IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 4 Issue 8, August 2017
ISSN (Online) 2348 – 7968 | Impact Factor (2016) – 5.264
www.ijiset.com

Effect of Drought and Salinity Stresses on Two Strawberry Cultivars during Their Regeneration *in Vitro*

Esam A. Hussein [1], Ahmed Y. El-Kerdany [2] and Mohamed K. Afifi [1]
[(1)] Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.
[(2)] Agricultural research center, Ministry of Agriculture, Giza, Egypt.
muhammed_saber22@yahoo.com

Abstract

In the present study, the runners of *Fragaria ananasa* cultivars Fortuna and Festival were surface sterilized. The apical meristems were isolated and cultured on hormone free Murashige and Skoog's culture media for 3 weeks to allow proliferation of shoot buds. The masses of shoot buds were transferred to MS culture media supplemented with 1.0 mg/L benzyl adenine and poly ethylene glycol (0.0, 0.5, 1.0, 1.5 and 2%) to study the effect of drought stress while for induction of salt stress the culture media were supplemented with sodium chloride (0.0, 500, 750 and 1000 mg/L). The treatments lasted for 3 weeks. Results of the present with Fortuna cultivar showed that drought stress reduced chlorophyll content from 3.20 to 0.57 mg/g; shoot length from 5.33 cm to1.50 cm; root length from 3.30 to 1.60 cm; and the number of leaves per explant from 4.33 to 1.33. On the other hand, the most stressful salt treatment (1000 mg/l NaCl) reduced the chlorophyll content from15.33to 10.13mg/g; the shoot length from 5.40 to 2.10 cm; the shoot dry biomass from 3.87 to 1.22 mg; the root length from 3.40 to 2.13 cm; the root dry biomass from 2.51 to 0.96 mg. The contents of proline, catalase and peroxidase increased from 0.53, 1.86 and 20.42 in the control plants to 3.95, 4.75and 20.42 µg/g in the stressed regenerants, respectively. The results obtained showed that the cultivar Festival exhibited almost the same behavior of Fortuna in response to both water and salt stress. The obtained results may help in assessing programs for selection of drought and salinity tolerant strawberry plants in future using *in vitro* techniques.

Keywords: *Fragaria ananasa*, Regeneration, Drought stress, Water stress, *In Vitro*.

Introduction

In vitro culture of plants, beside its beneficial role in micropropgation and improvement of plants, is regarded as a modern and effective tool for studying plant physiology away from the many interfering factors in the field (**Peak *et al*.,1988)**. Drought and salt stress are among the main problems restricting crop production in Egypt. Strawberry is cultivated all around the world, not only for its digestive and tonic properties, but because of the nutritional value of its fruits, important source of folate, vitamin C, fiber, potassium, flavonoids, autocianidin, phytochemicals and antioxidants (Nutritional values for 100 grams edible portion of strawberry according to **(USDA 2003)** is: Protein 0.61g, Sodium 1mg, Fat 0.37mg, Zinc 0.13mg, fiber 2.3g, Copper 0.049 mg, carbohydrates 7.02g, manganese 0.29mg, Calcium 14mg, Selenium

0.7 µg, Iron 0.38g, vitamin B-6 0.059mg, Magnesium 10mg, folate 17.7µg, Phosphorus 19mg, vitamin A, IU3µg and Potassium 166mg. according to **Seeram (2006)**, the anticancer effects of individual phytochemical constituents of strawberries, as well as whole strawberry extracts, have been demonstrated **.** These anticancer effects are exerted through multi-mechanistic means of action including the antioxidant actions of the berry's phenolic constituents by protecting DNA from damage, and also through effects exerted beyond antioxidation**.** The same author mentioned also that the biological activities of strawberry phytochemicals include the regulation of phase-II enzymes and the modulation of gene expression and subcellular signaling pathways of cell proliferation, angiogenesis and apoptosis (programmed cell death). Although there have been many published reports on the anticancer effects of individual phenolics known to be present in the strawberry fruit**.**

Water stress causes serious reduction in growth, quantity, and quality in many plants (**Pérez-Pérez *et al.*, 2008; , Tuna *et al.*, 2010**). It frequently occurs in both intensive fruit orchards and nurseries in many parts of the world. This situation directed researchers to make further investigations to reduce severe effects of water stress on different plant species. Therefore, new approaches including effective use of water, selection of drought resistant species, cultivars, and rootstocks have been considered to reduce the effects of water stress.

Salt stress is one of the most serious limiting factors for crop growth and production in the arid regions. About 23% of the world's cultivated lands is saline and 37% is sodic **(Khan and Duke, 2001).** Soils can be saline due to geo-historical processes or they can be man-made. The water and salt balance, just like in oceans and seas determine the formation of salty soils, where more salt comes in than goes out. Here, the incoming water from the land brings salts that remain because there is no outlet and the evaporation water does not contain salts. In semiarid and arid regions particularly during spring and summer months, the evaporative demand for the atmosphere results in significant drought stress in many crop plants, which is one of the most severe environmental stresses and affects almost all plant functions.

Materials and Methods

1- Plant materials

The mother plant of strawberry cultivars was grown in the Agricultural Research Center, Ministry of Agriculture, Egypt, used as a source of explants during this study. Runner tips of 1-2 cm long were taken from mother plants as that long was suitable for sterilization procedures.

Explants were rinsed under running tap water for 30 minutes. For surface sterilization, explants were immersed in different concentrations (5, 10, 15, 20 %) of sodium hypochlorite solution for 15 minutes. The plant materials were then, rinsed three times with distilled water. After sterilization, explants were shortened to remove the surfaces of explants and meristems of 3-5mm long were isolated as final explants.

2- Culture media

MS-hormone free the basal salts mixture of MS medium **(Murashige and Skoog, 1962)** was used for establishment experiments. The pH was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl. The media were solidified using agar at 7.5 g/L.

The prepared media were dispended at rate of 25 ml into 200 ml glass jars and covered with polypropylene lids and also glass tubes for shoot multiplication, rooting, and secondary metabolites production stages.

The media were then autoclaved at 1.5 kg/cm2 and 121 C^o for17 min then left to cool and harden for 24h before being used. The culture media was fortified with 1 mg/l BA for shoot bud proliferation.

2.1. Treatments used to study the effect of drought stress

Murashige and Skoog's medium supplemented with 1 mg\l BA with different concentrations of PEG (0.0, 0.5, 1.0, 1.5 and 2.0% (w/v) was prepared and poured in jars , each jar contained 35 ml of the described nutrient medium and plugged with polypropylene cap. All jars were incubated in growth room at 27 ±1°C under 16 hours daily exposure to low light intensity 1500 lux illumination.

2.2. Treatments used to study the effect of salinity stress

Murashige and Skoog (1962) basal medium supplemented with BA 1 mg\l and different concentrations of NaCl at(0.0, 500, 750,1000 mg/l were prepared and poured into jars , each jar contained 35 ml of the described nutrient medium and plugged with polypropylene cap. All jars were incubated in growth room at 27 ±1°C under 16 hours daily exposure to low light intensity 1500 lux illumination.

3- Analytical methods

Determination of chlorophyll:

The method used for the quantitative determination of chlorophyll was that of **(Vernon and Selly, 1966).** In such method, one gram aliquot of fresh leaves was cut into small pieces. The pigments were extracted by grinding the cut tissue with suitable amount of glass powder in mortar using 100 ml of 80% aqueous acetone (v/v). The homogenate was transferred quantitatively to a Buchner filter with Whatman No.1 filter paper. The filtrate was transferred quantitatively to 100 ml volumetric flask and made up to a total volume of 100 ml using 80% acetone.

The optical density of the plant extract was measured using spectrophotometer of two wave lengths (649 and 665 nm). These are positions in the spectrum where maximum absorption by chlorophyll (a) and (b) occurs. The concentrations of chlorophyll (a), (b) and total chlorophyll in plant tissue were calculated using the equations mentioned by **(Vernon and Selly, 1966)**.

Mg chlorophyll (a) / g tissue = 11.63(A665) – 2.39(A649).
Mg chlorophyll (b) / g tissue = 20.11(A649) – 5.18(A665).
Mg chlorophyll (a + b) / g tissue = 6.45 (A665) +17.72(A649).

Quantitative determination of free proline:

Contents of free proline were determined according to the method described by **(Bates *et al*., 1973).** In such method, 0.5 gm of fresh plants material was homogenized in 10 ml (3%) sulfosalicylic acid, the homogenate filtered through Whatman No.2 filter paper. Two mls of filtrate was reacted with two mls acidic ninhydrin (Prepared by warming 1.25 gm ninhydrine in 30 mls glacial acetic acid and 20 mls 6M phosphoric acid, with agitation, until dissolved, then kept cool) and two mls of glacial acetic acid in tet tube for one hour in a boiling water bath, then the reaction terminated in an ice bath. The reaction mixture was extracted with 4 mls toluene, mixed vigorously by attest tube stirrer for 15-20 sec. the chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read to 520 nm using UV- spectrophotometer (Unico 2000). Using toluene for a blank using a standard

curve of pure proline, the proline concentration was determined from a standered curve and calculated on a dry weight basis.
Proline content mg/g = x ppm x ml extract volume/ 2xsample dry weight x 100

Assay of catalase (CAT) and peroxidese (POX):

Extraction:

The plant materials used for estimation of catalase (CAT) and peroxidase (POX) enzymes were the first and second young leaves. In this regard, 2 g of the plant materials were homogenized with 10 ml of phosphate buffer pH 6.8 (0.1 M), then centrifuge at 2^{o}C for 20 min at 20000 rpm in a refrigerated centrifuge. The clear supernatant (containing the enzymes) was taken as the enzymes source **(MuKherjee and Choudhuri, 1983)**.

Determination:

Catalase (CAT) activity

Catalase activity was assayed according to the method of **(Chen *et al.*, 2000)**. The reaction mixture with final volume of 10 ml containing 40 µl enzyme extract was added to 9.96 ml H_2O_2 phosphate buffer pH 7.0 (0.16 ml of 30% H_2O_2 to 100 ml of 50 mM phosphate buffer). Catalase activity was determined by measuring the rate change of H_2O_2 absorbance in 60 second with a UV- spectrophotometer (Labomed, inc.23) at 250 nm. The blank sample was made by using buffer instead of enzyme extract. One unit of enzyme activity was defined as the amount of the enzyme that reduced 50% of the H_2O_2 in 60 second at 25^{0}C **(Kong *et al.*, 1998)**.

Peroxidase (POX) activity

Peroxidase activity was assayed using solution containing 5.8 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of the enzyme extract and 2 ml of 20 mM H_2O_2 after addition of 2 ml of 20 mM pyrogallol, the rate of increase in absorbance as pyrogallol was determined spectrophotometrically by UV- spectrophotometer (Labomed, inc.23) within 60 second at 470 nm and 25^{0}C **(Bergmeyer, 1974)**. One unit of enzyme activity was defined as the amount of the enzyme that catalyzed the conversion of one micromole of H_2O_2 per minute at 25^{0}C **(Kong *et al.*, 1998)**. The blank sample was made by using buffer instead of enzyme extract.

4- Statistical analysis

All of the experiments were arranged as a factorial experiment in a completely randomized design (2 cultivars X 4 treatments X 3 replicates). Duncan multiple range test at 5% level was used in the studied experiment to verify the differences between means of the treatments **(Snedecor and Cochran,1982) .**

Results

With respect to the water stress effects exerted by the application of effect of poly ethylene glycol on the two cultivars of strawberry adopted in this study, the results illustrated in **table (1)** may show that there was a general decrease in total chlorophyll contents, root length, shoot length, and the number of leaves with a concomitant increase in the amounts of proline determined. With respect to the cultivar Fortuna, the minimum amount of total chlorophyll (0.57 mg/g fresh weight of leaves) resulted from the treatment with 2% of PEG which is considered a significant decrease in comparison to the corresponding control (3.2 mg/g fresh leaf tissues). As regards to the cultivar Festival, the minimum amount of total chlorophyll (0.47 mg/g fresh weight of leaves) resulted from the treatment with 2% of PEG which is considered a significant decrease in comparison to the corresponding control (2.63 mg/g fresh leaf tissues).

In both cases there was an inverse correlation between the concentration of PEG and total chlorophyll content.

The shoot length of cultivar Fortuna decreased by almost half it length in response to the treatment with 0.5% PEG. The increase in PEG concentration was accompanied by a general decrease in shoot length. This general trend was observed also with cultivar Festival where the minimum shoot length (1.67 cm) was recorded in response to the treatment with 2% PEG.

With respect to root length, it has been observed that the root length decreased from 3.3 cm in control Fortuna plants to 1.6 in response to the treatment with 2% PEG. With respect to cultivar Festival, the root length decrease from 2.9 cm in control plants to 1.83 cm in response to the same concentration of PEG.

The number of leaves (in the best case) of Fortuna regenrants reached up 3.67 in response to the treatment with 0.5% of PEG which is also regarded as a significant decrease in comparison to the control plants, a result that indicates the deteriorative effect of water stress on straw berry and the results with cultivar Festival were almost similar to the results obtained with cultivar Fortuna.

In contrast to the above mentioned trend of results, the proline contents general increased in response to the increase in the PEG concentration. For example, in Fortuna cultivar, the amount of proline increased from 1.32 µg/g (in the control plants) to 3.65 µg/g in the stressed regenerants (2% PEG). Also, (in Festival cultivar), the amount of proline in the tested tissues increased from 1.75 µg/g in control plants to 3.9 µg/g in response to the most stressful PEG concentration.

An overview of table (2) may show that the salt stress created by incorporation of sodium chloride into the MS regeneration culture media had adversely affected the total chlorophyll content, shoot length, dry weight of shoot, root length, dry weight of root and the leaf number per regenerant too while in the great majority the amounts of proline, catalase and peroxidase activities increased with the increase in the severity of salt stress. This was observed in the two cultivars investigated.

As regards to the cultivar Fortuna and in comparison to the corresponding control, the total chlorophyll content decreased from 15.33 mg/g to 10.13 mg/g; the shoot length decreased from 5.40cm to 2.10 cm; the shoot dry biomass decreased from 3.87 mg to 1.22 mg; the root length decreased from 3.40 cm to 2.13 cm and the root dry biomass decreased from 2.51 mg to 0.96 mg and the leaf number decreased from 5.67 per regenerant to 2.0 leaves per regenerant in response to the treatment with 1000 mg/l sodium chloride. On the other hand, the results illustrated in table (2) indicate that the stressed regenerants had responded to the salt stress by elevating tissue contents of proline, catalase and peroxidase. For example, the proline content increased from 0.53 mg/g in the control plants to about 4 folds in the regenerants treated with 1000 mg/l NaCl. The amount of catalase and peroxidase increased from 1.86 µg/g and 20.42 µg/g to 4.75 and 24.51 µg/g in response to the treatment with 1000 mg/l NaCl respectively.

In the control regenerants of the cultivar Festival, the following values were recorded (total chlorophyll reached up 11.63 mg/g; shoot length 4.17 cm; shoot dry biomass 1.28 mg; root length 3.07 cm; root dry weight 0.98 mg; leaf number per regenerant 4.67; proline content 3.31 mg/g; catalase 1.72 µg/g and the peroxidase 19.81 µg/g.) while the most stressful sodium chloride treatment (1000 mg/l) resulted in the following values (total chlorophyll reached up 8.13 mg/g; shoot length 2.0 cm; shoot dry biomass 0.98 mg; root length 1.87 cm; root dry weight 0.27 mg; leaf number per regenerant 2.0; proline content 7.81 mg/g; catalase 4.31 µg/g and the peroxidase 21.27 µg/g.). The results indicate that salt stress activated proline

accumulation and catalase and peroxidase activity and adversely affected morphology and growth of the regenerants.

Table (1): Effect of treatment of strawberry with PEG

Cultivar	Conc. of PEG	Total chlorophyll	Shoot length (cm)	root Length (cm)	No. of leaves	Proline
Fortuna	0.0%	3.20 ^{a}	5.33 ^{a}	3.30 ^{a}	4.33 ^{a}	1.32 ^{j}
	0.5%	2.40 ^{c}	2.50 ^{d}	2.40 ^{e}	3.67 ^{b}	2.05 ^{h}
	1.0%	1.33 ^{f}	2.03 ^{e}	2.13 ^{f}	2.67 ^{cd}	2.56 ^{f}
	1.5%	0.77 ^{h}	1.73 ^{f}	1.93 ^{h}	2.33 ^{d}	3.25 ^{d}
	2.0%	0.57 ^{i}	1.50 ^{g}	1.60 ^{j}	1.33 ^{e}	3.56 ^{c}
Festival	0.0%	2.63 ^{b}	4.20 ^{b}	2.90 ^{b}	4.67 ^{a}	1.75 ^{i}
	0.5%	1.77 ^{d}	2.80 ^{c}	2.67 ^{c}	3.33 ^{bc}	2.34 ^{g}
	1.0%	1.50 ^{e}	2.47 ^{d}	2.47 ^{d}	3.00 ^{bcd}	2.85 ^{e}
	1.5%	0.87 ^{g}	2.00 ^{e}	2.07 ^{g}	2.33 ^{d}	3.93 ^{b}
	2.0%	0.47^{j}	1.67 ^{f}	1.83 ^{i}	1.33 ^{e}	3.98 ^{a}

Each value is a mean of three determinations**.** Means values within a column sharing the same subscript are not significantly different at P<0.05 according to Duncan's Multiple Range Test.

Table (2): Effect of treatment of strawberry with NaCl

Cultivar	Conc. of NaCl	Total chlorophyll	Shoot length (cm)	Shoot dry weight	Root Length (cm)	Root dry Weight	Leaf number	Proline	Catalase	Peroxidase
Fortuna	0.0	15.33 ^{a}	5.40 ^{a}	3.87 ^{a}	3.40 ^{a}	2.51 ^{a}	5.67 ^{a}	0.53 ^{h}	1.86 ^{g}	20.42 ^{e}
	500	12.87 ^{b}	3.83 ^{c}	2.16 ^{b}	2.67 ^{c}	1.71 ^{b}	4.33 ^{b}	1.07 ^{g}	2.12 ^{e}	22.31 ^{c}
	750	11.80 ^{c}	2.83 ^{e}	1.95 ^{c}	2.33 ^{d}	1.21 ^{c}	3.33 ^{c}	2.03 ^{f}	3.42 ^{c}	23.11 ^{b}
	1000	10.13 ^{d}	2.10 ^{g}	1.22 ^{d}	2.13 ^{e}	0.96 ^{d}	2.00 ^{d}	3.95 ^{d}	4.75 ^{a}	24.51^{a}
Festival	0.0	11.63 ^{e}	4.17 ^{b}	1.28 ^{de}	3.07 ^{b}	0.98 ^{e}	4.67 ^{b}	3.31 ^{e}	1.72 ^{h}	19.81 ^{h}
	500	9.40 ^{f}	3.57 ^{d}	1.16 ^{ef}	2.60 ^{c}	0.67 ^{f}	3.67 ^{c}	5.04 ^{c}	1.94 ^{f}	18.88 ^{f}
	750	8.70 ^{g}	2.50 ^{f}	1.11 ^{g}	2.27 ^{d}	0.56 ^{g}	3.33 ^{c}	5.71 ^{b}	2.24 ^{d}	20.40 ^{g}
	1000	8.13 ^{h}	2.00 ^{g}	0.98 ^{g}	1.87 ^{f}	0.27 ^{h}	2.00 ^{d}	7.81 ^{a}	4.31 ^{b}	21.27 ^{d}

Each value is a mean of three determinations**.** Means values within a column sharing the same subscript are not significantly different at *P*<0.05 according to Duncan's Multiple Range Test.

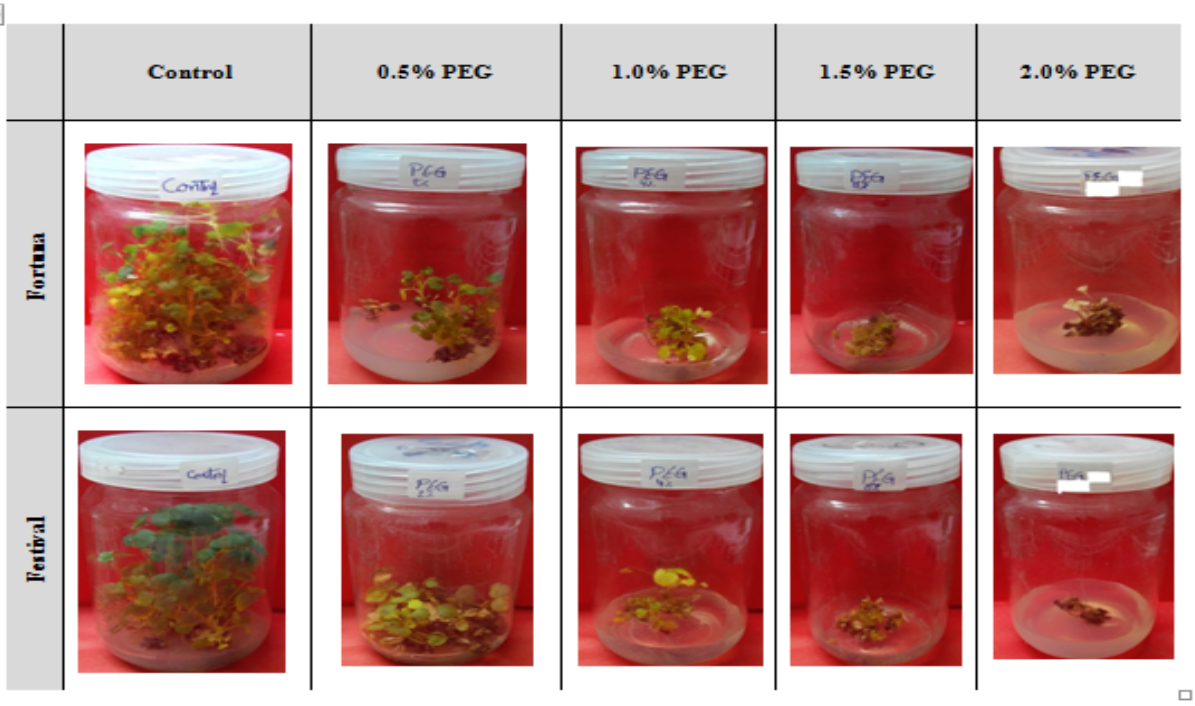

Figure (1): Effect of various concentrations of PEG on Fragaria ananasa cultivars Fortuna and Festival

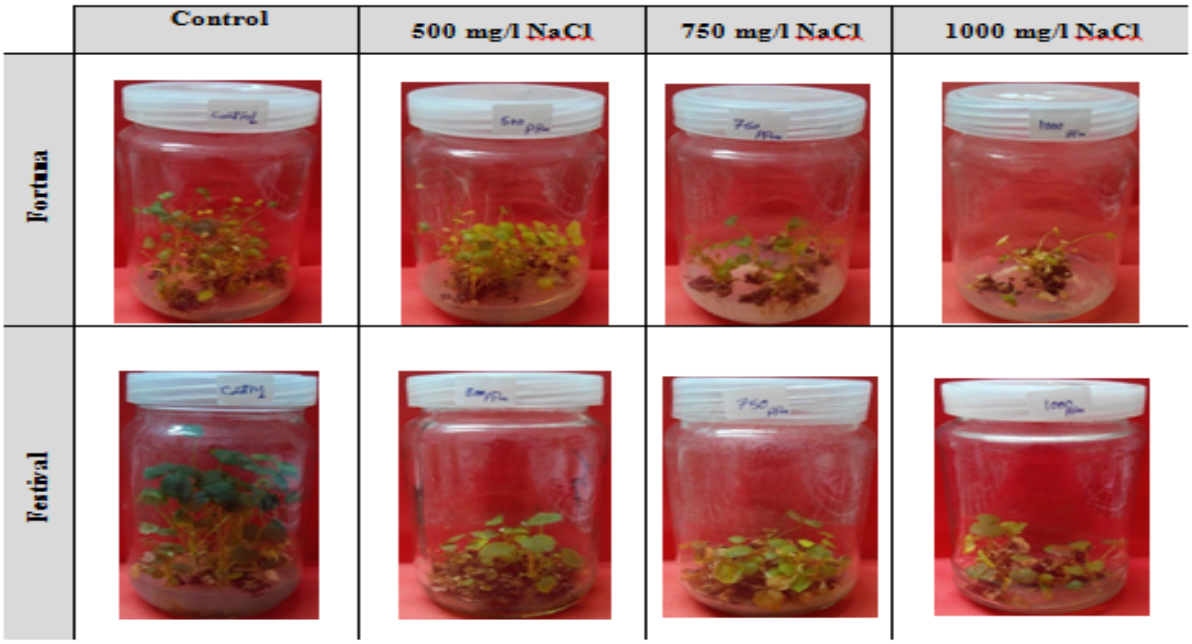

Figure (2) The effect of various concentration of NaCl on Fragaria ananasa cultivars Fortuna and Festival

Discussion

The results obtained in this study that water stress created by incorporating PEG into the culture media may adversely affect the chlorophyll contents, shoot length, root length and the number of leaves per regenerant and positively affected the contents of proline may agree and confirm the results obtained in earlier studies carried out on other plants. For example **Dami and Hughes (1995)** added polyethylene glycol (PEG) 2%,4% and 6% to the rooting medium of micropropagated grape shoots to induce water stress. Reduced growth and slow rooting were observed in treated plantlets with PEG as compared to control plantlets. In general, there was an inverse relationship between growth characteristics (plant height, number of leaves and number of roots) and PEG concentration in the culture medium. **Rajashekar *et al.*, (1995)** reported that significant growth differences were observed for two micropropagated *Prunus* accessions after 14 days in culture when 685 mM mannitol was included into Quoirin and Lepoivre (QL) nutrient medium. Addition of mannitol to the nutrient medium at 275 and 550 mM decreased explant fresh weights of *Marianna* 2624 to 36 and 28% of controls, respectively, at 28 days past initial culture. **Djibril *et al.*, (2005)** evaluated date palm *(Phoenix dactylifera L.*) tolerance to osmotic stress induced by polyethylene glycol (PEG) during the early stages of plant development where two varieties *Nakhla Hamra* (NHH) and *Tijib widely* cultivated in Mauritania were tested. NHH showed increasing in proline content when water deficit was induced by PEG. **Harb *et al.*, (2005)** studied the effects of polyethylene glycol (PEG; 0, 3, 6, 9, 12 and 15%) and gamma irradiation (0, 10, 20, 30, 40, 50 and 60 Gy), alone or in combination, on micro propagated banana cv. Williams determined *in vitro* and under greenhouse conditions. The gradual increase in PEG rates was negatively correlated with survival percentage, shoot and pseudo stem height, leaf and root number, shoot fresh and dry weights, leaf blade and midrib thickness, number and diameter of air cavities, number of midrib vascular bundles, root and vascular cylinder diameter, cortex width and number of vessels and photosynthetic pigments. On the other hand, PEG application was positively correlated with the total free amino acids, proline, total soluble phenols, sugars (reducing, non-reducing and total) as well as Na and Cl concentrations. N, P, K, Ca and Mg concentrations decreased under water stress conditions. In agreement with the results of this study but working with *Juniperus procera*, **Ibrahim *et al.*, (2014)** found out that both chlorophyll a and b were significantly reduced in response to water stress. **Budiman and Syamsuddin (2015)** mentioned that Prolonged water stress led to increases in the proline in some grass species. **Yuan *et al.*, (2016)** working with tomatoes reported that all levels of water stress caused a marked reduction of chlorophyll *a*, chlorophyll b, and total chlorophyll content in all developmental stages, while activities of antioxidant enzymes, such as superoxide dismutase, peroxidase, and catalase, and lipid peroxidation increased.

The results obtained in this study that salt stress may adversely affect the chlorophyll contents, shoot length, root length, shoot and root dry biomass and the number of leaves per regenerant and positively affected the contents of proline and the activity of catalase and peroxidase may be more or less similar to the results obtained by other investigators like **El-Said (1995)** investigated the effect of salinity levels (NaCl) on shoot tips cultures of two grape cvs King Ruby and Flame seedless. Shoot tips were cultured on MS medium containing BA (0.1, 0.2 and 0,5 mg/1) combined with (0.1 mg/l) NAA. Each of these concentration contained (0, 4000, 8000, 12000 or 16000 ppm) of NaCl. Shoot tip of King Ruby cv. was more tolerant than shoot tip of Flame seedless cv. cultured on MS medium containing different levels of NaCl. Increasing the concentration from 4000 to 12000 decreased the values of all growth

parameters (shoot number, shoot length and leaves number per explant). **Evers *et al.*, (1997)** studied the effect of NaCl on poplar (*Populus tremula x P. tremuloides)* shoots. *In vitro* stock proliferating cultures used as the source material. After 28 days of cultivation on media containing up to 300 mmol/ litre NaCl, the shoot and root lengths as well as the rooting percentage were smaller than in the control. Increasing salinity enhanced these effects. **Al-Naber *et al.*, (1998)** reported that growth behavior of *Atriplex nummularia* plantlets was studied under 3 concentrations of NaCl (0.1, 0.2 and 0.3 M) to induce and salinity stresses in Murashige and Skoog (MS) basal Medium. The growth of *A. nummularia* plantlets showed no significant differences for number or length of shoots, however, number of leaves per plantlet decreased with increasing levels of salinity stresses. **Darwesh *et al.*, (2006)** studied the effect of salinity at 6000, 10000 and 14000 p.p.m (NaCl + $CaCl_2$) on (*Phoenix dactylifera* L.) cv. Sakkoti propagated *in vitro* from shoot tip during proliferation and rooting stage. Increasing the levels of salinity decreased the shoot lets length cm, number of shoot lets leaves at proliferation stage and shoot lets length (cm), leaves number per plant let , number of roots ,root length/plantlet at rooting stage and survival percentage(68.2 % for control compared to 43% at 14000 ppm). **Nawel *et al.*, (2015**) showed that length and fresh weight of root and shoot were reduced significantly with salt treatment in two lettuce varieties. Regarding biochemical analysis in root increased in one of these varieties. **Alaa *et al.*, (2016**) reported that there is a positive relationship between salt tolerance and proline content and total peroxidase (POX) activity increased in tolerant cell lines as compared to control.

References

Alaa M.E, Mohamed S.A., Usama I.A., Hattem M.E. (2016): *In vitro* selection and characterization of salt tolerant cell lines in cassava plant (*Manihot esculenta* Crantz). International Journal of ChemTech Research; 9(5): 215-227.

AL-Naber G., Kafawin O. and Abu-Zanat M. (1998): Response of *Atriplex nummularia* plantlets to stimulated drought and salinity conditions in-vitro. Dirasat Agricultural Sciences; 25(1): 38-43.

Bates L., Waldren R.P. and Teare I.D. (1973): Rapid determination of free proline for water-stress studies. Plant and Soil; 39: 205-207.

Bergmeyer H.U., Bernt E., Schmidt F. and Stork H. (1974): in Methoden der enzymatischen Analyse (Bergmeyer, H.U., Hrsg.); 2:1241-1246; Verlag Chemie, Weinheim, and (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.); 3: 1196-1201.

Budiman N. and Syamsuddin N. (2015): Effect of water stress on growth, yield, proline and soluble sugars contents of Signal grass and Napier grass species 1Budiman Nohong and 2 Syamsuddin Nompo. American-Euroasian J. Sust. Agricul; 5:14-21.

Chen C., Yu R., Owuor E.D. and Kong A.N. (2000): Activation of antioxidant-responseelement (ARE), mitogen-activated protein kinases (MAPKs) and caspases by majorgreen tea polyphenol components during cell survival and death. Arch Pharm Res; 23:605–612.

Dami I. and Hughes H.G. (1995): Leaf anatomy and water loss of in vitro PEG-treated 'Valiant' grape. Plant Cell Tissue and Organ Culture; 42(2): 179-184.

Darwesh R.S.S., Abdallah A.E., Arafa A.M. and El-Banna A. (2006): Physiological studies on palms. Research Bulletin; 12: 1-11.

Djibril S., Kneyta M.O., FrançoisAbaye B. and Maurice S. (2005): Growth and development of date palm (*Phoenix dactylifera* L.) seedlings under drought and salinity stresses. African Journal of Biotechnology; 4 (9): 968-972.

El-Said H.M. (1995): Studies on micropropagation of grapevines. M.Sc. Thesis, Fac. Agric. Cairo Univ.

Evers D., Schmit C., Mailliet Y. and Hausman J.F. (1997): Growth characteristics and biochemical changes of popular shoots *in vitro* under sodium chloride stress. Journal of Plant Physiology; 15(1):748-753.

Harb E.M., El-Shihy O.M., Hanafy A.A. and Bayerly R.M.S. (2005): Effect of gamma irradiation on increasing water stress tolerance of micropropagated banana plants. Bulletin of Faculty of Agriculture, Cairo University; 56(1): 17-53.

Ibrahim A., Hashim E., Pervaiz K., Mohamed I., Mudawi E. and Abdalla A. (2014): Effect of water stress on relative water and chlorophyll contents of *Juniperus Procera* Hochst. Ex Endlicher in Saudi Arabia. Proceedings of the International Conference on Chemical, Agricultural and Medical Sciences; pp. 6-8, May 2-3, Antalya (Turkey).

Khan M.A. and Duke N.C. (2001): Halophytes- A resource for the future. Wetland Ecology and Management; 6:455-456.

Kong A.N, Yu R., Lei W., Mandlekar S., Tan T.H. and Ucker D.S. (1998): Differential activation of MAPK and ICE/Ced-3 protease in chemical-induced apoptosis: the role of oxidative stress in the regulation of mitogen-activated protein kinases (MAPKs) leading to gene expression and survival or activation of caspases leading to apoptosis. Restor Neurol Neurosci; 12:63–70.

Mukherjee S.P. and Choudhuri M.A. (1983): Implications of water stress-induced changes in the level of endogenous ascorbic and hydrogen peroxide in vigna seedlings.physiol.plant;58:166-170.

Murashige T. and Skoog F.A. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol; 15:473-479.

Nawel N., Issam S., Rym K. and Mokhtar L. (2015): Effect of Salinity on Germination, Seedling Growth and Acid Phosphatase Activity in Lettuce. American Journal of Plant Sciences; 6: 57-63.

Peak K.Y., Rhee W.Y., Wang J.K.H. and Chao Y.H. (1988): Effect of sodium chloride and sodium sulfate on growth and metabolite content in shoot tip culture of Chinese cabbage (*Brassica campestris* L. ssp. *Perinesis Rupr*). Journal of The Korean Society for Horticulture Science; 29 (3): 159-170.

Pérez-Pérez J.G., Romero P., Navarro J.M. and Botía P. (2008): "Response of sweet orange cv "Lane late" to deficit irrigation in two rootstocks. I: water relations, leaf gas exchange and vegetative growth," Irrigation Science; 26 (5): 415–425.

Rajashekar G., Palmquist D. and Ledbetter C.A. (1995): *In vitro* screening procedure for osmotic tolerance in Prunus. Plant Cell Tissue and Organ Culture.; 41(2): 159-164.

Seeram N.P. (2006): Strawberry Phytochemicals and Human Health: A Review, UCLA Center for Human Nutrition, David Geffen School of Medicine, University of California.

Snedecor G.W. and Cochran W.G. (1982): Statistical Methods. 7th Ed., The Iowa State University, Ames. IOWA, USA.

Tuna A.L., Kaya C. and Ashraf M. (2010): "Potassium sulfate improves water deficit tolerance in melon plants grown under glasshouse conditions," Journal of Plant Nutrition; 33 (9): 1276–1286.

USDA, (2003): Economics, Statistics, and Marketing Information.

Vernon L.P. and Selly G.R. (1966): The chlorophylls. Academic press. New York and London.

Yuan K.X., Yang Z.Q., Li Q. and Han Q. (2016): Effects of different levels of water stress on leaf photosynthetic characteristics and antioxidant enzyme activities of greenhouse tomato. Photosynthetica; 54(1): 28-39