Volume number 2
December 2009
Journal of Environmental studies 2: 73- 83.

العدد ٢
ديسمبر ٢٠٠٩
مجلة الدراسات البيئية ٢ : ٧٣ – ٨٣

Journal of Environmental Studies (JES)

Article Title

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Effect of dehydration on some physiological aspects of shade and light grown plant species using detached leaves

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Summary

Detached leaves were used to study the effect of hydration on some physiological aspects in shade (*Syngonium podophyllum*) as well as light grown (*Lycopersicon esculentum* Mill) plant species. In addition, these two plants were used to investigate the role of light to accelerate the effect of dehydration on peroxidase activity and chlorophyll degradation. Relative water content of both tomato and *S. podophyllum* (nephtytis) decreased drastically in three days under dehydration condition. Tomato leaves are thicker and fleshier than those of nephtytis; it may explain why the reduction in relative water content of tomato was higher than that of nephtytis, especially under dark condition. In three days, the reduction in total pigments of nephtytis was higher than those of tomato, especially under light condition. Both plant species showed reduction in total carbohydrate and protein contents resulted from the reduction of insoluble fractions. Under dehydration condition, both plant species showed disappearance (high in nephtytis) or appearance (high in tomato) of some polypeptides. In both plant species, increase of the soluble carbohydrate and protein fractions, enhance the peroxidase activity and expression of new polypeptides were three mechanisms to avoid the negative effects of dehydration but they were not enough to regulate the oxidative risks exerted by three days dehydration.

Keywords: Dehydration, detached leaves, nephtytis, protein analysis, peroxidase, SDS PAGE.

Introduction:

Nephtytis (*Syngonium podophyllum*) is an evergreen-shade plant. It can grow in pots for several years and reach ten meters in length under room condition under room condition. It has broad-arrowhead shape leaves. Therefore, it can be used to add a nice view for rooms and offices. Devlin and Witham (1986) reported that when the shade-plants are subjected to full sunlight, the leaves become chlorotic and die.

Tomato (*Lycopersicon esculentum* Mill) is important as a famous food all over the world and it needs sunlight to grow and yield well. It is grown in almost every country of the world. Its fruit is very important for human beans where it consists of many important chemical components. It is rich in vitamins (A and C), fiber, and it does not consists of cholesterol (Hobson and Davies, 1971).

Tomato fruit consists of approximately 20-50 mg of lycopene/100 g of fruit weight (Kalloo, 1991). Lycopene is an important member in carotenoid family which is natural compounds that cause colours of fruits and vegetables. Lycopene is the most powerful antioxidant in the carotenoid family and it protects human cells from free radicals that degrade many parts of the body; consequently, lycopene is considered as protective tool against cancer (Block et al., 1992; Gerster, 1997; Rao and Agarwal, 2000).

In green plants, the chloroplasts are considered the main site for light-dependent oxygen activation and production of reactive oxygen species (Mehler, 1951). This resulted in production of superoxide radicals which dismutate at physiological pH yielding H_2O_2. Accumulation of toxic concentration of H_2O_2 puts tissue at

oxidative risk due to the initiation of extremely aggressive hydroxyl radicals (Elstner and Osswald, 1994; Polle, 1995; Ogweno et al. 2009). It leads to chlorophyll degradation, leaf bleaching and even cell death, especially, in case of shade plants.

In this work, detached leaves were used to study the effect of dehydration on some physiological and molecular aspects in shade as well as light grown plant. In addition, these two plants were used to investigate the role of light to accelerate the effect of dehydration on peroxidase activity and chlorophyll degradation.

Materials and methods

Preparation of plant materials:

All experiments of this work were fulfilled using detached leaves. Tomato leaves were obtained from plants grown in pots containing 5 kilograms of clay soil and subjected to sunlight in April. On the other hand, nephtytis leaves were obtained from plants grown in pots containing 5 kilograms of clay soil but under room condition. Plants were watered every three day to reach the full water capacity in each time.

Exert dehydration on detached leaves

To study the effect of dehydration on physiological and molecular aspects using detached leaves, full expanded leaves was placed between a stack (1 cm high) of drying papers and thin glass plate under light (450 µmol m-2 s-1) or dark conditions at 25±2 0C. Under light condition, the effect of leaf dehydration was studied after 72 hr. Under dark condition, the effect of dehydration was studied after 12, 24, 48 and 72 hr.

Determination of photosynthetic pigments

The photosynthetic pigments (chlorophyll a, chlorophyll b, and carotinoids) were determined using spectrophotometric method recommended by Metzner et al. (1965). Photosynthetic pigments were extracted in 85 % (v/v) aqueous acetone from a known fresh weight of leaves. The extract was centrifuged at 4000 rpm for 10 min; the supernatant was diluted with 85% aqueous acetone to the suitable concentration for spectrophotometric measurements. The extinction was measured against a blank of 85% pure aqueous acetone at three wavelengths {(carotinoids at 452.5), (chlorophyll a at 644) and (chlorophyll b at 663 nm)}. Pigment fractions were calculated as mg/g dry weight.

Estimation of Carbohydrate contents

Carbohydrate content was determined in the aqueous solution with anthrone sulfuric acid reagent according to Fales (1951) and Schlegel (1956), using glucose as a standar. To extract water-soluble carbohydrates, a known weight of dry tissue powder was boiled in distilled water in a water bath for 1 h. For extraction of total carbohydrates, 50 mg of dry tissue powder was boiled in 1 N HCI in water bath for 1.5 h. Then the extracts were cooled and filtrated through a centered glass funnel. A total of 0.5 m1 of the extract was mixed with 4.5 ml of anthrone reagent. The mixture was then boiled in water bath for 7 min. After cooling, the developed blue green color was measured against blank by using Spekol Carl-Zeiss spectrocolorimeter at wavelength of 620 nm. Soluble and total carbohydrate contents were finally calculated as mg/g dw. The water insoluble carbohydrates were calculated as the difference between the amount of the total and water-soluble carbohydrates.

Estimation of Protein contents

Total protein contents of leaves were determined according to Lowry et al. (1951) using Bovine serum albumin as a standard. Total protein of 50 mg dry tissues were extracted in 10 m1 NaOH (0.1 N) for 2 h. The extracts were centrifuged and the supernatants were collected. One millimeter of extract was added to 5 m1 of alkaline reagent and mixed thoroughly then allowed to stand for 10 min. A total of 0.5 m1 of Folin reagent diluted 1:1 (v/v) was then added and mixed immediately. After 30 min, the extinction against appropriate blank was measured at 700 nm. Results were expressed as mg/g dry weight.

Determination of peroxidase activity

Estimation of relative peroxidase activity was measured and calculated according to Wakamatsu and Takahama (1993). For peroxidase analysis, 1 gm of tissue was ground at 4°C in a mortar in 1 ml. extraction buffer consisting of 0.1 M Tris- base pH 7 and containing 0.002 M cysteine. The homogenate was centrifuged at 13500 rpm for 20 min. The supernatant were collected for immediate peroxidase activity determination. The reaction mixture consisted 5 mM guaicol, 40 mM. potassium phosphate buffer, pH 7.2, 0.1 mM EDTA, 0.3 mM H_2O_2 and enzyme preparation (50 µl) in a final volume of 5 ml. The reaction was measured using spectrophotometer (470 nm), in room temperature.

Protein extraction for SDS PAGE

Proteins were extracted by grinding 1 gm. of plant materials with a mortar and pistil. The extraction was done in liquid nitrogen with extraction buffer (50 mM Tris-HCl buffer, pH 6.8, glycerol 10% w/v, ascorbic acid 0.1%, cysteine hydrochlode 0.1% w/v). After centrifugation at 13500 rpm fore 20 min., the samples were heated to 96 °C in a water path for 1.5 min. Ten volumes of protein extract mixed with one volume of Bromophenol blue solution (0.5 ml glycerin, 0.5 ml dist. water, a small crumb or Bromophenol blue) were loaded. Then, wells of gel were carefully filled up with electrophoresis buffer (0.025 M Tris, 192 mM glycine and 0.1% SDS). Electrophoresis was carried out overnight (10 mA per gel). The run was continued until the Bromophenol blue marker reached the last cm of the bottom of the slab. The gel was transferred to a tray containing the appropriate staining solution for 1.5 hr.

It was prepared by mixing the solutions of 0.2 (w/v) coomassie blue (CB) and 0.05% (w/v) bismark brown R (BBR) dissolved in 400 ml of de-staining solution. De-staining solution was consisted of 40% methanol, 7% acetic acid and 35% water. The gel was shaken gently in de-stain solution until the bands were easily visualized on clear background.

Results and discussion

Detached leaves were used to study the effect of dehydration on some physiological and molecular aspects of light sensitive (nephtytis) and light grown (tomato) plant species. Plant leaves were subjected for different periods of hydration under dark or light condition.

Under the influence of dehydration, relative water content of both tomato and nephtytis decreased drastically in three days (Table 1).

Table (1): Relative water content (mg/g fresh weight) of *Lycopersicon esculentum* and *Syngonium podophyllum* detached leaves under light or dark conditions calculated in relation to that of plant leaves cultivated in pots at room condition under full field capacity (control; 100%).

Treatment	Tomato (%)	Nephtytis (%)
Control	100	100
12 h in dark	64.17	83
24 h in dark	58.64	77.70
48 h in dark	33.11	72.72
72 h in dark	18.54	63.85
72 h in light	16.3	18.75

Subjecting tomato leaves to dehydration for three days under dark condition resulted in severe reduction in relative water content (18.54%) in comparison to that of control (100%). On the other hand, under these conditions nephtytis leaves showed moderate reduction in relative water content (63.85%). Tomato leaves are thicker and fleshier than those of nephtytis; it may explain why the reduction in relative water content of tomato was higher than that of nephtytis. Environmental stresses such as drought, and salinity result in reduction of the water content of the plant tissues, consequently, they markedly affect different criteria including water relation, plant growth parameters, pigment content and gene expression (Hassanein and El-Khatib 1998; El-Khatib et al., 1999; Hassanein 2004_a).

In three days, under dark or light conditions, the detached leaves of both plants under the influence of dehydration showed a clear sign about the damage of the

plant leaves. They became light green and began to dry due to the severe water loss especially in light (Hassanein 2004_b). Under these conditions, light induces the production of several reactive oxygen species, which results in significant damage to cellular constituents and even cell death, if protective mechanisms via antioxidation fail to detoxify these toxic oxygen species (Asada, 1994; Bowler et al., 1994; E1stner and Osswald, 1994; Polle, 1995; Low and Merida, 1996; Paolacci et al., 1997). In this work, the over production of reactive oxygen species was evaluated by dehydration of detached leaves under light or dark condition. Consequently, they caused leaf tissue damage but their effect was magnified by the presence of light as was reported by Paolacci et al. (1997).

The effect of leaf dehydration on photosynthetic pigments of *Lycopersicon esculentum* at light and dark conditions is included in Table 2. In general, chlorophylls a, and b, carotinoids, and consequently total pigments decreased when the tomato leaves were dehydrated at light or dark in comparison to that of control. Reduction the content of carotinoids under dark was higher than under light condition. The photosynthetic pigments were generally inhibited by water stress (El-Tayeb and Hassanein 2000) which may lead to a reduction the photosynthetic rate.

Table (2): photosynthetic pigment contents (mg/g dry weight) during dehydration of detached *Lycopersicon_esculentum* leaves for different periods under light or dark condition.

Treatments	Chl a	Chl b	Carotenoids	Total
Control	1.62	0.86	0.7	3.19
12 h in dark	1.51	0.79	0.74	3.05
24 h in dark	1.03*	0.6*	0.31*	2.00*
48 h in dark	0.7*	0.46*	0.37*	1.50*
72 h in dark	0.72*	0.39*	0.31*	1.42*
72 h in light	0.42*	0.40*	0.44*	1.22*

In case of nephtytis leaves, gradual decrease in chl a, chl b, and total pigments was detected when plant leaves were subjected for dehydration under light or dark condition (Table 3). On the other hand, dehydration stress resulted in increasing the carotenoid pigments in comparison to those of control plants. Increase the carotinoids may be used by the shade plant to control partially the oxidative damage.

Table (3): Photosynthetic pigment contents (mg/g dry weight) during dehydration of detached *Syngonium Podophyllum* leaves for different periods under light or dark condition.

Treatments	Chl a	Chl b	Carotenoids	Total
Control	1.88	1.05	0.23	3.16
12 h in dark	1.86	1.23	0.22	3.30
24 h in dark	0.96	0.64	0.46	2.06
48 h in dark	0.76	0.51	0.52	1.78
72 h in dark	0.73	0.43	0.52	1.68
72 h in light	0.63	0.40	0.79	1.83

Comparison between nephtytis and tomato indicated that while carotenoid content decreased in tomato detached leaves, it increased in case of nephtytis detached leaves especially under light condition. In addition, in three days, the reduction in total pigments of light sensitive plant species (61%) was higher than that of light grown plant (42%), especially under light condition. Chloroplasts are considered the main site for light-dependent oxygen activation and production of reactive oxygen species (Mehler, 1951). This resulted in production of superoxide radicals which dismutate at physiological pH yielding H_2O_2. Accumulation of toxic concentration of H_2O_2 puts tissue at oxidative risk due to the initiation of extremely aggressive hydroxyl radicals (Elstner and Osswald, 1994; Polle, 1995). It leads to chlorophyll degradation, leaf bleaching and even cell death especially in case of shade plants. Consequently, subjecting nephtytis plant for direct sun light for one day resulted in leaf bleaching. Rising the temperature or decreasing the soil moisture content in combination with full sunlight may magnify the toxic effect of activated oxygen species (Paolacci et al., 1997).

Soluble, insoluble, and total carbohydrates were estimated when tomato leaves were dehydrated under light or dark conditions. These parameters were shown in Table 4. Under the influence of dehydration, insoluble and total carbohydrate contents decreased in comparison to those of control. In three days, the reduction of insoluble and

total carbohydrate content in dark was higher than in light condition. On the other hand, soluble carbohydrate faction increased with the increasing of dehydration time but it was higher under light in comparison with the dark condition. The same results were obtained when nephtytis detached leaves were subjected for the same conditions (Table 5). In general, the reduction in total carbohydrate content resulted from the reduction of insoluble carbohydrate fractions. Under these conditions, either nephtytis or tomato plant species exert many mechanisms to avoid the negative effects of dehydration. One of these mechanisms was the increase of soluble carbohydrates. The decrease total carbohydrate values may be due to the corresponding decrease in photosynthesis, which could be attributed to the decrease in pigment content. Other reasons may be responsible for the photosynthesis reduction that the decrease of some important protein in the photosystem II (Kushnir et al. 1987).

Table (4): Carbohydrates contents during dehydration of detached *Lycopersicon esculentum* leaves for different periods under light or dark condition.

Treatment	Soluble carbohydrates	Insoluble carbohydrates	Total carbohydrates
Control	88.06	149.24	237.24
12 h in dark	77.80*	127.16*	204.96*
24 h in dark	81.71*	123.95*	205.66*
48 h in dark	116.45*	68.66*	185.11*
72 h in dark	98.54*	66.02*	164.56*
72 h in light	114.16*	60.28*	174.44*

Table (5): Carbohydrates contents during dehydration of detached *Syngonium Podophyllum* leaves for different periods under light or dark condition.

Treatment	Soluble carbohydrates	Insoluble carbohydrates	Total carbohydrates
Control	65.56	109.10	374.67
12 h in dark	73.70*	103.72	377.42
24 h in dark	65.56	102.33	367.89
48 h in dark	92.25*	95.16*	337.41*
72 h in dark	96.50*	75.07*	198.58*
72 h in light	75.59*	103.56*	209.16*

Total protein content of tomato and nephtytis leaves was influenced by dehydration of detached plant leaves under light or dark conditions as shown in Table 6 and Table 7. Insoluble and total protein content decreased when detached leaves of both investigated plants were dehydrated in light or dark. On the other side, soluble protein content increased when tomato or nephtytis leaves were dehydrated under light or dark condition. Plants accumulated soluble substances that are normal cell constituents particularly carbohydrates and nitrogen-containing compounds during a period of environmental stress (Joyce et al. 1992; Hervieu et al 1994; Hans et al. 1995).

Relative peroxidase activity in the two plant species was influenced by dehydration of plant leaves either in dark or light conditions. In general, relative peroxidase activities were high in all treatments in comparison to those of control (fresh leaves). The highest value of relative peroxidase activity of plant leaves was detected when nephtytis plant leaves dehydrated under light condition. Furthermore, relative peroxidase activity increased with the increase the dehydration time under dark condition. Relative peroxidase activity values in nephtytis (Table 8) were higher than that of tomato leaves. Studying of peroxidase is very important because it use phenolic compounds to detoxify reactive oxygen species (Elstner and Osswald, 1994). The antioxidative function of phenolic compounds is well known. They undergo an electrodonating reaction towards reactive radicals, creating phenoxy radicals

which may be more stable than the oxygen radical and await reduction by available electron donors such as ascorbate (Elstner and Osswald, 1994). In addition, the balance between pro- and antioxidative functions may, in turn, be regulated by phenol-oxidizing enzymes (phenol oxidases and peroxidases).

Protein pattern indicated that dehydration gave the same results under light or dark condition. However, shade plant (Fig. 1) was more sensitive than light grown plant (Fig. 2). While one new polypeptide band (148 KDa) was newly detected in nephtytis, five polypeptide bands (74, 54, 50, 28, and 24 KDa) were disappeared under dehydration condition.

Table (6): Protein content during dehydration of *Lycopersicon esculentum* leaves for different periods under light or dark condition.

Treatment	Soluble protein	Insoluble protein	Total protein
Control	95.5	218.1	313.6
12 h in dark	102.8	179.0	281.8
24 h in dark	110.0	150.2	260.2
48 h in dark	136.5*	65.5*	202.0*
72 h in dark	145.3*	108.9*	254.2*
72 h in light	146.4*	94.0*	240.4*

Table (7): protein content during dehydrating of Syngonium Podophyllum leaves for different periods under light or dark condition.

Treatment	Soluble protein	Insoluble protein	Total protein
Control	61.6	229.6	291.2
12 h in dark	83.5	201.3	284.8
24 h in dark	115.8	151.4	266.2
48 h in dark	94.6	162.2	256.8
72 h in dark	138.4	91.2	229.6
72 h in light	153.5	105.2	258.7

Table (8): Effect of hydration on relative peroxidase activity of Lycopersicon esculentum and Syngonium Podophyllum leaves under light and dark conditions.

Treatment	Relative peroxidase activity. tomato	Relative peroxidase activity. Nephtytis
Control	100	100
12 h in dark	265	126
24 h in dark	240	220
48 h in dark	316	366
72 h in dark	431	586
72 h in light	502	764

On the other side, in tomato, while five polypeptide bands (225, 183, 42, 37 and 21 KDa) were disappeared, eight new polypeptide bands (188, 186, 153, 150, 129, 98, 36 and 19 KDa) were detected under dehydration condition (Fig. 2).

Gene expression in shade plants was strongly affected by dehydration especially in the presence of light, where many polypeptide bands were disappeared. On the other side, gene expression in tomato leaves was changed in three days dehydration where some bands disappeared but other polypeptides were newly detected. In xeric tolerant plant species, both NaCI and mannitol exhibit the same gene expression but the response of tomato to NaCI and mannitol was different

(Hassanein 2004$_a$). Dehydrin proteins were induced by environmental stresses such as dehydration (Hu et al. 2010). In accordance with other reports, plants respond to environmental stresses by induction or repression in the synthesis of few polypeptides.

The induced polypeptides may play direct or indirect role in the cellular adaptation to abiotic stress (Ramagopal 1988; Hassanein 1999; Hassanein 2004$_c$).

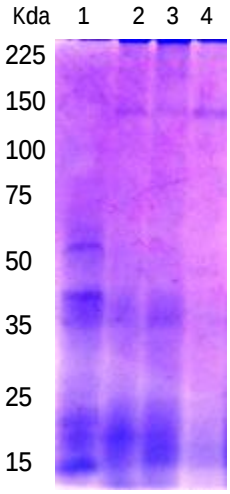

Fig. (1): Coommasseei blue- stained SDS-PAGE of polypeptides of nephtytis leaves under the influence of dehydration at light and dark conditions. The plant leaves were subjected for dehydration in dark for 48 (lane 2), and 72 hr (lane 3) as well as 72 hr in light (Lane 4). Control (lane 1): leaf obtained from plants grown under room condition under full field capacity.

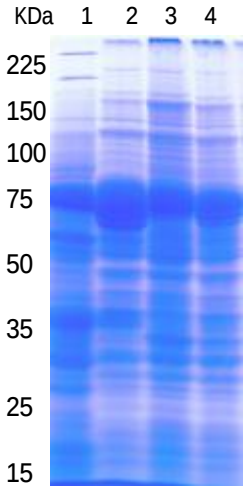

Fig. (2): Coommasseei blue- stained SDS-PAGE of polypeptides of tomato leaves under the influence of dehydration at light and dark conditions. The plant leaves were subjected for dehydration in dark for 48 (lane 2), and 72 hr (lane 3) as well as 72 hr in light (Lane 4). Control (lane 1): leaf obtained from plants grown under room condition under full field capacity.

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Journal of Environmental Studies (JES)

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