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In Vitro effect of exogenous NaCl on enzyme activity, chlorophyll and carotene accumulation in callus of chilli pepper (*Capsicum annuum*.L.) explants

El -Kaaby E A.J, Al-Anny J. A and Almaliky.E.S.

Department of Genetic Engineering, Biotechnology Center, Ministry of science and Technology, Baghdad 10001, Iraq

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Abstract

The present research was conducted in tissue culture laboratory at Genetic Engineering Department, during the years 2015-2016, to study the effect of salinity stress at level of (6, 8 and 10 dSm^{-1}) on enzymes activity of phenylalanine amonyalyse (PAL) peroxidase (POX), photosynthetic pigments chlorophyll and carotene in callus derived from shoots, roots, red and green fruits explants of chilli pepper Ampala hybrid. The results showed that, salinity stress induced changes in callus fresh and dry weight, activity of antioxidant enzymes, PAL and POX, total chlorophyll and carotene content.Mild and high salt level (8 and 10 dSm^{-1}) reduced callus growth with concomitant increase in PAL activity and carotene as well. Moreover, a positive response for carotene accumulation in callus initiated from red fruit explants reached 55.29 $\mu g.g^{-1}$ F.W at the level 10 dSm^{-1} as compared with chlorophyll pigment which record negative response at the same level for all explants.

Keywords: *Salt stress, callus, Pepper ,POX, PAL, Chlorophyll, carotene.*

1. Introduction

Under biotic or abiotic stresses, plants tend to accumulate large number of molecular compounds most of them have low molecular weight with nutritive and medicinal properties. Accumulation of such products often occurs when plants exposed to various stresses including elicitors or signal molecules [Ramakrishna and Ravishankar,2011]. Previous studies focused on the positive effect of salinity or drought stress in related to the increasing of anti oxidant enzyme activity [Sevengor et al,2011**;** Yasar, 2007; Gao et al, 2008**;** Chookhampaeng, 2011; Mani, 2015]or compatible solute like proline,amino acids [ALHattab et al ,2015]. Hussien and Aqlan[2011] remarked a reduction in growth under mild salt stress with concomitant increase in flavonoids, tannins and phenolic compounds in callus derived from hypocotyl explants of Fenugreek (*Trigonella foenum-graecum* L. Other study published by [El Kaaby,2016 a] found higher accumulation in proline,Capsaicin and ascorbic acid when shoots of chilli pepper cultured on MS media stressed by high concentration of polyethylene glycol (PEG). Also Navarro et al [2006] noticed an accumulation of total phenolic compound when red pepper exposed to a moderately saline levels. While Šutković et al [2011] found Positive correlations between the NaCl levels(150 mM or 200 mM) and accumulation of solasodine in calli produced from apical buds of *Solanum nigrum* plant. Taha [2014] found increase in oil content of Jojoba callus culture when expose to 1500 ppm of seawater.

Over the last few years plant cell tissue culture technique have been used widely to produce natural products like pigments, useful compound for their multi purposes such as antioxidants which reducing the risk of oxidation-related diseases [Islamian and Mehrali,2015] and pro-vitamin activities among others**.** According to Perez-Clementeand Gomez-Cadenas [2012], using in vitro technique offers a remarkable tool for studying physiological, biochemical and molecular regulation of plant development and stress response without interfering of other biotic or abiotic stress factors. The current study has focused on the enzyme activity and production of some pigments under salt stress conditions.

Materials and Methods

Mature seeds, green and red fruits of Ampala chilli pepper hybrid were surface sterilized according to [ElKaaby, 2016 b]. Seeds were germinated on MS [Murashige and Skoog,1962] medium supplemented with 2 $mg.L^{-1}$ GA3[El Kaaby et al, 2015].Three weeks later, shoot tips, roots were separated from chilli seedlings whereas green and red fruits were separated into small segments. For callus induction media all explants were cultured on MS medium supplemented with 2 $mg.L^{-1}$ 2, 4-D. Four weeks later, a constant weight (100) mg of callus was transferred to stress MS media at the levels of (6, 8

and 10 dSm^{-1}) of NaCl. Six weeks later,callus fresh and dry weight were determined, Phenylalanine ammonia-lyase (PAL) and peroxidase (POX) enzyme activity was assayed according to [Gao et al, 2008], chlorophyll and carotene were determined according to [Celik and Atak,2012].The experiments were design in completely randomized (C.R.D.) with 3 replicates of each parameter, means of each were compare at a level of 0.05 of probability and data analysis were performed according to GenStat software program.

Results and discussion

Effect of salt stress on callus biomass

Based on data analysis, the results in table (1) revealed significant reduction in fresh and dry callus biomass of all explants. However callus derived from Green fruits explants revealed maximum potential for salt tolerance in term of having a higher callus fresh and dry weight at the level of (8) dSm^{-1}which reached 507.30 mg and 58.12 mg respectively. In contrast to callus produced from root explants which proved to be sensitive due to the highly decrease at the same level reached 201.04 mg and 22.17mg for callus fresh and dry weight respectively.

Table 1: Callus fresh and dry weight (mg) exposed to different levels of NaCl

Explants	Callus fresh weight Salt levels dSm^{-1}			Callus dry weight Salt levels dSm^{-1}		
	6	8	10	6	8	10
Shoot tips	496.3	325.7	210.5	32.14	39.14	22.09
Roots	358.1	201.04	289.11	30.76	22.17	18.71
Green fruits	478.5	507.30	356.51	41.56	58.12	32.14
Red fruits	377.4	369.67	278.43	43.11	31.50	28.18
$L.S.D_{0.05}$	46.04			5.53		

*each number represent mean of 3 replicates

Peroxidase and phenylalanine amonyalyse activity

Results in table (2 and 3) revealed a significant increase in PAL and POX enzyme activity in callus subjected to NaCl stress. Maximum stimulation of PAL and POX enzymes activity were observed in callus of red fruits pepper explants under 10 dSm^{-1} level of salinity stress reached (42.15, 27.32 U/g F.W) as compare to minimum activity (14.17, 10.23 U/g F.W) respectively when callus of shoot tips were exposed to same level of NaCl in culture medium .

Table 2: Effect of salt stress on PAL enzyme activity (U/g F.W) in callus derived from different explants of chilli pepper hybrid

Explants	Salt levels dSm^{-1}		
	6	8	10
Shoot tips	12.14	12.2	14.17
Roots	13.43	15.3	21.09
Green fruits	18.09	25.81	31.09
Red fruits	28.7	34.14	42.15
$L.S.D_{0.05}$	2.71		

*each number represent mean of 3 replicates.

Table 3: Effect of salt stress on POX enzyme activity (U/g F.W) in callus derived from different explants of chilli pepper hybrid

Explants	Salt levels dSm^{-1}		
	6	8	10
Shoot tips	9.22	15.30	10.23
Roots	8.13	14.29	19.04
Green fruits	7.15	25.98	20.71
Red fruits	17.03	30.14	27.32
$L.S.D_{0.05}$	4.52		

*each number represent mean of 3 replicates.

Chlorophyll and Carotene pigments

The response of chlorophyll and carotene in callus originated from different explants of chilli pepper were determined .Based on data in table (4,5) higher concentration of chlorophyll were found in callus derived from shoot tips explants reached 44.13 $\mu g.g^{-1}$ F.W as compare to 0.15$\mu g.g^{-1}$ F.W in callus derived from roots explants in control treatment which represent the level 6 dSm^{-1} .However, all explants record a great reduction in Chlorophyll at (10 dSm^{-1}) level of salinity (table 4).Moreover, the response of explants were varied, roots and red fruits appears to be the less response when compare to Green fruits and Shoot tips. Conversely in case of carotene pigment. According to (table 5) carotene record a significant increase in callus initiated from red fruit explants reached 55.29 $\mu g.g^{-1}$ F.W at the level 10 dSm^{-1} On the other hand, carotene accumulations were found to be less in callus derived from the other vegetative explants.

Table 4: Effect of salt stress on chlorophyll ($\mu g.g^{-1}$ F.W) in callus derived from different explants of chilli pepper hybrid

Explants	Salt levels dSm^{-1}		
	6	8	10
Shoot tips	44.13	25.14	9.05
Roots	0.15	0.10	0.08
Green fruits	32.3	17.12	7.39
Red fruits	0.28	0.11	0.03
$L.S.D_{0.05}$	0.78		

*each number represent mean of 3 replicates

Table 5: Effect of salt stress on carotene (µg.g^{-1} F.W) in callus derived from different explants of chilli pepper hybrid

Explants	Salt levels dSm^{-1}		
	6	8	10
Shoot tips	0.02	1.42	2.36
Roots	0.15	0.33	0.75
Green fruits	21.14	36.17	22.51
Red fruits	38.15	41.13	55.29
L.S.D$_{0.05}$	8.14		

*each number represent mean of 3 replicates

Discussion

Salinity stress affected adversely on callus fresh and dry weight, previous study on pea plants by [Shahid et al, 2012] explain that, high salt stress decreases the water potential of the growth medium which leads to the reduction in cell turgor which in turn inhibits cell division and growth slows in (*Pisum sativum* L.) genotypes. Similar results in callus of tomato (*Lycopersicon esculentum***.** Mill**)** hybrids were found by [El Kaaby et al,2012] they explained the retardation of callus growth which might be due to lack the of endogenous growth regulators, which responsible for cell division as a results of break down, or lack of synthesis, which in turn may be caused a reduction in water availability in the culture medium. Also our results are agree with Nazif et al. [2000] who stated an adversely correlation between growth of cells in suspension culture of *Cassia acutifolia* plant and salt stress. On the other side, they remarked the positive role of salinity in production of anthraquinone in *Cassia acutifolia* plant.

PAL is the entry-point enzyme for phenylpropanoid pathway, which yields a various of phenolic compounds with structural and defense-related functions [El-Naggar.2012].Plant produce PAL, POX enzymes and carotene pigments as a part of defense mechanism against various stresses. In our research PAL activity increased in high level of stress this is agree with [Mani,2015] who found maximum activity of PAL reached (29.71 and 0.97 µmol/min/mg protein) in the placenta of pepper under water deficit treatment.

Conclusion

From the previous results in vitro callus culture is a promising technique to produce benefit compounds. Yet, further studies require to regenerate plants from stress callus.

References

[1]. Al Hattab.Z.N.;AL-Ajeel.S.A. and El Kaaby.E.A.2015. Effect of Salinity Stresson *Capsicum annuum* Callus Growth, Regeneration and Callus Content of Capsaicin,Phenylalanine, Proline and Ascorbic Acid.J. Life Science. 9: 304-310.

[2]. Celik .O and Atak.C.2012 The effect of salt stress on antioxidative enzymes and proline content of two Turkish tobacco varieties. Turk J Biol. 36: 339-356.

[3]. Chookhampaeng S 2011. The effect of salt stress on growth,chlorophyll content proline content and antioxidative enzymes of pepper (*Capsicum annuum* L.) seedling. Eur. J. Sci. Res., 49: 103-109.

[4]. EL-Kaaby. E. A.J.2016 **a**. Influence of Polyethylene glycol on some Morphological and Physiological characterization of two chilli pepper cultivars (Capsicum annuum L.) In Vitro. G. S. R. J.Multidisciplinary. 2(10):6-11.

[5]. El Kaaby.E.A.2016 **b**. Effect of Salinity Stress and the Addition of Some Precursors to Improve the Production of Capsaicin alkaloid in pepper *Capsicum annuum*.L in Vitro and the Detection of Genetic Stability Using DNA Markers.Ph.D. Dissertation. Biotechnology. College of Agriculture University of Kufa. Iraq.

[6]. El Kaaby Ekhlas A., Saadon.A. Al-Ajeel and Zahra N. Al Hattab.2015. Effect of Plant Hormones on Callus Induction from Fruit and Seedling Explants of Chilli Pepper (*Capsicum annuum* L.). J. Life Sciences. 9 (8):18-26.

[7]. ElKaaby,.E.A;ELanny,J,A;ALQaisy,S.A;ALAjeely,A.N;Ebraheem,H.A;Saleh,K.S,ALAubaidy,A.A.2012. Effect of Salinity stress on Callus induction and plant regeneration of three tomato hybrids*(Lycopersicon esculentum.* Mill) in vitro. The 1st Scientific AgriConference, 10-12th J. University of Duhok, 2012 Vol15.(1):457-461.

[8]. El-Naggar.H.M.2012. Phenylalanine ammonia-lyase (PAL) gene activity in response to proline and tyrosine in rosemary callus culture. African Journal of Biotechnology 11(1) : 159-163.

[9]. Gao. S.; Ouyang. C.; Wang. S.; Xu. Y.; Tang. Land Chen.F.2008. Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. Plant Soil Environ., 54 (9): 374–381.

[10]. Hussein**.**E.A and Aqlan.E.M.2011. Effect of mannitol and sodium chloride on some total secondary metabolites of fenugreek calli cultured *In vitro.* Plant Tissue Cult. & Biotech. 21(1): 35-43.

[11]. Islamian .J.Pand Mehrali.H .2015. Lycopene as A Carotenoid Provides Radioprotectant and Antioxidant Effects by Quenching Radiation-Induced Free Radical Singlet Oxygen: An Overview. Cell .J. 16(4): 386–391.

[12]. Mani.F.2015. Effect of water stress on some biochemical and physiological caracters of chili pepper *(Capsicum frutescens* L*.).* JECET. 4. (1): 75-84.

[13]. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant, 15: 473-497.

[14]. Navarro JM, Flores P, Garrido C, Martinez V. 2006.Changes in the contents of antioxidant compounds in pepper fruits at ripening stages, as affected by salinity. Food Chemistry 96:66–73.

[15]. Nazif NM, Rady MR and Seif El-Nasr MM .2000.Stimulation of anthraquinone production in suspension cultures of *Cassia acutifolia* by salt stress. Fitoterapia 71: 34-40.

[16]. Perez-Clemente.R.M. and Gomez-Cadenas.A.2012. *In vitro* Tissue Culture, a Tool for the Study and Breeding of Plants Subjected to Abiotic Stress Conditions.INTECH.Recent Advances in plant in vitro culture:Chapter (5): 91-108.

[17]. Ramakrishna.A and Ravishankar.G.A.2011. Influence of abiotic stress signals on secondary metabolites in plants.J. Plant Signal Behav. 6 (11): 1720–1731.

[18]. Sevengor. **S.;** Yasar. F.; Kusvuran.S and Ellialtioglu.S. 2011 The effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidative enzymes of pumpkin seedling Afri .J.Agric. Res.6(21): 4920-4924.

[19]. Shahid .M. A., Pervez .M. A., Balal .R. M., Abbas .T., Ayyub C. M., Mattson. N. S, A. Riaz and Iqbal. Z.2012. Screening of pea (*Pisum sativum* L.) genotypes for salt tolerance based on early growth stage attributes and leaf inorganic osmolytes. AJCS 6(9):1324-1331.

[20]. Sutković.J.; Ler .D and Abdel Gawwad.M.R.2011.*In Vitro* production of solasodine alkaloid in *Solanum nigrum* under salinity stress. J. Phytol 3(1): 43-49.

[21]. Taha.R.A.2014. Effect of Growth Regulators and Salinity Levels on *In vitro* Cultures of Jojoba Plants. World Applied .Sci. J. 31 (5): 751-758.

[22]. Yasar F (2007). Effects of salt stress on ion and lipid peroxidation content in green beans genotypes. Asian J. Biochem.19(2): 1165-1169.