International Journal of Multidisciplinary and Current Research ISSN: 2321-3124
Research Article Available at: http://ijmcr.com

Accumulation of secondary products in tomato callus *(Lycopersicon esculentum* Mill) under drought stress conditions

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Accepted 25 Nov 2016, Available online 27 Nov 2016, **Vol.4 (Nov/Dec 2016 issue)**

Abstract

Callus which initiated from shoot tips of tomato local variety (Lycopersicon esculentum. Mill) were exposed to Polyethylene glycol at levels of (-0.15, -0.29, -0.44, -0.59 MPa) to induce drought stress. Callus fresh and dry weight, amino acid proline, carotene and lycopene pigments were evaluated under drought stress condition In Vitro. Results revealed different response toward these indicators. Callus fresh weight recorded gradually increase started from levels of (-0.15, -0.29 up to -0.44 MPa) and remarkable reduction at level of (-0.59 MPa), unlike callus dry weight which gave positive response at (-0.44 and -0.59 MPa) levels. Proline and photosynthetic pigment (carotene, lycopene) also influence significantly by the drought. However, all physiological parameters recorded significantly higher accumulation at high level of drought stress.

Keywords: *Tomato, In Vitro, PEG, carotenoids, lycopene, proline*

Introduction

Although, drought stress have adversely effects on some vital biochemical markers like proteins and enzymes or physiological phenomena such as plant growth, yield and productivity, yet accumulation of useful compound with medical value and other variable uses viz, Glycosides, alkaloids and phenols were found to be active at the extremely conditions. Ramakrishna and Ravishankar (2011) reported that, secondary metabolites play a major role in the adaptation of plants to the environment and in overcoming stress conditions. Similarly, Ahmadizadeh (2013) cited the physiological and biochemical change in plant feature when exposed to drought stress.

Carotenoids are naturally occurring photosynthetic pigments with several biological activities, such as the protection from cancer or cardiovascular disease (Rao and Rao,2007) according to (Jaleel et al 2009) carotenes form a key part of the plant antioxidant defense system beside its provide additional value due to its activity as a pro-vitamin (Benítez-García et al .2014).On the other hand, plant generates several defenses enzymatic or non-enzymatic system to avoid oxidative damages (Al-Ghamdi, 2009) which caused by drought stress such as catalase, peroxidase and ascorbic acid (Prochazkova *et al.*, 2001). Hence, the research aim to evaluate some growth parameters and estimating the accumulation of carotene, lycopene and proline in stressed callus of local tomato cultivar invite.

Materials and Methods

The present study was conducted at Genetic Engineering laboratories/Ministry of Science and Technology/Directorate of Agricultural Research.

Seeds of local tomato cultivar were sterilized by soaking in Ethanol at concentration of (0.0, 70.0 and 90.0 %) for a period of 2 min followed by several concentrations (0.0, 1.0, 2.0, 4.0 and 6.0 %) of Sodium hypochlorite (NaOCl) for 15 min, washed three times with distilled deionizer (D.D) water. Seeds were cultured on MS (Murashige and Skoog, 1962) salts medium with ten replicates for each treatment .Seeds contamination percentage was recorded in this stage after 7 days.

To study the effect of drought stress on several parameters, shoot tips of tomato explants were cultured on callus induction media which described by(El Kaaby el al 2012). Callus with constant (100 mg) weight were cultured on MS salts supplemented Glycine, Nicotinic acid, Pyridoxine, Thiamine and Inositol at concentration of (2.0, 0.5, 0.5, 0.1 and 100 $mg.l^{-1}$), Sucrose and Agar (30, 6.0 $g.l^{-1}$) respectively.

Also, Polyethylene Glycol (PEG **6000**) were added to the media at the levels of (-0.15, -0.29, -0.44, -0.59) MPa.Each treatment was replicated 5 times. 8 weeks later, callus fresh and dry weights were recorded. Proline estimated according to (Jones and Gilli .1983), Lycopene and carotene were determination according to (Alminger et al 2012). The experiments were conducted in C.R.D (Completely Randomized Design) and data analysis

using GenStat program and means compared Least Significant Differences (L.S.D.). at 0.05% of probability.

Results and Discussions

Surface Sterilization

Data in (table 1) revealed a positive response in limiting seed contamination and no contamination percentage were recorded with high concentration of ethanol (70.0%, 90.0%) and (4.0%, 6.0 %) of NaOCl concentrations as compare with 100% in control treatment. surface sterilization is a critical stage which represent challenging step to get an aseptic explants used for *in vitro* experiments in next progressive experiments (Al-Mohammed et al 2014).Various protocols are used to eliminate invading pathogens such as sodium hypochlorite, mercuric chloride and ethanol or combination of each, sometimes additional antibiotics as well as fungicides. Many sterilants at specific concentration become toxic for the plant tissues, which in turn led to failure in related to seeds germinations. In our study, despite the last two treatments provide no contamination percentage, yet no seed germination was observed in (6.0% of NaOCl and 70.0% or 90% of Ethanol) concentration respectively. Our results are agree with (Sen et al 2013) in related to the negative effects of sterilizing substances at high levels on seed germination of Achyanthes aspera plants and disagree with (Al-Mohammed et al 2014) in the positive correlation of high concentrations with seeds viability of Borage plant.

Table (1): Effect of NaOCl and Ethanol concentration on seeds contamination percentage of Tomato local cultivar

% NaOCl	% Ethanol concentration		
	0.0	70	90
0.0	100	30.11	75.04
1.0	82.11	23.0	18.64
2.0	60.20	10.8	6.55
4.0	25.0	0.0	0.0
6.0	11.0	0.0	0.0
L.S .D $_{(0.05)}$	10.30		

Effect of drought stress on callus fresh and dry weight

Data in (table 2) showed adversely response for callus fresh weight at the high level of PEG (-0.59 MPa) which reached 277.18 mg as compare with 315.70 mg at the level (-0.15 MPa).Conversely, high level of PEG (-0.44 and-0.59 MPa) had positive effect on callus dry weight and at both levels no significant differences were observed. Our results are agree with (Khashan, 2016) that found significant decrease in callus fresh weight at high level of PEG, on the other hand disagree with the same researcher in case of callus dry weight. Moreover, (Jaleel *et al*. 2009) reported that a greater plant fresh and dry weight under limited condition considered as a desirable characters which in turn reflected positively on plant production while (Farooq et al, 2009) stated the adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production. Also agree with (Elkaaby 2016) who notice a remarkable increase in shoots dry weight and decrease in fresh weight of two tomato cultivars at level 70 gm.l^{-1} of PEG in stress media.

Table (2): Effect of PEG levels (MPa) on callus fresh and dry weight (mg) after 8 weeks of culture

PEG levels MPa	FW (mg)	DW(mg)
-0.15	315.70	41.09
-0.29	380.77	52.75
-0.44	423.05	61.11
-0.59	277.18	66.51
L.S.D $_{(0.05)}$	35.17	6.91

Data in table (3) indicate that, significant increase were found in accumulation of proline, Lycopene and Carotene with the increase of PEG levels started from (-0.29 MPa) up to (-0.59 MPa) despite non significant differences were found in Lycopene accumulation at level (-0.44, -0.59) MPa.

Under drought stress plants adapt various physiological and biochemical strategies, for example (Hussein and Aqlan 2011) improved production of total phenolics, tannins and flavonoids in fenugreek plants when subjecting the cultured plant cells to stress factors in vitro.(Kusvuran ,2012) remarked growth inhibition in shoot fresh , dry weight, plant height, leaf area and number of leaf in okra genotypes. Similar results were obtained by (Mansouri and Radhouane 2015) in Tunisian Barly genotypes.

Table (3): Effect of PEG levels (MPa) on accumulation of some secondary products

PEG levels MPa	Proline µg.g^{-1}	Lycopene mg.100g^{-1} f.w.	Carotene mg.100g^{-1} f.w.
-0.15	9.03	18.16	16.34
-0.29	14.5	32.17	28.14
-0.44	17.11	41.07	33.09
-0.59	22.03	44.51	52.15
L.S.D $_{(0.05)}$	3.21	5.12	2.78

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