



IMPACT OF OSMOTIC STRESS PEG AND SUCROSE ON CALLUS GROWTH AND ADVENTITIOUS MICRO SHOOTS REGENERATION OF (*CHRYSANTHEMUM HORTORUM* HORT. CV. DWARF) IN *IN VITRO* CONDITION

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Abstract

The responses of *Chrysanthemum hortorum* Hort. cv. dwarf callus which initiated from leaf bases of different concentration of two agent, polyethylene glycol (PEG) M_{w8000} 0.0, 30.0, 40.0, 60.0 $gm L^{-1}$ and sucrose 20.0, 30.0, 40.0, 60.0 $gm L^{-1}$ with 8 $mg L^{-1}$ BA+1.0 $mg L^{-1}$ NAA to induce osmotic stress including callus growth (fresh, dry weight), water content percentage and regeneration of micro-shoots. Results of this present study revealed that increasing the water stress which was initiated by increasing PEG concentration in MS medium caused gradual decrease in all characteristics, where as, a significant increase in fresh weight, dry weight, water content percentage and regeneration of adventitious shoots were noticed in 0.0 treatment reach 7.25gm, 0.70 gm, 90.24%, 9.60 micro shoot per 100 mg callus, 3.00 cm length, 6.80 leaf per branch and 0.28 cm of width leaf respectively. Result also showed that increasing sucrose concentrations in cultured media to 60.0 $gm L^{-1}$ caused increase fresh, dry weight of callus reach 6.38 gm, 2.35 gm respectively where as inhabited the water content percentage of callus to 63.28%. this treatment also increased regeneration of adventitious micro-shoots and gave the highest shoot and leaf numbers, length of shoots and leaf width reach 10.00 micro-shoot per 100 mg callus, 8.20 leaf per branch, 4.10 cm and 0.76 cm respectively.

Keywords : Callus, PEG₈₀₀₀, Sucrose, adventitious, micro shoots, (*Chrysanthemum hortorum* Hort.).

Introduction

Drought is an environmental stress which is a major barrier to productivity of agricultural crops throughout the world. Crops exposed to this stressful environment are observed initially to have reduced growth rates (Rains, 1989). Drought severely disturbs water balance of plant body causes alterations in water uptake patterns of plant (Imran *et al.*, 2012; Waraich *et al.*, 2011). *Chrysanthemum* (*Chrysanthemum hortorum*) belongs to Compositae (Asteraceae) family of plant (Chakravarty, 1976). Which is an important cut flower and pot plant throughout the world, The main problems faced by the *Chrysanthemum* grower are the different ranges of biotic and abiotic stress (Da silva, 2003; Song *et al.*, 2014). Regarding abiotic stresses, one major issue that hampers the production And quality of the cut flowers of *Chrysanthemum* is drought, which makes water unavailable to the *Chrysanthemum* plants, this condition produces stunted plant growth, a low number of flower buds, a small flower size, a low number of fully bloomed flowers and flower sticks with a short shelf life (Li *et al.*, 2015). *In vitro* selection technique has been used to improve abiotic environmental stresses such as drought tolerance (Gawande *et al.*, 2005). Also *in vitro* technique used to obtain drought-tolerance plant assuming that there is a correlation between cellular and *in vivo* plant response (Mohamed *et al.*, 2000). For *in vitro* drought stress condition, two of the most popular approaches are used with high molecular weight osmotic substances, like poly ethylene glycol (PEG), this substance cannot cross membrane and cannot get in to the cell to change its osmotic potential (Dragiiska *et al.*, 1996), Also PEG is nontoxic osmotic substance which is used to lower potential at the culture medium and it has been used to simulate drought stress in cultured plant tissues (Muhammad *et al.*, 2010). The other osmotic substance, sugar which is not only provides the required carbon sources for plant growth,

but in its original form, it also influences the osmotic potential in the culture medium (Shajahan *et al.*, 2016). The aim of this study was to assess the changes in growth and osmotic potential of calli and *in vitro* shoot regeneration of *Chrysanthemum hortorum*. Hort. cv. dwarf Which is affected by PEG and sucrose, to induce water stress.

Materials and Methods

The study was carried out in the plant tissue culture laboratory, college of Agriculture, University of Basra southern Iraq. Callus of (*Chrysanthemum hortorum* Hort. cv. dwarf) Plant which inducted from Leaf bases, (Fig. 1- A,B,C) 100 mg of callus (16 weeks age) were cultured in test tube in the prepared MS medium (Murashag and skoog, 1962), obtained from American Coisson Labs Company. Which added of 4.33 $gm L^{-1}$ of MS salt, Thiamine-HCl, Glycine, Nicotinic acid, pyridoxine were added at a concentration of 1 $mg L^{-1}$ for each of them, 170 $mg L^{-1}$ of sodium hydrogen orthophosphate, 40 $mg L^{-1}$ adenine sulfate and added growth regulators, benzyl adenine at a concentration of 8 $mg L^{-1}$ combination with 1.0 $mg L^{-1}$ naphthalene acetic acid. In first experiment drought was simulated by addition the osmotic agent with polyethylene glycol (PEG)₈₀₀₀ at concentrations 0.0, 30.0, 40.0, 60.0 $gm L^{-1}$ and the second experiment was supplemented with sucrose at concentrations 20.0, 30.0, 40.0, 60.0 $gm L^{-1}$. Two of osmotic agent were added to the MS medium prepared for the callus growth experiment and to induce adventitious micro-shoots in the second experiment. pH of each medium was adjusted at 5.7±0.1 using HCl and NaOH at one normality concentration before adding of 6-7 $gm L^{-1}$ Agar, and then MS medium was heated to 90C° using the hotplate magnetic stirrer. About 20 ml of MS media was added to culture tube and autoclaved at pressure of 1.0 $k.g.cm^{-2}$ for 20 min at 121C°. All cultures were maintained in an incubator at 25±2C° (24h light) for callus growth, The

following callus characteristics were measured under stress conditions after 8 weeks which included.

- 1- Fresh weight of Growth callus by the following formula:

$$FWGC = W_1 - W_0 / W_0 \text{ (Chen et al., 2006)}$$

Where W_0 is the weight of callus before treatment and W_1 is the final weight of callus after 8 weeks.

- 2- Dry weight of callus (DWC):

Fresh weight of callus in all concentrations of experiments was kept at 67C ° for 24h and then weighted.

- 3- Water content percentage: was Calculated by the following formula:

$WC\% = [(FW - DW) / FW] * 100$ where FW and DW are the fresh and dry weight of callus respectively.

Parts of test tubes contain grown callus in all treatments of PEG and sucrose were incubated under light condition to initiate micro-shoots for another 8 weeks under stress condition and number of micro-shoots formed per 100 mg callus, Length of shoot (cm) number leaf/branch and width of leaf/cm were calculated.

Experimental design and statistical analysis:

Simple experiments were designed for study according to complete Randomized Design. An analysis of Variance was performed and significant difference among treatment means were Calculated by the Least significant difference (LSD) test at a probability level of 0.01 (Al-Rawi and Khalaf Allah, 2000). Each experimental treatment repeated 10 times.

Results and Discussion

Effect of osmotic stress by PEG on the characteristics of callus:

100 mg of the callus tissue was initiated from leaf bases segments of *chrysanthemum hortorum* Hort. cv. dwarf. was cultured on MS medium supplemented with different concentration of water stress by PEG 0.0, 30.0, 40.0, 60.0 gm L⁻¹, with BA 8 mg L⁻¹ and 0.2 mg L⁻¹ NAA, after eight weeks of culture under light. Results of this study table.1 indicate that increasing of PEG₈₀₀₀ concentration from 0.0 to 60.0 gm L⁻¹ in to MS medium culture caused gradually decreases in callus FW, DW and WC percentage, it means there is a significant difference between examined treatments of drought by PEG₈₀₀₀ and control in all characteristics all values, The MS medium of 0.0 gm L⁻¹ PEG₈₀₀₀ (control) gave the highest FW, DW and WC percentage 7.25 gm, 0.70 gm, 90.24% respectively, while the lowest callus values was observed in MS medium supplemented with 60.0 gm L⁻¹ PEG₈₀₀₀ which reached to 1.32 gm, 0.48 gm, 63.50% respectively (Fig. 1, D) The reduction in callus growth may be related to the presence of PEG in the growth medium which in turn had an effect on the amount of endogenous growth regulators that response for cell division and cause reduced callus growth (FW, DW) (Aliyah et al., 2017). Or might be due that cells grown under stress may have more metabolic energy than those grown in the absence of stress, the extra energy is probably used up in regulating osmotic adjustment resulting in declined callus growth (Babu et al., 2007). The effect of water stress by PEG has been widely reported (Hassan et al., 2004; Al- Taha, 2013 and Berhan et al., 2016) in sunflower, sour orange and cactus pear respectively. They reported that, callus induction decreased under higher PEG level.

Table 1: Effect of different concentrations of PEG₈₀₀₀ gm L⁻¹ on characteristics of callus *Chrysanthemum hortorum*. Hort. cv. dwarf.

| Treatment PEG ₈₀₀₀ gmL ⁻¹ | Fresh weight growth callus gm/ | Dry weight of gm/callus | Water content percentag % |
|---|--------------------------------|-------------------------|---------------------------|
| 0.0 | 7.25 | 0.70 | 90.24 |
| 30.0 | 4.14 | 0.61 | 85.31 |
| 40.0 | 2.35 | 0.58 | 73.76 |
| 60.0 | 1.32 | 0.48 | 63.50 |
| L.S.D ≤ 0.01 | 1.67 | 0.16 | 8.60 |

Effect of osmotic stress by PEG on regeneration adventitious micro-shoots

Results in table 2 showed that calli regenerating micro shoots revealed a highly significant variation among the tested between different level of PEG₈₀₀₀ when added at concentrations 0.0, 30.0, 40.0 and 60.0 gm L⁻¹ in MS medium which started after 8 weeks of callus experiments.

At control (0.0 PEG₈₀₀₀) the highest value of numbers of micro-shoot per 100 mg callus, Length of micro-shoots per cm, number of leaves per branch and leaf width. Per cm were recorded 9.60 micro-shoot per 100 mg callus, 3.00 cm, 6.80 leaves per branch, 0.28 cm respectively, This treatment showed highly significant values as compared with other levels of PEG₈₀₀₀ which were lowest regenerated micro-shoots gradually when increased PEG₈₀₀₀ in MS medium from 30.0 to 60.0 gm L⁻¹ which reached 6.40 /per 100mg callus, 2.04cm, 5.40 /branch, 0.22 cm and 2.60 per 100 mg

callus, 0.22 cm, 2.40 branch, 0.10 cm respectively (Fig. 1-E).

The typical decrease in plant regeneration in callus cells of crop plants in response to water stress is due to water short age in the cells which leads to a decrease in cell turgor and eventually cell growth, In addition PEG in the medium causes cell dehydration by reducing water availability to cells, which leads to a loss of cell turgor and tience a loss of growth (Heyser and Nabors, 1981). Similar this findings in (Wani et al., 2010; Begum et al., 2011 and Mengesha et al., 2016) who reported that an increased PEG stress level in MS medium caused reduction in plant regeneration percent in Rice, Sugarcane, and Cactus pear respectively and also Kulkarui and Deshpande, 2007 reported that decrease in shoot length at tomato has been observed with increasing PEG concentrations.

Table 2: Effect of different concentrations of PEG₈₀₀₀ gm L⁻¹ on adventitious shoot formation from callus of *Chrysanthemum hortorum*. Hort. cv. dwarf.

| Treatment PEG ₈₀₀₀ gmL ⁻¹ | Numbers of micro-shoot per100 mg callus | Length of micro-shoots cm/ | Number of leaf branch/ | Width of the cm /leaf |
|---|---|----------------------------|------------------------|-----------------------|
| 0.0 | 9.60 | 3.00 | 6.80 | 0.28 |
| 30.0 | 6.40 | 2.04 | 5.40 | 0.22 |
| 40.0 | 4.80 | 0.38 | 3.60 | 0.14 |
| 60.0 | 2.60 | 0.22 | 2.40 | 0.10 |
| L.S.D≤0.01 | 1.34 | 0.66 | 1.17 | 0.10 |

Effect of osmotic stress by sucrose on characteristics of callus

The results of variance analysis related to effect of different level of sucrose 20.0, 30.0, 40.0, 60.0 gm L⁻¹ on FW, DW and WC percentage callus were shown in Table 3. A significant callus growth in all values characteristics was observed when the concentration of sucrose in MS medium was increased from 20.0 gm L⁻¹ to 60.0 gm L⁻¹, The highest FW, DW and WC percentage of callus was obtained in MS medium supplemented with 60.0 gm L⁻¹ sucrose and this level was significant as compared with the other treatments which reached to 6.38 gm, 2.35 gm, 63.28% respectively, but the lowest values of callus and water content percentage were observed in MS medium containing a least amount of sucrose

(20.0 gm L⁻¹) which reached to 3.71 gm, 0.33 gm, 90.98% respectively (Fig. 1- F). Carbohydrate concentration have been found to play important roles in different stages at the tissue culture processes, because plant cell, tissue or organ culture normally requires a Carbohydrate supply in order to satisfy energy demands (Amiri and Kazemitabar, 2011). Similar to this finding results were obtained by another worker (Al-Khayri and Al-Bahrany, 2001; Mandal and Datta, 2005; Abdul mujib *et al.*, 2009; Ibrahim *et al.*, 2012), on plants (*Citrus aurantifolia*, *Chrysanthemum morifolium*, *Digitalis lanata*, *Citrus sinensis* L. Osbeck) respectively they reported that callus growth when concentration of sucrose in MS medium was increased.

Table 3: Effect of different concentrations of sucrose gm L⁻¹ on characteristics of callus *Chrysanthemum hortorum*. Hort. cv. dwarf.

| Treatment Sucrose gm L ⁻¹ | Fresh weight growth callus gm/ | Dry weight of gm /callus | Water content percentag % |
|--------------------------------------|--------------------------------|--------------------------|---------------------------|
| 20.0 | 3.71 | 0.33 | 90.98 |
| 30.0 | 4.46 | 0.76 | 83.27 |
| 40.0 | 5.37 | 1.29 | 75.90 |
| 60.0 | 6.38 | 2.35 | 63.28 |
| L.S.D≤0.01 | 1.16 | 0.36 | 2.77 |

Effect of osmotic stress by Sucrose on regeneration adventitious micro shoots:-

Results in table 4 showed the effects of MS medium supplemented with vary levels of sucrose 20.0, 30.0, 40.0, 60.0 gm L⁻¹ which started after 8 weeks of callus experiment. A many new adventitious micro-shoots were regenerated at the upper and around of the callus in all treatments of osmotic stress by Sucrose (Fig.1- G, H, I, J). However, a significant difference increases in number of indirect adventitious micro-shoots per 100 mg of callus, length of micro shoots, number of leaf/branch and leaf width were observed in MS medium supplemented with 60.0 gm L⁻¹ sucrose 10.00 micro-shoot per100 mg callus, 4.10 cm, 8.20 per branch, 0.76 cm respectively. The lowest average in new shoots per 100 mg callus, length of micro shoot and number of leaves per branch were found in medium supplemented with low level of sucrose 20.0 gm L⁻¹ which reached to 1.60 micro-shoot per100 mg callus, 0.28cm, 2.60 per branch, 0.16 cm respectively (Fig.1- K). It means that the regeneration of

adventitious micro shoot per 100 mg callus and the other characteristics were increased due to increase in the level of osmotic stress by Sucrose in MS medium. Sucrose is widely used as a standard carbon source for plant tissue culture, In living plant cells, carbohydrates are necessary as a source of energy and a carbon substrate for biosynthesis. A continuous supply of carbohydrates to plants cultured *in vitro* is essential, since photosynthetic activity of *in vitro* growth tissue is usually reduced. These compounds are also necessary in media to influences the osmotic potential, For all these reasons, sugar have great potential effect on the physiology, growth and differentiation of cell (Gibson, 2000). This study is in agreement with Amutha *et al.*, (2003) who reported that sucrose evoked maximum regeneration frequency and increased the number of regenerating shoots, Nowak *et al.* (2004) reported that sucrose was a better carbon than glucose for organogenesis of *Wegierka zwykla*. Jala (2012) reported that 6% sugar gave the highest average new shoots and leaves per bunch of curcuma plant.

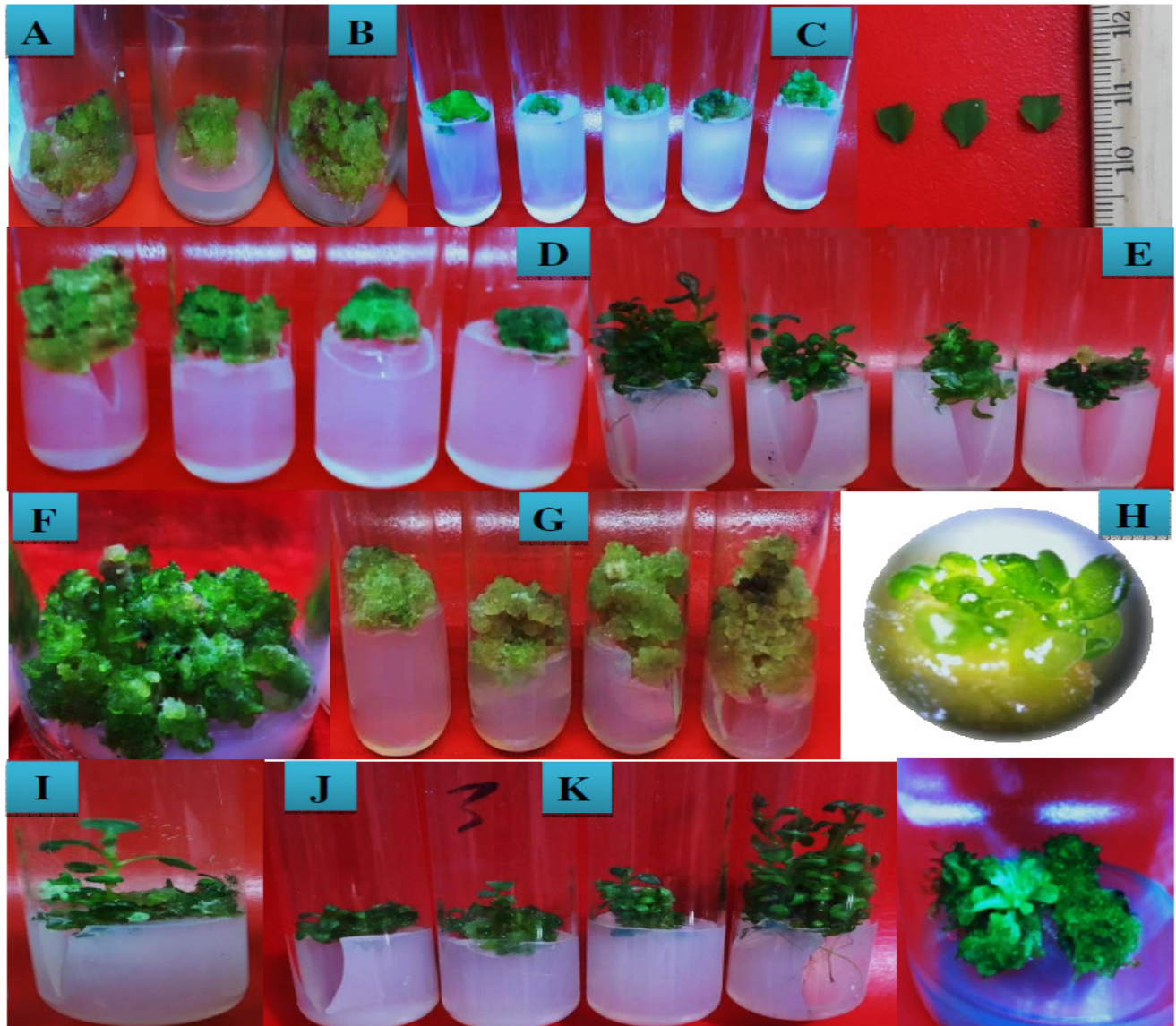
Table 4: Effect of different concentrations of sucrose gm L^{-1} on adventitious shoot formation from callus of *Chrysanthemum hortorum*. Hort. cv. dwarf.

| Treatment Sucrose gm L^{-1} | Numbers of micro-shoot per100 mg callus | Length of micro-shoots cm/ | Number of Leaves branch/ | Width of the cm /leaf |
|--------------------------------------|---|----------------------------|--------------------------|-----------------------|
| 20.0 | 1.60 | 0.28 | 2.60 | 0.16 |
| 30.0 | 2.40 | 0.94 | 3.80 | 0.26 |
| 40.0 | 5.00 | 1.20 | 5.00 | 0.44 |
| 60.0 | 10.00 | 4.10 | 8.20 | 0.76 |
| L.S.D \leq 0.01 | 1.17 | 0.60 | 1.37 | 0.10 |

Conclusions

This study has characterized the responses of callus which induced from base of leaf and regeneration adventitious micro-shoots of *Chrysanthemum hortorum* Hort. cv. dwarf to osmotic stress by PEG₈₀₀₀ and Sucrose. Results

showed that increasing the PEG₈₀₀₀ in MS media, inhibited FW, DW, WC percentage and regenerated adventitious micro-shoot, where as increasing Sucrose in MS media encourage FW, DW, while inhibited WC percentage of callus and increased regenerated of adventitious micro-shoots.

**(Fig. 1) Indirect organogenesis under osmotic stress.**

(A) base of leaf segment 0.4-0.6 cm (B) Initiated callus from base of segment on MS medium+BA at 0.0, 5.0, 6.0, 7.0, 0.8 mg L^{-1} +1.0 mg L^{-1} NAA after 8 weeks. (C) Reculture of callus on MS medium with 8 mg L^{-1} BA +1.0 mg L^{-1} NAA after 8 weeks (D) callus and (E) adventitious shoot grown on MS medium with PEG at 0.0, 30.0, 40.0, 60.0 gm L^{-1} . (F) callus grown on MS medium with sucrose at 20.0, 30.0, 40.0, 60.0 gm L^{-1} . (G) initiated small micro-shoot from callus on MS with 60.0 gm L^{-1} sucrose (microscope picture). (H, I, J) micro-shoots grown on MS + 60.0 gm L^{-1} . (K) adventitious shoots grown on MS + sucrose at 20.0, 30.0, 40.0, 60.0 gm L^{-1} after 8 weeks.

Abbreviations:

BA: 6-Benzyl adenine

NAA: α -Naphthalene acetic acid

MS: Murashige and Skoog salts

References

- Abdul Mujib, Z.F.; Fatima, S.; ARshi, A. and Vmar, S. (2009). Callus induction, biomass growth, and plant regeneration in *Digitalis lanata* Ehrh. Influence of plant growth regulators and carbohydrates. *Turk J. Bot.*, 33: 393-405.
- Aliyah, A.H.; El-Kaaby, E.A.; Mouhamad, R.S.; Mohammad, R.H. and Al-Auny, J.A. (2017). *In vitro* influence of drought on some physiological parameters in callus induced from seeds of four Rice (*Oryza sativa* L.). *International Journal of Multidisciplinary and Current Research*. Vol 5.
- Al-Khayri, J.M. and Al-BAhrany, A.M. (2001). *In vitro* micropropagation of *Citrus aurantifolia* lime. *Current Sci.*, 8: 1242-1246.
- Al-Rawi, K.M. and Khalaf Allah, A.M. (2000). Design and analysis of agricultural experiments. Dar Alkuteb for Press and Publishing, Mosul University, Iraq, 488 p.
- Al-Taha, H.A.K. (2013). Effect of shock and gradual drought by PEG callus growth and Proline accumulation in sour orange (*Citrus x aurantium*). *Advance in Agriculture & Botanic*. *Int. J. Bioflux Society*, 5(2): 77-83.
- Amiri, S. and Kazemitabar, S.K. (2011). Enhancement of callus induction and regeneration from embryo cultures of *Datura stramonium* by adjusting carbon sources and concentrations. *African Journal of Biotechnology*, 10(50): 10101-10107.
- Amutha, S.; Ganapathi, A. and Muruganantham, M. (2003). *In vitro* organogenesis and plant formation in *Vigna radiata* (L.) Wilczek. *Plant. Cell. Tissue Organ. Culture*, 72: 203-207.
- Babu, S.; Sheeba, A.; Yogameenakshi, P.; Anbumalarmathi, J. and Rangasamy, P. (2007). Effect of salt stress in the selection of salt tolerant hybrids in rice (*Oryza sativa* L.) under *in vitro* and *in vivo* condition. *Asian J. of Plant Sci.*, 6: 137-142.
- Begum, M.K.; Islam, M.O. Miah, M.A.S.; Hossain, M.A. and Islam, N. (2011). Production of somaclone *in vitro* for drought stress tolerant plantlet selection in sugarcane (*Saccharum officinarum* L.). *The Agric., Sci. J. of Krishi Foundation*, 9(1&2): 18-28.
- Chakravarty, H.L. (1976). *Plant wealth of Iraq*. (Dictionary of Economic Plants). Vol.one. Acharya prafulla Chandra road. Calcutta. printed in India. Ministry of Agriculture & Agrarian Reform. Iraq. pp: 121-127.
- Chen, J.J.; Yue, R.Q.; Xu, H.X. and Chen, X.J. (2006). Study on plant regeneration of Wheat mature embryos under endosperm supported culture. *Agric Sci China*, 5(8): 572-578.
- Da Silva, J.A.T. (2003). Chrysanthemum: Advances in tissue culture, cryopreservation, postharvest technology, genetics and transgenic biotechnology. *Biotechnol. Adv.*, 21: 715-766.
- Dragiiska, R.; Djilianov, D.; Denchev, P. and Atanassov, A. (1996). *In vitro* selection for osmotic tolerance in alfalfa. (*Medicago sativa*). *Bulg. J Plant Physiol.*, 22: 30-39.
- Gawande, N.D.; Mahurkar, D.G.T.; Rathod, H.; Jahagidar, S.W. and Shinde, M. (2005). *In vitro* screening of wheat genotypes for drought tolerance. *Annals Plant Physiol.*, 19: 162-168.
- Gibson, S. (2000). Plant sugar-response pathways. Part of a complex regulatory web. *Plant Physiol.* 124: 1532-1539.
- Hassan, N.S.; Shaaban, L.D.; Hashem, E.S.A. and Seleem, E.E. (2004). *In vitro* selection for water stress tolerant callus line of *Helianthus annuus* L. cv. myak. *Int. J. Agric. Biol.*, 6(1): 13-18.
- Heyser, J.W. and Nabors, M.W. (1981). Growth Water Content, and Solute Accumulation of Two Tobacco Cell Lines Cultured on Sodium Chloride, Dextran, and Polyethylene Glycol. *Plant Physiology*, 68: 1454-1459.
- Ibrahim, K.M.; Khalil, T.H. and Al-Hamyari, M.A. (2012). Effect of different concentrations of BA and Sucrose on callus induction and initiation of asexual embryos in Orange (*Citrus sinensis* L. Osbeck) nucellar seed tissues *in vitro*. *Iraqi Journal of Biotechnology*, 11(2): 196-208.
- Imran, M.; Abdul, R.; Ishfaq, A.H.; Shuaib, K.; Ahmad, A.K.; Abdul, Q. and Muhammad, A. (2012). Interaction of Callus Selection Media and Stress Duration for *in vitro* Selection of Drought Tolerant Callus of Wheat. *African Journal of Biotechnology*, 11: 4000-4006.
- Jala, A. (2012). Effects of NAA BA and Sucrose On Shoot Induction and Rapid Micropropagation by Trimming Shoot Of *Curcuma Longa* L. *Int. Trans. J. Eng. Manag. Sci. & Tech.*, 3(2): 101-109.
- Kulkarni, M. and Deshpande, U. (2007). *In Vitro* Screening of Tomato Genotypes for Drought Resistance Using Polyethylene glycol. *African Journal of Biotechnology*, 6: 691-696.
- Li, P.; Song, A.; Gao, C.; Wang, L.; Wang, Y.; Sun, J.; Jiang, J.; Chen, F. and Chen, S. (2015). Chrysanthemum WRKY gene CmWRKY17 negatively regulates salt stress tolerance in transgenic chrysanthemum and *Arabidopsis* plants. *Plant Cell Rep.*, 34: 1365-1378.
- Mandal A.K.A. and Datta, S.K. (2005). Direct somatic embryogenesis and plant regeneration from ray florets of chrysanthemum. *Floriculture Section, National Botanical Research Institute, Lucknow-26001, U.P., India, Biologia Plant Tarum*, 49(1): 29-33.
- Mengesha, B.; Mekbib, F. and Abraha, E. (2016). *In Vitro* Screening of Cactus [*Opuntia ficus-indicia* (L.) Mill] Genotypes for Drought Tolerance. *American Journal of Plant Sciences*, 7: 1741-1758.
- Mohamed, M.A.H.; Harris, P.J.C. and Henderson, J. (2000). *In vitro* Selection and Characterization of a Drought Tolerant Clone of *Tagetes minuta*. *Plant Science*, 159: 213-222.
- Muhammad, H.; Khan, S.A.; Shinwari, Z.K.; Khan, A.L.; Ahmad, N. and Lee, J. (2010). Effect of Polyethylene Glycol Induced Drought Stress on Physio-Hormonal Attributes of Soybean. *Pakistan Journal of Botany*, 42: 977-986.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, 15: 473-492.
- Nowak, B.; Miczyński, K. and Hudy, L. (2004). Sugar uptake and utilization during adventitious bud differentiation on *in vitro* leaf explants of 'Węgierka Zwyczajka' plum (*Prunus domestica*). *Plant Cell Tissue Organ. Culture*, 76: 255-260.
- Rains, D.W. (1989). Plant tissue and protoplast culture: applications to stress physiology and biochemistry. In : plant under stress. Hamlyn C.J.T.J. Flowers and M. B.

- Jones (ed): 181-96. Cambridge Univ. Press, New York, Port Chester, Melbourne, Sydney.
- Shajahan, A.; Rajo, C.S.; Thilip, C.; Varutharaju, K.; Faizal, K.; Mehaboob, V.M. and Aslam, A. (2016). Direct and Indirect Somatic Embryogenesis in Mango Ginger (*Curcuma amada* Roxb.). Plant Molecular Biology Laboratory, Department of Botany, Jamal Mohamed College Tiruchirappalli 620020, India, Springer International Publishing Switzerland, Fundamental Aspects and Applications, DOI 10.1007/978-3-319-33705-0-20, 367-379.
- Song, A.; An, J.; Guan, Z.; Jiang, J.; Chen, F.; Lou, W.; Fang, W.; Liu, Z. and Chen, S. (2014). The constitutive expression of a two transgene construct enhances the abiotic stress tolerance of chrysanthemum. Plant Physiol. Biochem., 80: 114–120.
- Wani, S.H.; Sofi, P.A.; Gosal, S.S. and Singh, N.B. (2010). *In Vitro* Screening of Rice (*Oryza sativa* L.) Callus for Drought Tolerance. Communications in Biometry and Crop Science, 5: 108-115.
- Waraich, E.A.; Ahmad, R.; Ashraf, M.Y. and Saifullah, E.H. (2011). Role of Mineral Nutrition in Alleviation of Drought Stress in Plants. Australian Journal of Crop Science, 5: 764-777.