International Journal of Multidisciplinary and Current Research

Research Article

ISSN: 2321-3124
Available at: http://ijmcr.com

In Vitro Regeneration of Tomato *(Lycopersicon esculentum)* Plants under Drought Stress

Zahra N. Al Hattab, Wajida K. Al Qaudhy, Ashwaq Abdul Razaq, Ekhlas A. Al Kaaby and Jenan A. Al Ani

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

Accepted 22 Nov 2015, Available online 28 Nov 2015, **Vol.3 (Nov/Dec 2015 issue)**

Abstract

This experiment was conducted to regenerate tomato plants in the presence of polyethylene glycol (PEG). Calli were induced from cotyledonal leaf, shoot tip, stem, and leaf of two tomato cultivars on MS medium supplemented with 1 mg /L BA and 0.6 mg /L IBA. The produced calli were subjected to drought stress on MS media supplemented with 0, 20, 40, 60 gm /L PEG. The results showed no significant differences between the two cultivars in the calli fresh weights of the different explants. However, there were differences among the explants. Stem callus showed the highest followed by the leaves. Also Super Regina's stems showed significantly higher callus fresh weight than Falcon's stems. Leaf's and stem's calli dry weights were equal and they were significantly different from the cotyledonal leaves and the shoot tips. Callus fresh and dry weights of both cultivars were reduced as PEG increased. The highest callus fresh weight was for Super Regina in 0 g/L PEG and the lowest was for Falcon in 60 g/L PEG. At 20 g/L PEG, Falcon callus fresh weight was significantly higher than Super Regina. In all PEG treatments Falcon's callus dry weight was significantly higher than Super Regina's except at 40 g/L PEG they were equal. Plant regeneration in the presence of PEG from both cultivars was severely affected. The number of regenerated plants was reduced on 60 g/L PEG, whereas 0 g/L PEG and 40 g/L PEG showed the highest number of regenerated plant/explant.

Keywords: *Tomato, PEG, Drought, Tissue culture*

Introduction

Tomato is one of the most important economical vegetable crops in the Solanaceae family grown throughout the world (M. Devi, *et al*., 2008). It is recognized as a highly valuable, medicinal, industrial and nutritious food. Moreover it has been grown in a wide range of environmental conditions such as tropical, sub-tropical and temperate areas (P. Bahati, *et al*., 2004). However productivity of tomato has been low due to many biotic and abiotic stresses.

In Iraq among the different abiotic stresses, drought is a major agronomic problem that profoundly affects crop growth during the last decade. This problem also affects the productivity world wide especially in arid and semi-arid areas (K. Yamaguchi-Shinozaki and K. Shinozaki, 2006; H. D. Adams *et al*., 2009). Limited water resources combined with an increase in aridity of semi-arid regions have led to an urgent demand for improving crop drought resistances (J. Passioura, 2007). The development of crop varieties with increased tolerance to drought is an important strategy to meet global food demands with water shortage.

In vitro provides a uniform environment for induction and selection of drought tolerant plants from the somoclonal variants. Polyethylene glycol has been used as osmotic regulator in the medium for the selection of drought tolerant plants (A. T. Abdul-Raheem 2007).

Many tissue culture studies have been conducted on tomato and different explants sources have been used for callogenesis and regeneration. Various hormonal combinations are used to induce callus and regeneration such as BAP, IAA and Kin (M. Devi *et al*., 2008). Successful callus induction and regeneration from shoot apex, nodal segments and root segments were reported (Z. Chaudhry *et al*., 2010). The effect of variety as well as hormones on callus proliferation and regeneration of tomato cultivars have also been reported (S. Ishag *et al*., 2009). The present study was conducted to explore the ability of two tomato cultivars to induce calli and regenerate plants under drought stress using PEG as osmotic regulator.

Materials and Methods

This research was conducted at the tissue culture laboratory of The Ministry of Science and Technology/ Directorate of Agriculture /Baghdad, Iraq. Two imported Tomato cultivars namely Falcon and Super Regina produced by American company were used in this experiment.

Mature seeds were washed thoroughly with distilled water and immersed in 95% Ethanol for 2 mints, rinsed with sterilized distilled water and treated with 2.4% Sodium Hypochlorite for 10 mints and washed three times with sterilized distilled water 5 mints each. All the above steps were done in a laminar air flow hood. Sterilized seeds were germinated on hormone free MS medium (T. Murashige and F. Skoog 1962) and incubated in the culture room at 25± 1 C and 16 h light. Shoot tips, cotyledon leaves, stems and leaves from the seedlings were cultured separately on MS medium supplemented with 1 mg/L BA and 0.6 mg/L IBA and incubated in the culture room under the same condition mentioned above.

After four weeks in culture thirty replications with 100 mg calli/ replication from all explants were cultured on new callus induction medium supplemented with different concentrations of Poly Ethylene Glycol **(**PEG) (0, 20, 40, 60 gm/L). Forty days from culture, ten replications from each treatment were selected randomly for calli fresh weight measurements. Then calli were dried in the oven at 70 C for 72 h to measure their dry weight.

Calli from all treatments (20 replications) were transferred to regeneration media which was MS medium supplemented with 2 mg/L IAA and 1mg/L Kin. Regenerated plants were transferred to small pots for acclimatization and transplanted to the plastic house for seed production.

All experiments were conducted in CRD and they were analyzed using L.S.D at P= 0.05.

Super Regina Falcon

Figure 1 Seed germination of Falcon and Super Regina Tomato cultivars on free hormone MS medium

Results

The results showed that seeds of the two cultivars were very viable and gave 100% germination on free hormone MS medium (Fig. 1).

Super Regina

Falcon

Figure 2 Callus induction from different explants of Falcon and Super Regina Tomato cultivars. From left: Super Regina: (Cotyledonal leaves, Leaves, Stems) Falcon: (Stems, Leaves, Cotyledonal leaves)

Table 1 Explant effect on callus fresh weight (g) of two Tomato cultivars Super Regina and Falcon

Cultivars	Explant				Mean
	Shoot tips	Cotyledon leaves	leaves	stem	
Super regina	0.62	0.58	1.02	1.13	0.84
Falcon	0.69	0.58	1.02	1.09	0.85
Mean	0.66	0.58	1.02	1.11	
$L.S.D_{0.05}$	Explant 0.05		Interaction 0.07		Cultivars N.S

Table 2 Explant effect on callus dry weight (g) of two tomato cultivars Super Regina and Falcon

Cultivars	Explant				Mean
	Shoot tips	Cotyledon leaves	leaves	Stem	
Super Regina	0.08	0.05	0.06	0.07	0.06
Falcon	0.07	0.08	0.13	0.11	0.10
Mean	0.07	0.07	0.09	0.09	
$L.S.D_{0.05}$	Explant 0.014		Interaction 0.02		Cultivars 0.01

Table 3 Effect of PEG concentrations on callus fresh weight of two tomato cultivars Super Regina and Falcon

Cultivars	PEG Concentration (g/L)				Mean
	0	20	40	60	
Super Regina	2.26	0.64	0.31	0.15	0.84
Falcon	2.21	0.77	0.26	0.14	0.83
Mean	2.24	0.71	0.29	0.15	
$L.S.D_{0.05}$	PEG Conc 0.05		Interaction 0.07	Cultivars N.S	

Table 4 Effect of PEG concentrations on callus dry weight (g) of two tomato cultivars Super Regina and Falcon

Cultivars	PEG Concentration (g/L)				Mean
	0	20	40	60	
Super Regina	0.11	0.06	0.04	0.04	0.06
Falcon	0.17	0.11	0.04	0.07	0.10
Mean	0.14	0.08	0.04	0.06	
$L.S.D_{0.05}$	PEG Conc 0.014		Interaction 0.02	Cultivars 0.01	

Table 5 Effect of different concentrations of PEG on callus fresh weight (g) of different tomato explants

Explant	PEG Concentration (g/L)				Mean
	0	20	40	60	
Shoot tips	1.76	0.65	0.13	0.13	0.67
Cotyledon leaves	1.87	0.58	0.13	0.13	0.68
Leaves	2.50	1.02	0.51	0.17	1.05
Stem	2.80	1.11	0.36	0.14	1.10
Mean	2.23	0.70	0.28	0.14	
$L.S.D_{0.05}$	PEG Conc 0.05		Interaction 0.09	Explants 0.05	

In Vitro seed germination provides sterilized seedlings that were used as explant source. All explants induced calli within the first two weeks and callus color ranged from pale yellow to pale green depending on the explant type (Fig 2).

The results (Table 1) showed no significant differences between the two cultivars in the mean of calli fresh weight produced from the different explants. However there were differences among the explants of each cultivar in the mean of the fresh weight. Stem's callus fresh weight was the highest (1.11 g) followed by the leave's (1.02 g), the shoot tip's (0.66 g) which are significantly higher than the Cotyledon leaves (0.58 g). Super Regina's stems gave the highest fresh weight mean which was significantly higher than Falcon stems (Table 1).

For calli dry weight (table 2), the results showed that Falcon was significantly higher than Super Regina and they were 0.1g and 0.06 g respectively. Moreover, the stems and the leaves gave the highest mean of dry weight (0.09 g for both) which was significantly higher than the Cotyledon leaves and the shoot tips. For cultivar and explant interaction, Falcon leaves gave significantly higher dry weight than the other combinations (0.13g) (table 2).

PEG effect on Falcon and Super Regina calli growth were studied after 40 days from culture. The results showed negative effect of PEG concentrations on callus fresh and dry weight of both cultivars. They were reduced as the PEG concentration increased (Table 3 and 4). There was no significant difference between the two cultivars in the fresh weight with a mean of 0.84 g and 0.83 g for Super Regina and Falcon respectively (Table 3). Callus fresh weight was the highest for Super Regina in 0 g/L PEG treatment (2.26 g) and the lowest was for Falcon in 60 g/L PEG treatment (0.14 g). At 20 g/L PEG treatment Falcon fresh weight was significantly higher than that for Super Regina (0.77g and 0.64 g respectively). On the other hand, Falcon dry weight was significantly higher than that of Super Regina with a mean of 0.10 g and 0.06 g respectively (Table 4). In all PEG treatments Falcon's dry weight was significantly higher than Super Regina's except at 40 g/L PEG they were equal (0.04 g) .

The effect of different concentrations of PEG on callus fresh weight induced from different tomato explants is shown in Table 5. The mean of fresh weight induced from the leaves and the stems were 1.05 g and 1.10 g respectively and they were significantly different than that of the shoot tips and the Cotyledon leaves (0.67 and 0.68 g respectively). Calli induced from shoot tips and Cotyledon leaves were affected by PEG concentrations. They gave the same fresh weight in 40g and 60 g PEG treatments (0.13 g) which was the lowest compared with the other treatments.

Moreover, calli dry weight produced from the leaves and the stems were equal (0.09 g) and were significantly higher than that produced from the shoot tips and the Cotyledon leaves (0.07 g) (Table 6). However the highest mean dry weight was for calli produced from the Cotyledon leaves and the stem in 0 g/L PEG treatment (0.15 g).

Table 6 Effect of different concentrations of PEG on callus dry weight (g) of different tomato explants

Explant	PEG Concentration (g/L)				Mean
	0	20	40	60	
Shoot tips	0.14	0.06	0.04	0.05	0.07
Cotyledon leaves	0.15	0.03	0.03	0.06	0.07
Leaves	0.12	0.12	0.04	0.09	0.09
Stem	0.15	0.12	0.05	0.03	0.09
Mean	0.14	0.08	0.04	0.06	
$L.S.D_{0.05}$ PEG Conc 0.014 Interaction 0.028 Explants 0.014					

Table 7 Explant on plant regeneration (plant/explant) of two tomato cultivars Super Regina and Falcon

Cultivars	Explant			Mean
	leaves	Soot Tip	Cotyledonal Leaves	
Super regina	2.65	2.70	4.10	3.15
falcon	1.70	2.08	2.43	2.07
Mean	2.18	2.39	3.26	
$L.S.D_{0.05}$ Explant 0.568 Interaction 0.803 Cultivars 0.464				

Table 8 Effect of PEG concentrations on plant regeneration (plant/explant) of two tomato cultivars Super Regina and Falcon

Cultivars	PEG concentrations (g/L)				Mean
	0	20	40	60	
Super Regina	4.33	2.50	3.20	2.57	3.15
Falcon	2.33	2.20	2.40	1.33	2.07
Mean	3.33	2.35	2.80	1.95	
$L.S.D_{0.05}$ PEGConc 0.656 Interaction 0.928 Cultivars 0.464					

The dry weight of leaves calli were not affected by 20 g/L PEG treatment; it was the same as the control with an average of 0.12g. Also it was the highest in 60 g/L PEG treatment (0.09 g) compared with the other explants calli (Table 6).

Plant regeneration was affected by cultivar as well as the explant type (Table7). Although Super Regina gave less calli dry weight than Falcon as was shown previously, it gave significantly higher number of regenerated plants with an average of 3.15 plants /explant. The cotyledonal leaves gave the significantly higher number of plants than other explants with an average of 3.26 plants /explant.

Plant regeneration in the presence of PEG from both cultivars under investigation was severely affected (Table 8). Calli grown on regeneration media supplemented with 60 g/L PEG changed to brown color and the number of regenerated plants were reduced (Fig 3), whereas 0 g/L PEG treatments and 40 g/L PEG treatments gave the highest number of regenerated plants (3.33 and 2.80 respectively) which were significant higher than the other treatments (Fig 4). For the interaction of the cultivars and the PEG, the results showed that Super Regina at 0 PEG treatment gave significantly higher number of plants/explant compared with the other treatments and the lowest number was (1.95) from Falcon grown on 60 g/L PEG treatment. However both cultivars gave higher number of regenerated plants on 40 g/L PEG than the 20 g/L PEG.

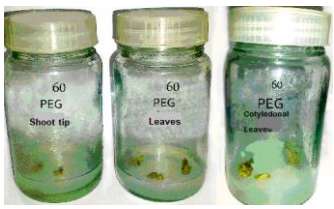

Figure 3 Calli produced from different explants from left (Shoot tip, leaves, and cotyledonal leaves) grown on regeneration medium supplemented with 60 g/L PEG

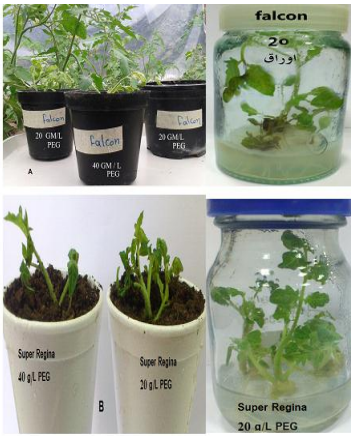

Figure (4) Plants regenerated from calli grown in the presence of 20 and 40 g/L (A- Falcon at the flowering stage, B- Super Regina)

Discussion

The results of the current experiment showed that all explants induced calli which is in agreement with Many researchers who reported succesfull callus induction from various tomato explants in the precence of growth regulators (M. Devi, *et al*., 2008; S. Ishag, *et al*., 2009). Variations in the callus induction and growth among Tomato cultivars induced from different explants and the effect of hormones were reported by many researchers (A. T. Abdel-Raheem *et al*., 2007; P. Adams *et al*., 1992; P. Bhatia *et al*., 2004).

The results of the current experiment showed that there were no significant differences between the two cultivars in the fresh weight, while there were significant differences in the dry weight. These results indicated that Super Regina's callus cells have less mass than that of Falcon under drought condition. Cell divisions of Super Regina's callus might be stopped or slowed down in the presence of PEG in media. Moreover other cell activities might also stopped. However their higher fresh weight might occur because of water imbibitions from the medium. The ability of cells to control the water movement through their membranes under drought stress and continue to grow normally might explain their ability to tolerate drought. These findings are in agreement with what was reported by other researchers (R. A. Bressan *et al*. 1981; M.K. Rai *et al*., 2011).

Although PEG had severe impact on callus induction and growth, plants were regenerated from calli grown on media with high level of drought stress. These results might be due to the sonaclonal variation which gave cell lines that tolerate the PEG effect and did not lose the regeneration ability. Callus induction and plant regeneration are two different traits which are controlled by different genes and they can be improved by breeding (G. Pinto, *et al*. 2008).

All the produced plants were acclimatized in the green house and transferred to the field and they gave fruits. Some of the plants that regenerated from drought tolerant calli might be drought tolerant too. Plant tissue culture technique allows opportunities for the researcher to improve plants against abiotic stress factors with the *in vitro* selection method (S. M. Jain, 2001). Callus cells grown on media supplemented with PEG might be drought tolerant. PEG imposes water stress on the callus cells. This environment mimics water deficiency in the soil. Researches in this field showed that callus cells tolerate water stress in the media produce plants with the same tolerant (A. T. Abdel-Raheem, et. al, 2007; M. A. Mohamed et. al, 2000). Additionally it is possible that the regenerated plants from the different treatments in the present study are drought tolerant. Even without PEG in the medium few cells might be drought tolerant due to the somaclonal variations that occur in the cultured cells (A. Safarnejad, 2004). Plant breeders depend on this phenomenon to produce plants with desirable traits (R. A. Azevedo, 2011; Z. Chaudhry, et. al, 2010; Z. N. Hashim et. al, 1990; M. K. Rai et. al, 2011).

In conclusion it has been possible to regenerate tomato plants from calli grown under drought stress. However, field test under water stress condition of the regenerated plant is an important step to confirm their drought tolerant ability. Therefore, all the fruits were collected and will be grown in water stress conditions for evaluation and selection.

References

[1]. A.T. Abdel-Raheem, A. R. Ragab, Z.A. Kasem, F. D. Omar, and A.M. Samera 2007. *In Vitro* Selection for Tomato Plants for Drought Tolerance via Callus Culture under Polyethylene Glycol (PEG) and Mannitol Treatments. African Crop Science Conference Proceedings Vol. 8 PP. 2027- 2032.

[2]. H.D. Adams, M. Guardiola-Claramonte, G.A. Barron-Gafford, J.C. Villegas, D.D. Breshears, C.B. Zou, P.A. Troch and T.E.Huxman. 2009. Temperature Sensitivity of Drought-Induced Tree Mortality Portends Increased Regional Die-Off under Global-Change-Type Drought Proceedings of the National Academy of Sciences, USA 106 7063–7066.

[3]. P. Adams, J.C. Thomas, D.M. Vernon, H.J. Bohnert and R.G. Jensen. 1992. Distinct Cellular and Organismic Responses to Salt Stress. Plant Cell Physiol. 33: 1215-1223.

[4]. R. A. Azevedo , R. F. Carvalho, M. C. Cia and P. L. Gratao. 2011. Sugarcane under Pressure: An Overview of Biochemical and Physiological Studies of Abiotic Stress Tropical plant biology, Vol 4 (1): pp 42-51

[5]. P. Bahatia, N. Ashwath, T. Senaratna and D. Midmore 2004. Tissue Culture Studies of Tomato *(Lycopersicon esculentum)*. *Plant Cell, Tissue and Organ culture* vol. 78 : 1-21

[6]. R.A. Bressan, P.M. Hasegawa and A.K. Handa. 1981. Resistance of Cultured Higer Plant Cells to Polyethylene Glycol Induced Water Stress. Plant Sci Lett. Vol. 21: 23-30.

[7]. Z. Chaudhry, S. Abbas, A. Yasmin, H. Rashid, H. Ahmed and M.A. Anjum. 2010. Tissue Culture Studies in Tomato *(Lycopersicon esculentum)* Var. Moneymaker Pak. J. Bot., 42(1): 155-163, 2010.

[8]. M. Devi, M. S. Dhaliwal, A. Kaur, and S. S. , Gosal. 2008. Effect of Growth Regulators on In Vitro Morphogenic Response of Tomato. Indian Journal of Biotechnology 7: 526-530.

[9]. Z.N. Hashim, W.F. Campbell and J.G. Carman. 1990. Morphological Analyese of Spring Wheat (CIMMYT cv. PCYT-10) Somaclons. *Plant Cell, Tissue and Organ Culture* vol. 20 (2): p. 95 – 99

[10]. S. Ishag, M.G. Osman, and M.M. Khalafalla. 2009. Effects of Growth Regulators, Explant and Genotype on Shoot Regeneration in Tomato (*Lycopersicon esculentum* CV.Omdurman) Int. J. Sustain Crop Prod. 4(6): 7-13.

[11]. S.M. Jain. 2001. Tissue Culture- Derived Variation in Crop Improvement, *Euphytica*, 118:153-166.

[12]. M.A. Mohamed, P.J.C. Harris and J. Henderson. 2000. *In Vitro* Selection and Characterization of a Drought Tolerant Clone of *Tagetes minuta*, *Plant Sci*., 159: 213-222.

[13]. T. Murashige and F. Skoog. 1962. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Culture. Phsiol. Plant 15: 473-497.

[14]. J. Passioura. 2007. The Drought Environment: Physical, Biological and Agricultural Perspectives. Journal of Experimental Botany 58:113–117.

[15]. G. Pinto, Y.S. Park, L. Neves, C. Aranjo and C.Santos. 2008. Genetic Control of Somatic Embryogenesis Induction in *Eucalyptus globules* Labill. Plant Cell Reports. Vol 27(6): 1093-1101.

[16]. M.K. Rai, R.K. Kalia, R. Singh, M.P. Gangola and A.K. Dhawan. 2011. Developing Stress Tolerant Plants Through *In Vitro* Selection-an Overview of The Recent Progress, *Env. Expr. Botany,* 71: 89-98.

[17]. A. Safarnejad. 2004, Characterization of Somaclones of *Medicago sativa* L. for Drought Tolerance, *J. Agric. Sci. Technol.,* 6: 121-127.

[18]. K.Yamaguchi-Shinozaki and K. Shinozaki. 2006. Transcriptional Regulatory Networks in Cellular Responses and Tolerance to Dehydration and Cold Stresses. *Annu. Rev. Plant Biol.* **57**, 781–803.