.3^{c}	3.7^{c}	1.2^{c}	0^{d}

In the column, values followed by same letters are not significant different according to the LSD at $p \geq 0.05$.

cultivars (Figure 1b). The osmotic potential (ψ_s) was - 0.4, - 0.6 and - 0.8 MPa in the presence of PEG at 200,270 and 295 gl^{-1}, respectively. The sap extract of non-stressed calli showed the lowest osmotic potential values in all the cultivars compared to the stressed ones (Figure 1c). In all treatments, the osmotic potential was lower in the case of cv. PS-10 than that of others. The PEG treated calli showed higher levels of proline compared to control. Highest amount of proline accumulated in callus of cv. Roma than that of cv. PS-10 and cv. Peto (Figure 1d). The proline accumulation was significantly different in control medium compared to medium supplemented with PEG. Among the calli obtained from the cultivars, there were no significant difference in -0.4 MPa of osmotic potential.

Result showed that the shoot form number from cotyledonary nodal explants in these tomato cultivars was decreased with increasing PEG concentrations (Table 1).

At all PEG treatments, the number of shoots in cv. PS-10 and Peto was more than cv. Roma and cv. Nora. In all the cultivars, the number of shoots in each explant was significantly decreased as PEG level increa-sed; however, in -0.8 MPa treatments, this parameter was more decreased in each explants.

DISCUSSION

The addition of PEG to the culture media decreased the water potential of the media, thereby inducing water stress that adversely affected the callus growth and *in vitro* regeneration capacity of the tomato cultivars. Several authors reported the use of PEG for *in vitro* drought screening in crop plants (Gopal and Iwama, 2007; Danson et al., 2006). Callus growing in the presence of increasing PEG concentrations increased their percent dry matter content and reduced RGR in all tomato cultivars. The decrease in osmotic potential is considered a potential cellular mechanism of drought resistance as it enables turgor maintenance and growth continuation (Bajji et al., 2000; Mohamed et al., 2000). In this study, cv. PS-10 showed low osmotic potential at all PEG treatments and thus it turned to be a better drought tolerant cultivar than Roma while cv. Peto and Nora showed average drought tolerance. Turgor has been found not to be a restrictive factor to growth during stress in the intact plant (Chandler and Thorpe, 1989), tomato cells adapted to PEG (Kulkarni and Deshpande, 2007; Singh and Sharma, 2008). Highest amount of proline accumulated in cv. Roma, the culture media that was supplement with PEG. Proline has been shown to be accumulated in a number of plant tissues in response to different types of osmotic stresses (Barakt and Abdel-Latif, 1995; Purushotham et al., 1998). In some cases, drought sensitive cultivars accumulated more proline than tolerant ones (Mansour, 2000; Crusciol et al., 2009). The presence of PEG in the regeneration medium had a detrimental effect upon most parameters associated with plantlet regeneration. Thus decrease in the total number of viable plantlets regenerated from tomato explants. A similar decrease in plants led to regeneration under *in vitro* stress conditions as reported in potato (Gopal and Iuama, 2007), rice (Binh et al., 1992) and tomato (Kulkarni and Deshpand, 2007).

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