

Response of irradiated chilli pepper shoots (*Capsicum annum. L.*) propagated in vitro to drought stress tolerance

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Abstract

Drought stress in plants caused by water deficiency. In Vitro selection with mutation techniques offered stress tolerance in plants. Shoot tips of chilli pepper (*Capsicum annum. L.*) exposed for gamma radiations at the doses (0, 10 and 20 Gy). Irradiated shoots were exposed to stress tolerance in the presence of Polyethylene Glycol (PEG 6000) in MS medium. Analysis of variance results for all characters studied were found to be significant, highest dose 20 Gy interaction with 40 g.l⁻¹ of PEG gave highest number 6.2 shoot. explant⁻¹ whereas 10 Gy and 40 g.l⁻¹ of PEG gave highest plant height 6.04 cm. Further, higher accumulation of proline reached 26.97 mg. g⁻¹ at interaction of 20 Gy+ 60 g.l⁻¹ PEG while highest accumulation of CHO found at 20 Gy+ 40 g.l⁻¹ PEG.

Keywords: In Vitro, PEG, Gamma, proline, chilli

Introduction

Drought stress is a major environmental stresses that threatens the economic of many countries due to its adversely affected plant growth and productivity in many parts of the world [Shao *et al.*, 2006]. Researchers in the agricultural sciences and food technology sectors elucidated the importance of produced plant yield and productivity under stress condition [Silva *et al.*, 2016]. Polyethylene glycol (PEG) imposing low water potential. Further, several researchers cited the assessing of (PEG) to induce osmotic stress through in vitro for different crops for example, chilli pepper [El –Kaaby.;2016], rice [Wu *et al.*, 2015.; Atiya *et al.*,2017.;Yousif *et al.*,2017], tomato [Manoj, K. and Deshpande.2005.; George *et al.*, 2015] castor bean [Silva *et al.*, 2016], Polynesian arrowroot [Martin *et al.*,2018] and eggplant [Gobo *et al.*,2017] eggplant [Gobu *et al.*, 2014]., soybean [Sakthivelu *et al.*, 2008]. Chilli pepper (*Capsicum annum. L.*) proved its efficiency in nutritional and farma properties for its high quality accumulation of Capsaicin alkaloid, antioxidant compounds like ascorbic acid and the osmo protecting proline. El Kaaby [2016] found high accumulation of Capsaicin alkaloid in shoots of chilli pepper in vitro cultured on stressed PEG media. Previous studies remarked the positive side of production some useful compounds under salt or drought stresses. For example, increasing of anti oxidant enzyme activity [Sevengor *et al.*,2011; Chookhampaeng, 2011; Mani, 2015]

Gamma radiation supports many beneficial like induce desire mutation in plants.

Drought tolerance screening In Vitro under comparing to field conditions which needs environmental influences and time consuming in addition the impossible for a number of plant species [Ehsanpour and Razavizadeh, 2005].Using Gamma radiation and cell culture in vitro techniques enhanced many and adapted genetic variability [Mahfouze *et al.*,2012] and speed up breeding program in short period un depending on growth seasons [Salman.; *et al.*, 2018] and climatic changes. The aim of this study is in vitro gamma rays improve chilli pepper plants to drought tolerance.

Materials and Methods

The experiment was conducted in Agricultural Research Directorate/ Genetic engineering department/ Ministry of Science and Technology. Seeds of chilli pepper (local variety) were surface sterilized using and germinated on Murashige and Skoog 1962. (MS) basal salts liquid media supplemented with 2 mg. l⁻¹GA3 [El Kaaby *et al.*, 2015]. 2 weeks later, shoots with (1 cm) height were irradiated with Gamma radiation (CO⁶⁰) at different doses (0, 10, 20) Gry. For shoots multiplications, Irradiated shoots were transferred to MS media supplemented with 2 mg.L-1 GA3[El Kaaby *et al.*, 2015].Media were stresses with Polyethylene Glycol (6000) at concentrations (0,40,60 g. l⁻¹) with osmotic potential (ψ_s) of -0.30, -0.58 and -0.74 MPa [Sakthivelu *et al.*, 2008]. Six weeks later, growth

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parameters such as number of shoots, and shoots height were recorded. Moreover, physiological parameters such proline, carbohydrates were recorded also. Proline was determined according to Bates *et al*, [1973], carbohydrate concentrations (Total sugar content) was determined according to Herbert *et al* [1971].

Statistical analysis

The experiments were design in completely randomized (C.R.D) with three replicates for each parameter; Means were compared according to Duncan’s Multiple Range (DMRT) test at level $p \leq 0.05$.

Results and Discussion

Data in table (1) revealed that addition of PEG affected significantly on number of shoots and shoots height (table 2). Clear inhibition in numbers of shoots at higher concentration 60 g.l⁻¹ of PEG 1.80 shoot. explant⁻¹ compare to 5.5 shoot. explant⁻¹ in media free PEG. At contrary, higher dose 20 Gy of gamma rays affected positively on numbers of shoots 4.67 shoot. explant⁻¹ compare to 2.8 shoot. explant⁻¹ at control non irradiated treatment table(1). Same results was shown in table (2) in the absence of PEG stress (0 treatment) chilli shoots recorded highest height 4.24 cm explant⁻¹ comparing to 2.073 cm at 60 g.l⁻¹ of PEG. Regarding to Gamma effects, data documented in table (2) referred that 10 Gy gave 5.153 cm. On the other hand, the interactions between gamma doses and PEG Concentrations in table (1 and 2) were greatly affected on these parameters, high dose 20 Gy and 40 g.l⁻¹ of PEG were superior to give highest number 6.2 shoot. explant⁻¹ whereas 10 Gy and 40 g.l⁻¹ of PEG were superior to give highest plant height 6.04 cm. Our results are agree with El-Shafey *et al*. [2009] who stated 10 and 20 Gy alleviated the harmful effect of drought stress in callus of rice crop also similar results were found by [Al-Mousa *et al*, 2016] for optimum 20 Gy of Gamma affected positively on rooting of grape (*Vitis vinifera* L.) shoots .

Table 1: Gamma radiation and Poly Ethylene effects on number of shoots. explant⁻¹ of chilli pepper

Gamma Dose (Gy)	PEG Concentration (g. l ⁻¹)			Mean
	0	40	60	
0	5.0 a	2.4 b	1.0 c	2.8 c
10	5.8 a	3.2 b	2.4 b	3.8 b
20	5.8 a	6.2 a	2.0 bc	4.67 a
Mean	5.53 a	3.93 b	1.80 c	

*Means with different letters are significantly different from each other within the variants at 5% level according to Duncan’s multiple range test

For accumulation of proline and carbohydrates, Data in table (3,4) clarified that, at high doses of Gamma rays (20 Gy) positively accumulation were found (21.26 mg. g⁻¹ and 4.121 mg.g⁻¹ for proline and CHO respectively. At 40

and 60 g.l⁻¹ PEG a significant increase in proline accumulation reached (19.78 mg. g⁻¹, 19.89 mg. g⁻¹) respectively (table 3), whereas 40 g.l⁻¹ of PEG caused significant increase in CHO accumulation reached 3.568 mg. g⁻¹ (table 4). Moreover, higher accumulation of proline reached 26.97 mg. g⁻¹ found when the interaction was 20 Gy+ 60 g.l⁻¹ PEG (table 3).

Table 2: Gamma radiation and Poly Ethylene effects on shoots height (cm) of chilli pepper

Gamma Dose (Gy)	PEG Concentration (g.l ⁻¹)			Mean
	0	40	60	
0	3.56 c	2.504 d	1.52 e	2.528 b
10	5.48 b	6.04 a	3.94 c	5.153 a
20	3.68 c	1.08 ef	0.76 f	1.84 c
Mean	4.24 a	3.208 b	2.073 c	

*Means with different letters are significantly different from each other within the at 5% level according to Duncan’s multiple range test

Table 3: Proline accumulation (mg.g⁻¹) in irradiated shoots exposed to PEG-Stress in vitro

Gamma Dose (Gy)	PEG Concentration (g.l ⁻¹)			Mean
	0	40	60	
0	0.61 d	21.78 b	14.58 c	12.32 b
10	18.81 bc	19.42 bc	18.12 bc	18.78 b
20	18.69 bc	18.13 bc	26.97 a	21.26 a
Mean	12.7 b	19.78 a	19.89 a	

*Means with different letters are significantly different from each other within the variants at 5% level according to Duncan’s multiple range test

While highest accumulation of CHO was found at 20 Gy+ 40 g.l⁻¹ PEG (table 4) with significant reduction at 60 g.l⁻¹ PEG this is in agree with Yousif [2002] who explained the reduction in carbohydrate may be due to the use of carbohydrate as an energy source for tolerant cell.

Conclusion

Further studied are recommended associated with field performance for shoots propagated In Vitro and assessing genetic variability by using DNA molecular marker which would be useful for identifying better dose of gamma rays effected on chilli pepper drought tolerance.

Table 4: Carbohydrate accumulation (mg.g⁻¹) in irradiated shoots exposed to PEG-Stress in vitro

Gamma Dose(Gy)	PEG Concentration (g.l ⁻¹)			Mean
	0	40	60	
0	0.124 f	2.348 d	2.26 d	1.577 c
10	3.942 b	3.29 c	1.634 e	2.955 b
20	4.23 b	5.066 a	3.066 c	4.121 a
Mean	2.765 b	3.568 a	2.32 c	

*Means with different letters are significantly different from each other within the variants at 5% level according to Duncan’s multiple range test

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