# *In vitro* Drought Tolerant of Rootstock Apple (*Malus domestica* Borkh.) and Pear (*Pyrus calleryana*)

**Gharbia H. Danial[*] , Diaa A. Ibrahim[**], Belan M. Khalil and Vyan M. Musa.**
*Dept. Scientific Research, Faculty of Science, University of Duhok Duhok, Iraq, Email: gha.2008@yahoo.com
** Dept. Scientific Research, Faculty of Science, University of Duhok Duhok, Iraq
*** Dept. Scientific Research, Faculty of Science, University of Duhok Duhok, Iraq

## Abstract:

**The apple and pear explants were cultured in MS multiplication media supplemented with different concentrations of polyethylene glycol (6000), (0.0, 2%, 4%, and 6%). The results revealed that PEG at 6% was more effective than 2% and 4% by producing significantly lower average number of branches per explants (3.58) in apple and (3.62) in pear as compared to the highest average number of branches (6.06) in apple and (9.04) in pear achieved by control treatment. On the other hand, the shoot length and leaves number were reduced in both apple and pear at all PEG levels. After then, the plantlets were rooted in MS media supplemented with 1.0 mg/l NAA, and acclimatized in greenhouse. Under *in vitro* conditions callus induction from apple, the response of calli to elevated levels of PEG was recorded as fresh weight. These results indicated that increased levels of PEG were used to create water stress. There was a reduction in callus induction ability with increasing levels of PEG. And the control was superior compared to all other treatments.**

**KEYWORDS.** *In vitro*, Drought tolerant, Apple, Pear, Polyethylene glycol.

## I. Introduction:

Drought resistance is become of increasing importance in rootstock selection under actual field conditions due to low heritability and required time. Selection in tissue culture is thought to be one way to improve selection efficiency, but this requires standardized protocols. Drought is considered as the most threatening factor that faces agriculture in Kurdistan region. To face drought an initiated program to produce apple and pear plants characterized with low water using polyethylene glycol in multiplication stage**.** A biotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment. A biotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% [1]. One of the most important a biotic factors limiting plant germination and early seedling

stages is water stress brought about by drought and salinity [2]. Drought is one of the most common environmental stresses affecting plant growth and productivity [1]. Water deficit, extreme temperatures and low atmospheric humidity lead to drought, which is one of the most limiting factors for better plant performance and higher crop yield [3]; [4]. Increasing human population and global climate change make the situation more serious [5]. The repercussions of water deficit include its adverse effects on plant phenology, phasic development, growth, carbon assimilation, assimilate partitioning and plant reproduction processes [6].

Drought tolerant cultivars can be achieved by using a high molecular weight compound known as polyethylene glycol. PEG (polyethylene glycol) has been used in vitro to induce water stress in plants [7,8]. PEG is a non-penetrating inert osmotic that lowers the osmotic potential of nutrient solutions, but it is not taken and is not phytotoxic [9]. PEG stimulates water stress in cultured plant cells in the same way it does in the cells of intact plants [10]. Producing sustainable and profitable crops under drought conditions required technological and biological approaches, including; selection of new, more drought tolerant cultivars of named plants using conventional breeding programs or tissue culture techniques [11].

Breeding of drought tolerant crops should be given a high research priority in all future plant biotechnology programs [12]. Hence, Plant tissue culture plays an important role in the production of agricultural and ornamental plants and in the manipulation of plants for improved agronomic performance. *In vitro* culture of plant cells and tissues has attracted considerable interest over recent years because it provides the means to study plant physiological and genetic processes in addition to offering the potential to assist in the breeding of improved cultivars by increasing genetic variability.

The aims of present study are to produce apple and pear plants characterized with low water (i.e. drought tolerant) requirements. To achieve such an objective callus (i.e. group or mass of cells) cultures were initiated from apple leaf segments grown on nutrient media supplemented with 2,4-D . The callus was subcultured onto media that contains different concentrations of PEG of Mw. of 6000 as drought stress agent where only tolerant cells can grow. The optimal concentration of PEG will be determined followed by plant regeneration.

## II. Materials and Methods:

This study has been conducted in Plant Tissue Culture Laboratory at Scientific Research Center in University of Duhok. during the period from June , 2011 to February , 2012. Auxiliary buds of MM 106 apple rootstock and *Pyrus calleryana* L. pear grown *in vitro* on MS media [13] were used as explants .The cultures were incubated at 25± 2°C under 16 hrs daily exposures to 1000 lux of cool white light. The following levels of PEG (MW 6000) were added to proliferation media at 0, 2, 4, and 6% . Healthy single shoots of both apple and pear were cultured on Murashige and Skoog (1962) medium supplemented with 2.0 mg/l BA, 1.0 mg/l IBA and 1.0 mg/l $GA_3$ , thiamine HCl 0.4 mg/l , inositol 100 mg /l, sucrose 30g./l [14] . After pH was adjusted

to 5.7 using NaOH or HCl, 7 g./l, agar was added to the media, then dispensed at 25 ml rates, into 250 ml Mason jars and capped with colorless PVP covers and fitted with rubber bunds. The media was autoclaved at 121°C and 1.05 $kg.m^{-2}$ for 20 minutes and allowed to solidify under room temperature. Three single axillary buds of apple and pear were cultured in each culture jar.

Five replications were assigned for each level of treatment and the experiment was designed according Completely Randomized Design (CRD). The results were recorded six weeks after incubation including shoot numbers, shoot lengths and leaves numbers. After then the explants cultured in MS rooting media containing 1.0 mg/l NAA [14]. For acclimatization stage, a number of successfully rooted plantlets were removed from culture vessels and their roots were washed with distilled water and immersed in Benlate fungicide (0.1% for 10 minutes). They were transferred to pots containing a steam sterilized soil mix (peatmoss+ loam+ Styrofoam 1:1:0.5, v:v:v) under tightly controlled atmosphere of the greenhouse.

For callus induction, leaf discs ($1\times1$ $cm^2$) from apple were excised under aseptic conditions and cultured on MS media supplemented with 2 mg/l 2,4-D [12] . Three explants were cultured in each vessel with five replications. The results were recorded after 8 weeks of culture. The data were statistically analyzed by one way ANOVA test with SPSS v. 17.

## III. Results and Discussion:

Adding different concentrations of polyethylene glycol to proliferation media played an important role on growth and development of both apple and pear. The effect of PEG on rootstocks is shown in Table (I). The presence of PEG in the medium caused decreasing in all measured parameters for both apple and pear (Figures 1, A and D). For apple shoot numbers, the highest mean number of shoots (6.06 shoots/explants) were recorded in media devoid of PEG (Fig. 1, B), but it showed no significant difference with those containing 2% and 4% PEG and did not differ significantly with 6% PEG (3.58) shoots/explants (Fig.1 ,C). Although, there is no significant differences between 2% and 4% of PEG, but one can see that shoot numbers in 4% PEG was greater compared to 2%PEG. For mean shoot lengths and leaf numbers, similar results were observed, with highest mean shoot length of branch(1.36 cm) and the highest number of leaves per explants (27.04) in control treatment ( 0.0% of PEG ) contained media, and lesser values in all other media supplemented with PEG. Where as the lowest mean shoot length was found in (4% of PEG) 0.79cm and the lowest leaves number was found for the (6% of PEG) 15.58 leaf/explants.

Table.I: Effects of different concentrations of PEG on shoot number, shoot length and leaf number of both apple and pear.

| PEG conc. % | Apple | | | Pear | | |
|---|---|---|---|---|---|---|
| | Shoot No. mean±(S.E.) | Shoot length(cm.) mean±(S.E.) | Leaf No. mean±(S.E.) | Shoot No. mean±(S.E.) | Shoot length(cm.) mean±(S.E.) | Leaf No. mean±(S.E.) |
| 0.0 | 6.06±1.03 a | 1.36±0.29 a | 27.04±8.24 a | 9.04±3.17 a | 1.41±0.15 a | 29.82±6.78 a |
| 2.0 | 4.17±1.13 ab | 1.00±0.16 b | 18.58±3.43 b | 3.90±1.82 b | 0.95±0.13 b | 17.36±3.53 b |
| 4.0 | 4.92±2.04 ab | 0.79±0.22 b | 16.76±2.65 b | 4.10±0.58 b | 0.68±0.06 c | 15.18±3.19 b |
| 6.0 | 3.58±1.63 b | 0.90±0.11 b | 15.58±5.58 b | 3.62±044 b | 0.92±0.13 b | 15.66±2.05 b |

*Numbers followed by the same letter are statistically not different at 0.05 level of probability.

For pear the mean shoot number was the greatest in control treatment (0.0%PEG) where it recorded 9.04 shoots/explants which were significantly different from the other treatments (Fig.1, E). PEG caused a remarkable reduction in the mean lengths of branches and leaves numbers as compared to the control treatment from 1.41 cm (control) to only 0.68 cm (4%PEG). The highest number of leaves per explants 29.82 obtained at control, which was significantly higher when compared to (2%, 4% and 6% PEG) concentrations .These results for apple and pear an observation was similarly reported by [6] in soybean, [15] in Date palm, [11] in Pear.

In all cases, the high concentrations of PEG reduced the shoot numbers, and [16] showed that germination percentage in *Eremosparto songoricum* decreased with an increase of polyethylene glycol concentration. The results showed that PEG (MW6000) induced osmotic stress by possessing significant effect on both apple and pear growth. PEG treatments reduced shoot multiplication and inhibited growth;

they were influenced by increasing PEG concentrations from 2 to 6%. The effect of PEG may be attributed to the osmotic changes in the nutrient media, which force the cultured plantlets to lose water due to plant drought and reducing ability up taking of water and minerals [11].

Then the tolerant explants transfer to rooting media containing 1.0 mg/l NAA, after sufficient development of roots, plantlets were successfully transplanted in pots and finally established to the field condition (Fig. 1, G). After 10 days of culture, callus initiation revealed at leaf margins. All PEG concentrations initiated callus cultures, while the callus weight differed according to PEG content. The effect of PEG on callus initiation from apple leaf explants is illustrated in Table (II).

Table.II: Effects of PEG concentrations on apple leaf derived callus initiation and weight.

| PEG conc. % | Callus percentage % | Callus weight (g) mean±(S.E.) |
|---|---|---|
| 0.0 | 100 | 0.45±0.10 a |
| 2.0 | 100 | 0.28±0.02 b |
| 4.0 | 100 | 0.34±0.04 b |
| 6.0 | 100 | 0.27±0.01 b |

*Numbers followed by the same letter are statistically not different at 0.05 level of probability.

As showed in the table the callus initiation was not affected by PEG concentrations, while its weight with increasing level of PEG the callus weight decreased differently. The greatest callus weight was recorded in 0.0% PEG supplied media (0.45 g) and it was significant different from all other treatments and the low callus weight was (0.27g) at 6%PEG (Figure 1, H and J). However, no significances were observed between other PEG contained media. It could be concluded that the weight of callus was gradually decreased by increasing PEG concentrations. Lowering water potential of the media decreases the cell division and callus growth [17; 18]. The quality of callus also varied according to PEG supply in the media. At the end of culture the no or low PEG contained media has a bigger, brighter, and slightly green callus, while it become yellow to brown and tough mass of cell in high PEG content media. Similar results were observed by [12], when using 2% PEG that caused severe reduction in callus weight.

[2] determined that the presence of PEG in the media diminishes a favoring water

movement into cell and if the concentration was too high, the gradient would be reversed and causes the water to leave the cell. Callus growth reduction is probably due to reduction of cytoplasm and vascular volume resulting from removal of water from cytoplasm by lowering cellular water potential [19].

## IV. Conclusion:

Statistical analysis showed that at both apple and pear shoots number increased at 4% PEG concentration; therefore, they were considered tolerant plants to water deficiency as compared with other concentrations and adding 4% PEG to the callus induction medium gave the callus the best drought tolerance than other treatments and the protocol can potentially be employed to develop drought tolerant plants *in vitro*.

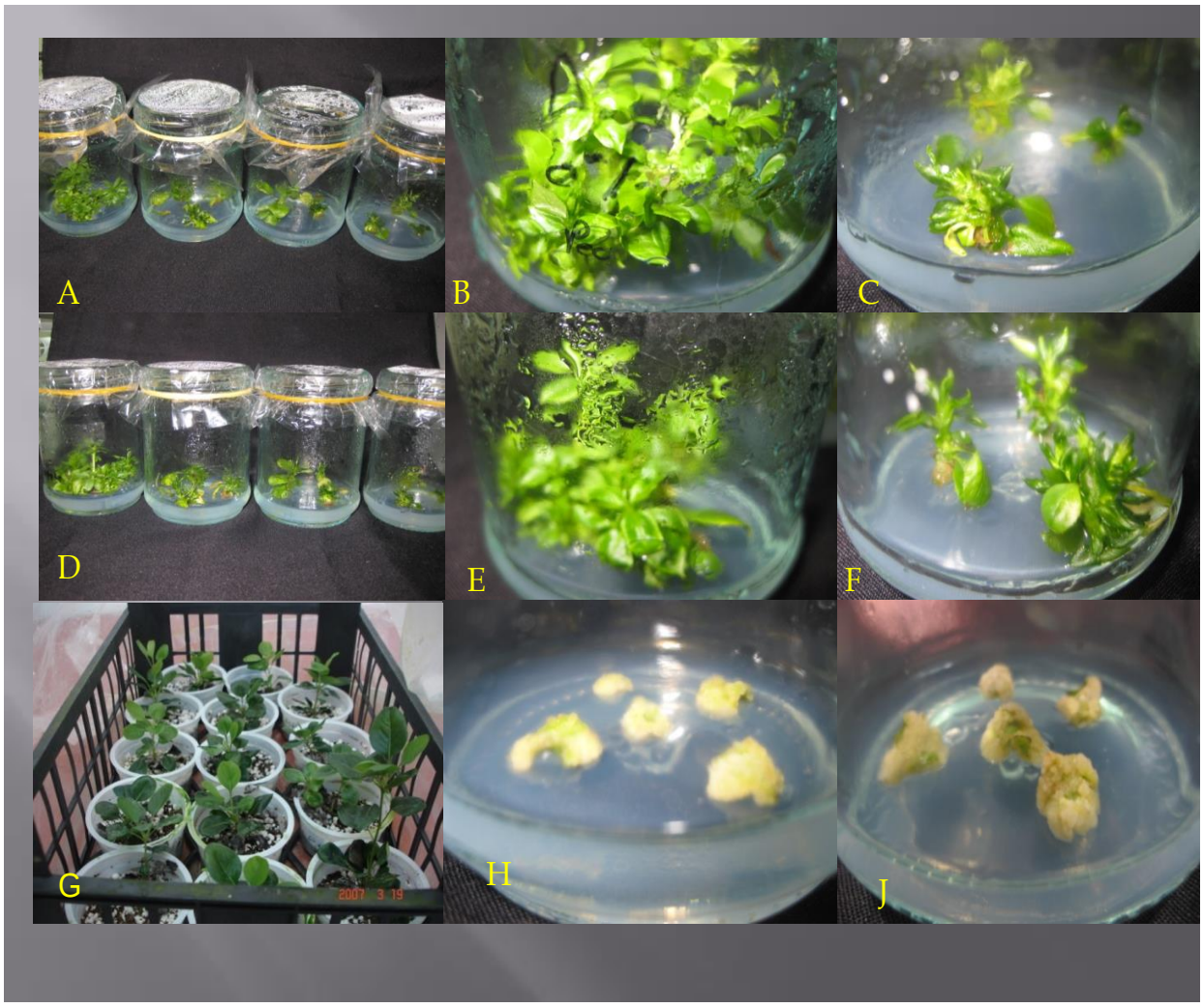


Fig.1: Effects of different PEG concentrations on micropropagation of apple and pear and callus induction of apple. A. Apple culture after 6 weeks, B. 0.0% PEG, C. 6% PEG, D. Pear cultures after 6 weeks, E. 0.0% PEG, F. 6.0% PEG, G. Acclimated plantlets , H. Callus induction after 3 weeks, J. Callus cultures after 6 weeks.

## References

[1] J.S. Boyer, 1982. "Plant Productivity and Environment", Sci., 218, PP. 443-448.

[2] M. J. Almansouri, M. Kinet and S. Lutts " Effect of Salt and Osmotic Stresses on Germination in Durum Wheat (*Triticum durum* Desf.) ", Plant Soil, 231, PP. 243-254. (2001).

[3] L. Szilgyi, " Influence of Drought on Seed Yield Components in Common Bean", Bulg. J. Plant Physiol., Special Issue, 320-330, (2003).

[4] H. Hirt and K. Shinozaki, "Plant Responses to a Biotic Stress", Springer-Verlag, Berlin Heidelberg, (2003).

[5] S. Hongbo, L. Zongsuo, S. Mingan, " Changes of Anti-Oxidative Enzymes and MDA Content Under Soil Water Deficits Among 10 Wheat (*Triticum aestivum* L.) Genotypes at Maturation Stage", *Colloids and Surfaces B: Biointerfaces*, 45, PP.7–13, (2005).

[6] M. Hamayun, S. A. Khan , Z. K. Shinwari , A. L. Khan , N. Ahmed and I. Lee " Effect of Polyethylene Glycol Induced Drought Stress on Physio-Hormonal Attributes of Soybean" .Pak. J. Bot., 42(2), PP. 977-986,(2010).

[7] R.H. Ruf, E.R. Eckard and R.O. Gifford, "Compounds of Osmotic Adjustment of Plants to Rapid Changes in Root Medium Osmotic Pressure", Soil Sci., 104, PP. 159-162, (1967).

[8] M.R. Kaufman and A.N. Eckard, "Evaluation of Water Stress Control by Polyethylene Glycols by Analysis of Guttation", Plant Physiol., 47, PP. 453-456, (1971).

[9] D.W. Lawlor, "Absorption of Polyethylene Glycols by Plants and Their Effects on Plant Growth", New Phytol., 69,PP. 501-513. (1970).

[10] N. Munir and F. Aftab, "The Role of Polyethylene Glycol (PEG) Pretreatment in Improving Sugarcane's Salt (NaCl) Tolerance", Turk. J. Bot., 33, PP. 407-415, (2009).

[11] A.M.F. Al-Ansary, A. A. Rizkalla and A.M. Badr-Elden, "Micropropagation and Biochemical Genetic Markers Detection for Drought and Salt Tolerance of Pear Rootstock" , Australian J. of Basic and Applied Sciences, 1(4), PP. 625-636, ( 2007).

[12] S. A. Yassen, "*In Vitro* Propagation of Apple *( Malus domestica* Borkh.) and Pear ( *Pyrus calleryana*) Rootstocks", M.Sc. dissertation in plant tissue culture. University of Duhok, college of Agriculture, Iraq, (2010).

[13] T. Murashige and F. Skoog, "A revised Medium for Rapid Growth and Bioassay with Tobacco Tissue Cultures", Physiol. Plant, 15, PP.473-497, (1962).
[14] R. S. Toma, "Micropropagation and *in Vitro* Shoot-Tips Grafting of Apple (*Malus domestica* Borkh.) and Pear (*Pyrus sp*.L.) ", Ph.D Dissertation in plant tissue culture. University of Duhok, College of Agriculture, Iraq, (2009).

[15] T.A. Al-Mansoori, M.N. Alaa-Eldeen and P.D.S. Caligari, "Evaluation of *in Vitro* Screening Techniques for Salt Tolerance in Date Palm", Acta Hort., 736, PP.301-307, (2007).

[16] H. Li, X. Li, D. Zhang, H. Liu and K. Guan, "Effect of Drought Stress on the Seed Germination and Early Seedling Growth of the Endemic Desert Plant *Eremosparton songoricum* (Fabaceae)" ,Excli Journal, 12, PP.89-101, (2013).

[17] A.A. Ehsanpour and R. Razavizadeh ¨ Rffect of UV-C on Drought Tolerance of Alfalfa (*Medicago sativa*) Callus", Amer. J. of Biot. 1(2), PP.107-110, (2005).

[18] I. Mahmood, A. Razzaq, M. Ashraf, I.A. Hafiz, S. Kaleem, A. Qayyum, and M. Ahmad, "*In Vitro* Selection of Tissue Culture Induced Somaclonal Variants of Wheat for Drought Tolerance", J.Agric., 50(2), PP.177-188, (2012).

[19] G. Sakthivelu, M. K. Akitha Devi, P. Giridhar, T. Rajasekaran, G. A. Ravishankar, T. Nedev and G. Kosturkova, "Drought-Induced Alterations in Growth, Osmotic Potential and in Vitro Regeneration of Soybean ", Plant physiology, 34(1-2), PP.103-112, (2008).