Journal of Applied Horticulture, 8(2): 129-134, July-December, 2006

Effect of exogenous application of anti-stress substances and elemental sulphur on growth and stress tolerance of tissue culture derived plantlets of date palm (*Phoenix dactylifera* L.) cv. 'Khalas' during acclimatization

Mohamed. A. Awad[1]*, A.A. Soaud and S.M. El-Konaissi

*Department of Aridland Agriculture, College of Food and Agriculture, UAE University, P.O.Box. 17555 Al-Ain, United Arab Emirates. *E-mail: Mohamedawad@uaeu.ac.ae; [1]Permanent address: Faculty of Agriculture, Pomology Department, Mansoura University, El-Mansoura/Egypt.*

Abstract

There is a high demand for date palm plantlets regenerated via tissue culture techniques. However, such plantlets require a long acclimatization period extending 12-18 months before transplanting in the open field. The effect of foliar and soil application of anti-stress substances and elemental sulphur, respectively, on growth and survival percentage of tissue culture-derived 'Khalas' date palm plantlets during acclimatization were studied. The results showed that application of salicylic acid, acetyl salicylic acid (aspirin), elemental sulphur, plantacur-E (a vitamin E formulation containing 25% α-tocopherol) at 1%, and oleic acid at 100 ppm, significantly increased plantlet survival percentages compared to the control. In this respect, gamma aminobutyric acid (GABA) at 20 mM was the most effective treatment compared to 10 mM and the control. Salicylic acid, aspirin, elemental sulphur and plantacur-E (at 2%) significantly increased the concentrations of Fe, Mn, Zn, and Cu in leaflets compared to the control. However, the macro nutrients showed no clear response to the applied treatments. Application of 250 ppm of the ethylene biosynthesis blocker, ABG-3168 (ABG), inhibited the growth of plantlets, and completely suppressed growth at 500 ppm, suggesting the potential role of ethylene biosynthesis in subsequent plantlet development. Irrigation with 10,000 ppm sea water for two months decreased chlorophyll concentration and increased electrolyte leakage by 2-3 fold compared to the control and the other treatments. GABA at 20 mM significantly increased chlorophyll concentration and decreased electrolyte leakage of leaflets compared to all the saline water treatments. In contrast, ABG at 250 ppm significantly decreased chlorophyll concentration and increased electrolyte leakage of leaflets by about 3-fold compared to all the saline water treatments. These results show potential role of GABA, salicylic acid, aspirin and oleic acid conducive for improved survival percentage of plantlets and stress tolerance during acclimatization.

Key words: Tissue culture, acclimatization, elemental sulphur, gamma aminobutyric acid, salicylic acid, aspirin, vitamin E, oleic acid, ABG-3168, *Phoenix dactylifera* L.

Introduction

The number of naturally produced offshoots by fruiting palms are not sufficient to meet the high demand for the new plantations. Thus, there is a special interest in date palm plants regenerated via tissue culture since this technique can provide a large number of homogenous plants that are true to type and free of diseases and can be produced in large scale (Zaid and de Wet, 1999). Generally, these plantlets require an acclimatization period of about 12-18 months before transplanting in open field conditions. The VP1-stage (*in vitro* plant in a stage-1) is the most sensitive and critical stage due to the stress caused by moving the plantlets from the controlled laboratory conditions and transplanting in pots under greenhouse conditions for 2 to 4 months. During this period, the survival rate of plantlets is low which might, in the long run, lead to economic losses (Zaid and de Wet, 1999; Farag *et al.*, 2002).

Anti-stress substances may enhance the plantlets tolerance to environmental stresses thus increasing the survival rate (Sreenivasulu *et al.*, 2000). Salicylic acid has been found, among many other functions, to control ion uptake by roots, stomata conductivity and to increase the antioxidant capacity of plants (Raskin, 1992). Alpha tocopherol (vitamin E) and ascorbic acid (vitamin C) are antioxidant substances concentrated in the chloroplast and protect the photosynthetic apparatus when a plant is subjected to stress, by scavenging the excessively reactive oxygen species known as free radicals (Fryer, 1992; Kranner *et al.*, 2002). Environmental stresses *e.g.* heat, drought, salt and mechanical stress increase GABA accumulation in plant tissues. The mechanical damage in soybean leaves increased GABA levels by 10 to 25-folds within 1 to 4 min of the start of the stimulus (Kathiresan *et al.*, 1997). Also, it has been reported that the kinetics of GABA accumulation in plants reveals a stress-specific pattern of accumulation that is consistent with a physiological role for GABA in stress mitigation (Kinnersley and Turano, 2000). Increase in free unsaturated fatty acids, *e.g.* oleic acid, due to hydrolysis of membrane phospholipids, often occur during plant senescence and under adverse conditions including wounding, freezing, drought, salt, and pathogen elicitation. The oleate-stimulated phospholipase-D and phosphatidic acid decreased H_2O_2-induced cell death in arabidopsis (Wang and Wang, 2001; Zhang *et al.*, 2003). Ethylene has a significant effect on plant development and influences the stages from

seed germination to organ senescence. However, because of the diversity of ethylene action, it was difficult to assign a definitive role for it in growth responses (Naqvi, 1994). Generally, salinity and drought stress increase ethylene level in vegetative plant tissues (Nilsen and Orcutt, 1996). Elemental sulphur is strongly involved in improving nutrient assimilation and in stimulating the anti-oxidative defense system of plants through its metabolite glutathione (Gondent and Ullman, 2000; Tausz *et al.*, 2000). Also, the acidity produced during elemental sulphur oxidation increases the availability of nutrients such as P, Mn, Ca, and SO_4 in soils which may enhance growth performance of plants (Marschner, 1995).

The aim of the present study was to investigate the effect of exogenous application of anti-stress substances and elemental sulphur on survival percentage, nutrient uptake and salinity tolerance of tissue culture derived date palm plantlets cv. 'Khalas' during the acclimatization.

Materials and methods

Plant materials and experimental procedure: This experiment was conducted during the period from 2004 to 2005 on tissue culture-derived plantlets of the commercially important 'Khalas' date palm cultivar at the Date Palm Research and Development Unit (DPRDU), and the Horticulture Laboratory, Department of Aridland Agriculture, College of Food and Agriculture, UAE University, Al-Ain, UAE. Healthy and uniform tissue culture-derived plantlets were transplanted in paper pots (9 x 8 cm), maintained in greenhouse under controlled temperature (28°C and 80-90% relative humidity) and received the normal acclimatization program (irrigation and fertilization, pest and disease control) developed by DPRDU. The plantlets were subjected to one of the following treatments with elemental sulphur alone as soil application and the other treatments as foliar spray. A complete randomized design with 3 replicates (15 plantlets each) per treatment was adopted (Steel and Torrie, 1980). Elemental sulphur was applied as fine particles in pots at three different levels of 0.0283, 0.1415, or 0.283g/pot (these rates correspond to 1, 5 and 10 ton/ha, respectively). Salicylic acid and acetyl salicylic acid were applied at 0.5 mM or 1.0 mM. Plantacur-E was applied at 1.0 or 2.0%. Oleic acid was applied at 100 ppm. GABA was applied at 10 mM or 20 mM, only in the second experiment (GABA was not available at the time of conducting experiment 1). ABG was applied at 250 ppm or 500 ppm. Elemental sulphur was applied once at transplanting, whereas, the other chemicals were applied twice, at 2-days and at 2-weeks from transplanting. All treatments, except elemental sulphur, were combined with 0.1% Tween-20 (polyoxyethylene sorbitan monolaurate) as a wetting agent. In control, plantlets were sprayed with only water plus 0.1% Tween-20. This experiment was repeated to confirm the obtained results.

During the VP1-stage of acclimatization (*in vitro* plantlets under acclimatization procedure extending 0-4 months at 28°C according to the definition provided by the DPRDU), the number of survived, well rooted plantlets and suitable to move to VP2-stage were recorded at 2 and 4 months from transplanting. The survived plantlets were transplanted in plastic pots (25 x 16 cm). At the end of the VP2-stage (*in vitro* plantlets under acclimatization procedure extending 4-12 months at 30°C), all the plantlets in all treatments survived. These plantlets were transplanted in larger plastic pots (40 x 24 cm). During the VP3-stage (*in vitro* plantlets under the acclimatization procedure extending 12-15 months at about 35°C), leaflets at middle age were excised from plantlets and transferred to the horticulture laboratory for nutrient analysis.

During the VP3 stage, the plants of each treatment were irrigated with sea water (diluted to 10,000 ppm) (800ml/pot/week) for two months. The control treatment was subdivided into two equal groups, one group irrigated with diluted sea water and the other group irrigated with normal water. At the end of the two months, leaflets at middle age were excised from the plantlets and immediately transferred to the horticulture laboratory for chlorophyll and electrolyte leakage measurements.

Nutrient analysis of the leaflets: During the VP3-stage, before the start of sea water irrigation, random leaflet samples from only experiment 1 were collected from each replicate of each treatment for nutrient analysis. The collected leaflets were washed with deionized water and oven dried for 48 h at 65°C. The samples were crushed and passed through 20-mesh stainless steel sieve. Samples were digested by the dry ashing method as described by Jones and Case (1990). Total content of micronutrients (Fe, Mn and Zn) were determined by the atomic absorption spectrophotometer Varian, model SpectrAA 220 FS. Sulphur content was measured using ICPAES, Varain model Vista MPX. Phosphorus was determined colorimetrically according to the method described by Kuo (1996). Total nitrogen concentration in leaves was determined, after the wet digestion according to Jones and Case (1990), by steam distillation using the semi automatic Kjeldahl method.

Total chlorophyll measurement: Total chlorophyll was measured in leaflets by the procedure of Hiscox and Israelstam (1979). Two hundred milligrams of leaf tissue in fractions was placed in a vial containing 14 mL dimethyl sulphoxide (DMSO) and chlorophyll was extracted into the fluid without grinding at 65°C by incubating for 8 hours. The extract was then transferred to a graduate tube and made up to a total volume of 20 mL with DMSO. The OD values at 645 and 663 nm were read in Beckman DBG spectrophotometer against a DMSO blank.

Electrolyte leakage measurement: During the VP3-stage two leaflets of middle age, each from different plantlet, were collected for each replicate/treatment. The collected leaflets were directly washed with tap water and rinsed in deionized water to remove the dust and electrolytes adhering to the surfaces and then lightly cleaned with tissue papers. Leaf segments of 3 x 3 cm were cut from the middle of each leaflet (pinna), cut into 2-pieces, placed in each test tube containing 40 mL of deionized water then loosely covered with aluminum foil. Three replicates, each with two leaflets were used for each treatment. The tubes were then incubated in the refrigerator at 6°C over night before electrical conductivity of each solution was determined. In the next day, the tubes were taken from the refrigerator, warmed up to room temperature (22°C±2), placed in a shaker (Gesllschaft FurLabortechnik, GFL, mbh-model 3015, Germany) for one hour to diffuse electrolytes, vortexed for a few seconds. Electrolyte leakage before killing was measured with digital electrical conductivity meter (Orion- model 150- USA). The leaflet

samples were killed by autoclaving (JKA-J.39 Autoclave, Japan) at 121°C for 20 min to release all electrolytes, cooled to 22±2°C after which they were left on the shaker for 1 hour, vortexed for a few seconds. Total electrolyte leakage was measured by using the same digital conductivity meter. Percentage of electrolyte leakage was calculated for each sample using the ratio of the initial (before killing) to the final (after killing) measurements (Ingram and Buchanan, 1984; Jiang and Huang, 2001; Farag *et al.*, 2002).

Statistical analysis: The data were subjected to analysis of variance (ANOVA) using the statistical package MSTATC Program (Michigan State University, East Lansing, MI). Comparisons between means were made by least significant differences (LSD) at 5% level.

Results

Percentage of successful plantlets at VP1-stage: Most of the applied substances positively influenced 'Khalas' plantlets growth during the VP1-stage of the acclimatization period (estimated as the percentage of successful plantlets that were ready to move to the next stage of the acclimatization program or VP2-stage) (Table 1). In experiment 1, elemental sulphur application, especially at the low and the moderate levels, increased plantlets growth after 2 months during the VP1-stage. Aspirin and salicylic acid were the most effective treatments, especially at 0.5 mM, in increasing growth and development of plantlets both after 2 and 4 months. However there was no significant difference between the high and the low concentration of aspirin and salicylic acid on the survival percentage of plantlets. The ABG treatment at 250 ppm significantly inhibited subsequent plantlet growth and completely prevented plantlet development at 500 ppm. Similarly, plantacur-E and oleic acid also had no significant effect on the survival percentage of plantlets both after 2 and 4 months (Table 1). In experiment 2, a similar trend was observed, however, the positive effect of sulphur, aspirin and salicylic acid was less pronounced on the survival percentage of plantlets than in experiment 1. In contrast to the results of experiment 1, plantacur-E (especially at 1%) and oleic acid at 100 ppm application had significant positive effects on the survival percentage of plantlets both after 2 and 4 months. Interestingly, GABA at both concentrations (10 and 20 mM) showed a clear positive effect on the survival percentage of plantlets. In this respect, GABA at the high concentration of 20 mM was more effective (91% survived plantlets) than at the low concentration of 10 mM (73% survived plantlets) (Table 1). At the end of the VP2-stage all plantlets in all the treatments survived.

Table 1. Successful plantlet percentage of 'Khalas' date palm during the VP1-stage of the acclimatization period as affected by elemental sulphur and anti-stress substances

Treatments	Successful plantlet (%) during the VP1-stage			
	Experiment 1		Experiment 2	
	After 2 months	After 4 months	After 2 months	After 4 months
Control	47.0	64.4	44.4	62.2
Sluphur at 0.0283g sulphur/pot	62.2	71.1	44.4	65.5
Sulphur at 0.1415g sulphur/pot	64.4	74.6	55.6	62.2
Sulphur at 0.283g sulphur/pot	57.8	73.3	55.6	67.7
Aspirin at 0.5 mM	60.0	80	48.9	68.9
Aspirin at 1.0 mM	73.3	77.8	42.2	67.7
Salcylic acid at 0.5 mM	66.7	84.4	57.8	66.7
Salcylic acid at 1.0 mM	64.4	80	51.1	77.8
Plantacur-E at 1%	42.2	57.8	73.3	75.5
Plantacur-E at 2%	37.8	53.3	64.4	68.9
ABG at 250 ppm	14.4	35.5	18.7	32
ABG at 500 ppm	0.0	0.0	0.0	0.0
Oleic acid 100 ppm	48.9	62.2	75.5	86.7
GABA 10 mM	-	-	62.2	73.3
GABA 20 mM	-	-	82.2	91.1
F-test	***	***	***	***
LSD (*P*=0.05)	10.8	13.1	10.9	9.6

*** significant at level $P = 0.001$; - not calculated.

Nutrient concentration of leaflets at the VP3 stage: Salicylic acid and its derivative acetyl salicylic acid (aspirin) application, especially at the lower concentration, significantly increased the concentrations of Fe, Mn, Zn, and Cu in the leaflets compared to the control (Table 2). Also, elemental sulphur application significantly increased the concentration of Fe, Mn, Zn and Cu. However, the concentration of Cu was significantly decreased at the highest level of sulphur application compared to the control. Plantacur-E application, only at the higher concentration, significantly increased the concentration of Fe, Mn, Zn, and Cu in the leaflets compared to the control (Table 2). Oleic acid application significantly increased the concentration of N, Fe and Mn but the other nutrients were not affected. The untreated (control) plantlets contained significantly higher concentration of sulphur than all other treatments. However the concentrations of N, P and K were only slightly affected by the applied treatments (Table 2).

Chlorophyll concentration and electrolyte leakage of leaflets after 2-months of irrigation with sea water: After two months irrigation with sea water (diluted to 10,000 ppm salinity) all plants survived in all treatments including the control. However, the irrigation with sea water significantly decreased the concentration of chlorophyll in all the treatments compared to the control in both experiments (Table 3). ABG application at 250 ppm significantly decreased chlorophyll concentration compared with the control and most of the other treatments in both experiments. Plantlets treated with 0.5 mM aspirin contained significantly higher concentration of chlorophyll than those treated with 1.0 mM in only experiment 1. There was no significant difference in chlorophyll concentration between the different sulphur treatments in both the experiments. GABA application at 20 mM significantly increased the concentration of chlorophyll compared to the sea water treatment and most of the other treatments (Table 3).

Electrolyte leakage of leaflets significantly increased (2-3 fold) by sea water treatment in both experiments (Table 3). ABG application at 250 ppm significantly increased electrolyte leakage (about 3-fold) compared with the control and the other treatments in both experiments. In experiment 1, elemental sulphur at both low and high levels significantly decreased electrolyte leakage compared to the sea water treatment. Plantlets treated with aspirin and salicylic acid at both 0.5 mM and 1.0 mM showed significantly lower percentages of electrolyte leakage than those

Table 2. Nutrient concentration of 'Khalas' date palm plant leaflets at the VP3-stage of the acclimatization period as affected by elemental sulphur and anti-stress substances

Treatments	N%	P%	K%	S%	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Control	1.70	0.33	1.84	0.34	26.52	5.22	3.13	1.38
Sluphur at 0.0283g sulphur/pot	1.67	0.34	1.95	0.32	34.01	5.35	3.23	1.67
Sulphur at 0.1415g sulphur/pot	1.78	0.28	1.81	0.32	38.01	6.53	4.14	1.73
Sulphur at 0.283g sulphur/pot	1.83	0.27	2.03	0.32	36.22	6.13	4.36	1.48
Aspirin at 0.5 mM	1.72	0.29	1.82	0.27	35.53	7.16	4.08	2.08
Aspirin at 1.0 mM	1.79	0.3	1.88	0.3	31.6	6.74	4.39	1.67
Salcylic acid at 0.5 mM	1.73	0.24	1.81	0.32	31.81	5.89	4.34	2.05
Salcylic acid at 1.0 mM	1.8	0.28	1.83	0.3	29.06	6.2	3.95	1.47
Plantacur-E at 1%	1.66	0.28	1.96	0.28	29.7	5.13	3.23	1.6
Plantacur-E at 2%	1.79	0.31	1.78	0.27	34.15	5.87	4.34	1.83
Oleic acid 100 ppm	1.83	0.3	1.76	0.26	33.02	6.54	3.27	1.35
F-test	***	***	***	***	***	***	***	***
LSD (*P*=0.05)	0.05	0.05	0.09	0.05	2.27	0.43	0.48	0.26

(***)-significant at level *P* = 0.001; (-)-not calculated.

Table 3. Total chlorophyll concentration and electrolyte leakage (%) of 'Khalas' date palm plant leaflets treated with sea water at the VP3-stage of the acclimatization period as affected by elemental sulphur and some anti-stress substances

Treatments	Experiment (1)		Experiment (2)	
	Chlorophyll (mg/g fw)	Electrolyte leakage (%)	Chlorophyll (mg/g fw)	Electrolyte leakage (%)
Control (normal water)	1.68	2.69	1.55	2.87
Sea water	1.19	4.98	0.91	4.91
Sluphur at 0.0283g sulphur/pot	1.25	4.26	1.11	4.51
Sulphur at 0.1415g sulphur/pot	1.31	4.44	1.14	4.87
Sulphur at 0.283g sulphur/pot	1.26	4.17	1.12	4.10
Aspirin at 0.5 mM	1.39	4.18	0.94	4.72
Aspirin at 1.0 mM	1.24	4.30	0.91	4.77
Salcylic acid at 0.5 mM	1.24	4.63	1.19	4.78
Salcylic acid at 1.0 mM	1.21	4.68	1.10	4.53
Plantacur-E at 1%	1.25	4.84	1.19	4.56
Plantacur-E at 2%	1.18	4.97	1.10	4.85
ABG at 250 ppm	1.11	6.74	0.92	6.25
Oleic acid 100 ppm	1.31	3.88	1.11	4.50
GABA 10 mM	-	-	1.06	4.90
GABA 20 mM	-	-	1.25	4.10
F-test	***	***	***	***
LSD (*P*=0.05)	0.14	0.68	0.15	0.63

(***)-significant at level *P* = 0.001; (-)not calculated.

treated with only sea water in experiment 1. Among all the treatments, oleic acid significantly decreased electrolyte leakage. In experiment 2, elemental sulphur at the high level significantly decreased electrolyte leakage compared to the sea water treatment. Interestingly, GABA application at 20 mM significantly decreased electrolyte leakage than the sea water and most of the other treatments (Table 3).

Discussion

Our results showed that the application of anti-stress substances GABA, plantacur-E, salicylic acid, aspirin, oleic acid, and elemental sulphur increased survival percentage of the plantlets during the VP1-satge of the acclimatization period which would otherwise lead to economic loss (Table 1). The plantlets during VP1-stage was most sensitive to stress since during both VP2 and VP3 stages all the plantlets survived in all the treatments including the control. The results showed that, in both experiments, irrigation with sea water for two months significantly decreased the concentration of chlorophyll and increased electrolyte leakage of the plant leaflets compared to the control (Table 3). In this context, most of the applied substances especially GABA, salicylic acid and aspirin increased chlorophyll concentration and clearly decreased the electrolyte leakage in the plant leaflets compared to those treated only with sea water (Table 3). Such effects might be due to protecting the endogenous anti-oxidant systems often correlated with increased resistance to oxidative stress and/or controlling the level of free radicals within plant tissues (Sreenivasulu *et al.*, 2000). It is also possible that the anti-stress substances were effective in maintaining the membrane integrity to reduce the leakage of electrolyte through its positive effect on the antioxidant enzymes system as has been demonstrated by Pinhero and Fletcher (1994) in corn seedlings. It is generally known that most of environmental stresses have in common a similar mechanism in affecting plant growth and performance. Under stress conditions, the generation of free radicals and low nutrient uptake are believed to be the main cause for damaging and dis-functioning of plant cells (Bohnert *et al.*, 1995; Sreenivasulu *et al.*, 2000). Elemental sulphur is strongly involved in improving nutrient assimilation and in the anti-oxidative defense systems of plants through its metabolite glutathione. Glutathione is also necessary for the efficient-functioning of other defense systems such as ascorbate and α-tocopherol regeneration and carotenoids and protein thiols conservation (Gondent and Ullman, 2000; Tausz *et al.*, 2000). Hence, in the present study we propose that the protection confined by elemental sulphur was due to a similar mechanism

of enhanced free radical scavenging systems in the plant.

Our results showed positive effects of most of the applied anti-stress substances especially salicylic acid, aspirin and elemental sulphur on the concentrations of Fe, Mn, Zn, and Cu in the leaflets compared to the control (Table 2). These nutrients have been reported to be connected with the improved tolerance of several plants (Kinnersley and Turano, 2000). Elemental sulphur also increased the level of these nutrients in the leaflets probably by increasing their availability and uptake via modifying soil pH as reported by Marschner (1995). The results on salicylic acid and aspirin are in accordance with those of Raskin (1992) who reported that salicylic acid, among many other functions, controls ion uptake by roots and stomatal conductivity and increases the antioxidant capacity of plants. These results suggest salicylic acid and aspirin as mediator of mineral acquisition in stress-related metabolism. It has been reported that salicylic acid and its derivative aspirin induced multiple stress tolerance in bean and tomato plants against drought, heat, chilling, and salinity when seeds imbibed in aqueous solutions (0.1-0.5 mM) or when applied to the plants as foliar spray or even as soil drenches (Senaratna *et al.*, 2000). Moreover, salicylic acid has been found to protect wheat plants from drought and salinity stress, diminished the alteration of phytohormones, and increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth (Shakirova *et al.*, 2003). Our results on the positive effects of GABA application (Tables 1 and 3) confirm those of Kinnersley and Turano (2000) who reported that the kinetics of GABA accumulation in plants reveals a stress-specific pattern of accumulation that is consistent with a physiological role for GABA in stress mitigation. To the best of our knowledge, this is a pioneer study investigating the role of GABA as a potential anti-stress substance for improving growth and stress tolerance ability of date palm plantlets (Tables 1 and 3). Such positive effects of GABA have been confirmed on 'Khadrawy' date palm plantlets, however, the lower concentration (10 mM) was more effective than the higher one (20 mM), indicating a cultivar dependent concentration (unpublished data).

The application of the ethylene biosynthesis blocker, ABG at 250 ppm greatly inhibited and completely suppressed subsequent plantlet development and growth at 500 ppm (Table 1). Also, the ABG-treated plantlets contained a lower chlorophyll concentration and showed a higher electrolyte leakage, after sea water irrigation, than the control (Table 3). These results suggested an important role of ethylene in subsequent plantlet development. This study might provide certain recommendations to benefit both the tissue culture-based date palm industry and growers.

Acknowledgements

This work was financially supported by the Research Affairs at the UAE University under the contract No.01- 01-6-11/04. We thank Mr. Abou-Mossalam Azab, Rashid Abdel-Fattah, Saad Khalil and Azhari Omer, College of Food and Agriculture, UAE University for their indispensable technical support. We also thank the staff of CLU, UAE University for their support in sulphur analysis. The contribution of Date Palm Research and Development Unit, UAE University is greatly appreciated.

References

Bohnert, H., E.D. Nelson and R.G. Jensen, 1995. Adaptation to environmental stresses. *The Plant Cell*, 7: 1099-1111.

Farag, K.M., S. Al-Konanissi and A. Zaid, 2002. Tolerance of Barhi and Khalas date palm plants reproduced by tissue culture to heat and salt stresses and treatments to enhance their tolerance. *Proceedings of the Fourth Annual UAE University Research Conference, UAE*, pp. 13-17.

Fryer, M.J. 1992. The antioxidant effects of thylakoid vitamin E (α-tocopherol). *Plant Cell Environ*., 15: 381-392.

Gondent, L. and P. Ullman, 2000. Glutathione metabolism in plants in relation to stress tolerance. Plant Sulfur Research in Europe, Cost Action 829, Instit. Plant Nutri. Soil Science, Federal Agric. Res. Centre, Braunschweig, Germany, pp. 37-38.

Hiscox, J.D. and G.F. Israelstam, 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.,* 57: 1332-1334.

Ingram, D.L. and D. Buchanan, 1984. Lethal high temperatures for roots of three citrus rootstocks. *J. Amer. Soc. Hort. Sci.,* 109: 189-193.

Jiang, Y. and B. Huang, 2001. Physiological responses to heat stress alone or in combination with drought: A comparison between tall Fescue and perennial Ryegrass. *HortScience*, 36: 682-686.

Jones, J.B. and V.W. Case, 1990. Sampling, Handling, and Analyzing Plant Tissue Samples. In: *Soil Testing and Plant Analysis,* Westerman (ed.). Book Series no. 3. Soil Science Society America, Madison WI, pp. 389-427.

Kathiresan, A., P. Tung, C.C. Chinnappa and D.M. Reid, 1997. Gamma aminobutyric acid stimulates ethylene biosynthesis in sunflower. *Plant Physiol*., 115: 129-135.

Kinnersley, A.M. and F.J. Turano, 2000. Gamma aminobutyric acid (GABA) and plant responses to stress. *Criti. Rev. Plant Sci*., 19: 479-509.

Kranner, I., R.P. Beckett, S. Wornik, M. Zorn and H.W. Pfeifhofer, 2002. Revival of a resurrection plant correlates with its antioxidant status. *Plant. J*., 31: 13-20.

Kuo, S. 1996. Phosphorus. In: *Methods of Soil Analysis*, part 3, D.L. Sparks (ed.). Chemical Methods. Soil Sci. Soc. Am., Book Series no. 5 Madison, WI, USA.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants.* 2nd Edition. Academic Press, London.

Naqvi, S.S.M. 1994. Plant hormones and stress phenomena. In: *Handbook of Plant and Crop Stress*, M. Pessarakli (ed.), Marcel Dekker, New York - Basel, Hong Kong, p.383-400.

Nilsen, E. T. and D.M. Orcutt, 1996. *Physiology of plants under stress; abiotic factors.* John Wiley & Sons, INC., pp. 83-198.

Pinhero, R.G. and R.A. Fletcher, 1994. Paclobutrazol and ancymidol protect corn seedlings from high and low temperature stress. *Plant Growth Reg.*, 15: 47-53.

Raskin, I. 1992. Role of salicylic acid in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* 43: 439-463.

Senaratna, T., D. Touchell, E. Bunn and K. Dixon, 2000. Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regul*., 30: 157-161.

Shakirova, F.M., A.R. Sakhabutdinova, M.V. Bezrukova, R.A. Fatkhutdinova and D.R. Fatkhutdinova, 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci*., 164: 317-322.

Sreenivasulu, N., B. Grimm, U. Wobus and W. Weschke, 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). *Physiologia Plantarum*, 109: 435-440.

Steel, R.G.D. and J.H. Torrie, 1980. *Principles and procedures of statistics, A biometrical approach.* 2nd Edition, McGraw-Hill, New York, USA, 1980.

Tausz, M., A. Wonishch, M. Muller and D. Grill, 2000. The role of glutathione in the development of stress and damage to plants. Plant Sulfur Research in Europe, Cost Action 829, Instit. Plant Nutri. Soil Science, Federal Agric. Res. Centre, Braunschweig, Germany, pp. 101-104.

Wang, C. and X. Wang, 2001. A novel phospholipase D of Arabidopsis that is activated by oleic acid and associated with the plasma membrane. *Plant Physiol.*, 127: 1102-1112.

Zaid, A. and P.F. de Wet, 1999. Date palm propagation. In: *Date palm cultivation,* A. Zaid and E.J. Arias (eds.), FAO plant production and protection paper, No. 156. p. 74-106.

Zhang, W., C. Wang, C. Qin, T. Wood, G. Olafsdottir, R. Welti and X. Wang, 2003. The oleate-stimulated phospholipase D, PLDs, and phosphatidic acid decrease H_2O_2-induced cell death in Arabidopsis. *The Plant Cell*, 15: 2285-2295.