

COMPARATIVE EFFECTS OF STRESSFUL FACTORS ON GROWTH AND CARBOHYDRATE METABOLISM OF BROAD BEAN PLANTS

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ABSTRACT

The effects of heat, UV-irradiation and salinity on growth and metabolism of broad bean plants were investigated. Exposure of bean plants to these stressful factors induced variable significant decreases in the levels of growth parameters throughout the experimental period, as compared with control plants. In relation to controls, direct exposure of broad bean plants to heat, UV-irradiation and salinity stresses, induced significant variable changes in the soluble carbohydrate components. Concurrently with carbohydrate changes, significant variable increases in the activities of both invertase and α -amylase of broad bean plants were maintained throughout the entire period of the experiment.

INTRODUCTION

Adverse plant responses to stress conditions depend on the osmotic and toxic effects of the stressful factors and on the level and duration of the stress (Shalata and Neumann, 2001). Responses range from germination and growth inhibition and accelerated leaf senescence under moderate stress to permanent wilting of shoots with subsequent plant death under severe stress (Munns, 1993).

Plants require sunlight for photosynthesis and thus are constantly also exposed to potentially damaging ultraviolet radiation (UV) that is present in sunlight (Kucera *et al.*, 2003). Ultraviolet radiation is categorized into several components based on different wavelength ranges (UV-A 320-400 nm, UV-B 280-320 nm and UV-C shorter than 280 nm) in the biological sciences. Of these UV-B and UV-C have been known to affect most organisms harmfully. The negative effects of UV-A and UV-C radiation of various plant species result in deformed morphological parameters; decreased length of radicle, length of plumule, decreased leaf area per unit plant, biomass and dry mass accumulation (Weih *et al.*, 1998; Krizek *et al.*, 1998 and Zuk-Golaszewska *et al.*, 2003); and significant decrease in total carbohydrate content (Musil, 1996 and Saleh *et al.*, 2006).

Soil salinity, one of the major abiotic stresses reducing agricultural productivity, affects large terrestrial areas of the world (Yeo, 1999). The response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism (Mass and Hoffman, 1977 and Tarakcioglu and Inal, 2002).

Supra-optimal temperatures cause a major stress on crops (Singla *et al.*, 1997). Adverse effects of high temperature stress have been noted during both vegetative and reproductive stages in various crop plants. Processes

leading to floral development and quality of seeds are critically affected by high temperature stress (Grover *et al.*, 2000).

The present paper describes the modifications maintained in growth parameters, and the associated changes in carbohydrate metabolism in broad bean plants as a result of treatment with heat, UV- irradiation or salinity stresses.

MATERIALS AND METHODS

Plant material and growth conditions

In this investigation, a series of experiments, embodied in three separate sections were carried out. A homogeneously-sized lot of *Vicia faba* (cv. Egypt 1) seeds was selected and surface sterilized by soaking in 10^{-3} M mercuric chloride solution for 3 min, washed thoroughly with sterile distilled water and then soaked for 24 h in sterile distilled water at $25 \pm 1^\circ\text{C}$, with aeration to avoid anaerobiosis as a complicating factor. This lot of seeds was then divided into 3 groups; one group being used for each of the above mentioned stressful factors.

For the UV and heat experimental set up, the first two groups of seeds were sown in small plastic pots (12 cm in diameter), each containing 500 g clay-loamy soil (2:1, v/v). The pots were irrigated with tap water according to the usual practice and maintained at room temperature.

After 16 days from sowing the seeds, the first group of pots was subdivided into three sets; one being left without treatment and the other two sets were UV- irradiated for two hours, every other day, for 10 days; pots being quickly returned back to the original growth condition, after each treatment. The treatment scheme adopted can be summarized as follows:

- 1- Control.
- 2- Exposure of seedlings to UV-C radiation (254 nm).
- 3- Exposure of seedlings to UV-A radiation (365 nm).

All the exposure treatments were performed in UV light boxes (50 x 100 x 50 cm) in which UV radiation was supplied from a compact UV lamp, 254 nm and 365 nm (8 Wm^{-2}) (UVP factory, USA) which was suspended above and perpendicular to the germination boxes at a distance of 100 cm.

Also, after 16 days from sowing the seeds, the second group of pots was subdivided into three sets, one being left without treatment and the other two sets were subjected either to low (20°C) or high (40°C) temperatures. Treatment to either of the low or the high temperatures was carried out for 2 hours, every other day, for 10 days; pots being returned back to the original growth conditions, after each treatment. All the heat treatments were performed in growth chambers at controlled temperatures. The treatment scheme adopted can be summarized as follows:

- 1- Control.
- 2- Exposure of seedlings at low (20°C) temperature.
- 3- Exposure of seedlings at high (40°C) temperature.

After treatment, the remaining seedlings were transplanted into large pots (30 cm in diameter) containing 9 kg clay: loamy soil; 2:1, v/v) and left to grow

outdoor in the botanical garden. Uniform watering was carried out according to the usual practice and triplicate samples representing vegetative, flowering and fruiting stages were taken for analyses after 43, 60 and 85 days, respectively.

As for salinity treatment, the seeds of the 3rd group were sown in large pots (30 cm in diameter) containing equal amounts (9 kg) of homogeneous clay-loamy soil (2:1; v/v). After 16 days from sowing the seeds, this 3rd group of pots was divided into 3 sets, one being left as control and the other two sets were treated with NaCl to maintain salinity levels at 6 and 12 mmhos. Thus, appropriate amounts of NaCl were calculated and added with the irrigation water so as to maintain the required salinity levels. All pots were uniformly irrigated with tap water every three days so as to maintain the soil at the field capacity throughout the experimental period. For control as well as for the differently salinized plants, super-phosphate (0.8 g pot⁻¹) was applied with irrigation water every three weeks. Triplicate samples representing the vegetative, flowering and fruiting stages were taken for analyses after 43, 60 and 85 days from the date of sowing the seeds respectively.

It should be mentioned that the results obtained from the analyses of duplicate determinations and triplicate samples were remarkably close, thus the data presented in the corresponding tables are the means of triplicate samples.

Estimation of carbohydrates

The method of extraction of different carbohydrate fractions (glucose, fructose and sucrose), used was patterned after those adopted by Yemm and Willis (1954) and Van Handel (1968). In the ethanolic extract of the broad bean plants, glucose was estimated using the o-toluidine procedure of Feteris (1965). Fructose was estimated in the ethanolic extract using the resorcinol method of Roe (1934) as described by Devi (2002). Sucrose was determined by first degrading reactive sucrose present in 0.1 cm³ extract with 0.1 cm³ 5.4 N KOH at 97 °C for 10 min. Three cm³ of freshly prepared anthrone reagent [150 mg anthrone + 100 cm³ 72% (w/w) H₂SO₄] were then added to the cooled reaction products and the mixture was heated at 97 °C for 5 min, cooled and the developed colour was read at 620 nm, using spectrophotometer (Van Handel 1968).

Invertase activity

Extraction of crude invertase was performed using the method of Pressey and Avants (1980). The reaction mixture for the invertase assay consisted of 0.1 cm³ enzyme preparation, 0.2 cm³ of 0.1 M sodium acetate (pH 5), 0.1 cm³ of 0.15 M NaCl, and 0.1 cm³ of 0.73 M sucrose. The enzyme preparation was diluted with 0.15 M NaCl to produce approximately 1- μ mol reducing groups. The blank prepared for each sample was the same as the assay mixture but was heated before the addition of sucrose (Nelson 1944).

α -amylase activity

α -amylase was extracted and assayed according to the methods adopted by Gibbs (1955) and Street and Close (1956).

RESULTS AND DISCUSSION

Changes in growth parameters

Heat stress induced by exposure to low and high temperatures led to significant decreases in the growth parameters determined in broad bean plants at vegetative, flowering and fruiting growth stages, below the control values; the magnitude of decrease being higher with the high temperature (Table 1). Thus, it should be also mentioned that plants treated with the high temperature appeared to wilt at the vegetative stage and were dead at the flowering and fruiting stages and no samples were available for examination.

Irradiation of broad bean plants with UV-A and UV-C, in general, induced a progressive significant decrease in all the determined growth parameters, throughout the three successive stages of growth and development, as compared with control unstressed plants. The magnitude of decrease was higher with UV-C than with UV-A (Table 1).

On the other hand, broad bean plants stressed with the low concentration of NaCl was found either not to change or to show slight, if any negative or positive changes, as compared with control levels throughout the duration of the experimental period. With the high concentration of NaCl, all growth parameters determined showed significant marked decreases, throughout the three successive stages of growth and development, as compared with control levels (see table 1).

Younis *et al.* (2012) point out that because plants must be exposed to sunlight to possess photosynthesis, they are also exposed to high levels of UV radiations in the biosphere which might damage the performance of many crop species (Caldwell *et al.*, 2003). These induced negative alterations in all growth parameters may be attributed to photomorphogenetic mechanisms. Photomorphogenetic UV-radiation effects may be associated with changes in cell division and/ or cell elongation (Gehrke, 1999).

The negative effects of UV-A and UV-C radiations results in deformed morphological parameters. Exposure to UV-A and UV-C decreased length of root and length of shoot of whole plants and dry matter accumulation (Zuk-Golaszewska *et al.*, 2003 and Younis *et al.* 2012). Dai *et al.* (1995) and Younis *et al.* (2011) reported that after a few weeks of UV-B, UV-A and UV-C exposure, leaf area and plant dry weight of rice and broad bean were significantly reduced.

In accordance with the present results concerning the effects of salinity on growth parameters, Abd El-Samad *et al.* (2010) observed that dry mass, water content and leaf area of maize and broad bean plants decreased with increasing salinity. Munns (2002) stated that salinity reduces the ability of plants to take up water, and this quickly causes reductions in growth rate. High NaCl concentrations caused a great reduction in growth parameters such as leaf area, fresh and dry weight of leaves and roots of multigerm varieties of sugar beet (Ghoulam *et al.*, 2001).

Heat stress due to increased temperature is an agricultural problem in many areas in the world. Transitory or constantly high temperatures cause an array of morpho-anatomical, physiological and biochemical changes in plants, which affect plant growth and development and may lead to a drastic reduction in economic yield (Wahid *et al.*, 2007).

Heat stress affects plant growth throughout its ontogeny, though heat-threshold level varies considerably at different developmental stages. For instance, during seed germination, high temperature may slow down or totally inhibit germination, depending on plant species and the intensity of the stress. At later stages, high temperature may adversely affect photosynthesis, respiration, water relations and membrane stability, and also modulate levels of hormones and primary and secondary metabolites (Wahid *et al.*, 2007).

High temperatures caused significant declines in shoot dry mass, relative growth rate and net assimilation rate in maize, pearl millet and sugarcane, though leaf expansion was minimally affected (Ashraf and Hafeez, 2004; Wahid, 2007).

Major impact of high temperatures on shoot growth is a severe reduction in the first internode length resulting in premature death of plants (Hall, 1992).

Changes in carbohydrates and carbohydrate related enzymes

It is apparent from the patterns of changes reported in table 2, that treatment of broad bean plants with low and high degrees of temperature induced a progressive significant decrease in all soluble carbohydrate components detected below the control levels. The magnitude of response being more operative with the high temperature and also being most operative with the glucose component, at all stages of growth and development of the plants. For all carbohydrate components detected in plants under low and high salinity regimes, an opposite pattern of changes was maintained throughout the experimental period (Table 2).

Irradiation of broad bean plants with UV-A induced a progressive significant decrease in glucose, fructose, sucrose and total soluble saccharides content below the control levels, throughout the entire period of the experiment. On the other hand UV-C irradiation of broad bean plants induced a progressive significant increase in all carbohydrate fractions determined as well as in the total saccharides content, above the control levels, throughout the three successive growth stages (Table 2).

The changes in the activities of invertase and α -amylase in the variously treated broad bean plants, throughout the entire period of the experiment, are recorded in table 3. Broad bean plants treated with low temperature showed an appreciable significant increase in both invertase and α -amylase activities, above the control levels, throughout the experimental period. On the other hand, marked significant decreases in both invertase and α -amylase activities were obtained, at the vegetative stage, in response to treatment with high temperature; afterwards no samples were available because of death of treated plants.

Irradiation of broad bean plants with UV-A and UV-C induced a significant marked increase in both invertase and α -amylase activities, above the control levels, throughout the entire period of the experiment. The magnitude of increase was higher in plants irradiated with UV-C than in those plants irradiated with UV-A.

As for salinity treatments, plants treated with 6 mmhos NaCl were found to have increased levels of invertase and amylase activities above those of control levels. On the other hand, 12 mmhos NaCl induced a decrease in the activities of both enzymes. Both responses being insignificant at the vegetative stage, whereas significant responses were observed at flowering and fruiting stages.

In accord with the obtained results (NaCl – stressful factor), the most significant response of lupin plants to NaCl excess is the increase of sucrose content in leaves, which is partially due to increase in sucrose synthase activity under salinity (Fernandes *et al.*, 2004). Furthermore, Timpa *et al.* (1986) found that the salt-stressed cotton plants showed two to three times greater amounts of carbohydrates (glucose and sucrose) over the values determined for the control samples. A possible explanation for the increased total saccharide contents of salt treated broad bean plants should contribute to the maintenance of osmotic pressure in the rapidly expanding cells of broad bean (Pfeiffer and Kutschera, 1996).

The soluble sugars of broad bean shoots were decreased by heat. The contents of total saccharids were decreased significantly by heat stress (Hamada, 2001). With the demand for carbohydrates starch degradation increased, but that was not strictly associated with low levels of sucrose, glucose and fructose (Baur-Hoch *et al.* 1990). According to the theory of Miinch, the rate of translocation between the source leaf and the various sinks is determined by the sucrose gradient between the source and the sink regions (Hitz and Giaquinta, 1987). This reduction was associated with higher levels of sucrose and lower proportions of starch, thus suggesting a temperature-related decrease in the rate of starch synthesis (Wolf *et al.*, 1991).

Saleh *et al.* (2006), Abdel-Aziz (2008) and Younis *et al.* (2012), indicated that increasing supplemental doses of UV radiation significantly decreased the contents of total carbohydrates of certain plant species. Also in a long term study enhanced UV-radiation significantly decreased the ratio of storage starch to chloroplast area in field-grown silver birch leaves (Kostina *et al.*, 2001).

Broeckling *et al.* (2005) reported increases in the activities of some carbohydrate-related enzymes due to exposed of certain plant species to UV-radiation. Furthermore, Darbelley *et al.* (1997) investigated the changes in both invertase and α -amylase activities and in starch and free sugar contents in correlation with lipid mobilization in *Helianthus annuus*.

Generally we can possibly point out that, under stress conditions there are gradually increasing requirements to the plant to adjust itself to the environment and this is initiated by disturbance of normal metabolic processes. In some cases plants are able to restore their normal functions, which results in an augmented resistance to the respective stress factor

(adaptation). On the other hand, if the stress impact is superior to the adaptive capacity of the plant, then permanent damage occurs. So the nature of the stress is dual it is often destructive, but also under some circumstances it could be constructive, and the last appears to be the driving force of the adaptive evolution of the plant (Lichtenthaler, 1996; Alexieva *et al.*, 2003 and Younis *et al.*, 2012).

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مقارنة تأثير عوامل الاجهاد المختلفة على نمو و ايض الكربوهيدرات لنبات الفول
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يهدف البحث الى دراسة تأثير عوامل اجهاد مختلفة كالاجهاد الحراري ، الاجهاد الاشعاعي ، الاجهاد الملحي على نمو و ايض نبات الفول . حيث ادى تعرض نبات الفول لعوامل الاجهاد الى نقص معنوي في دلالات النمو المختلفة خلال فترة التجربة مقارنة بالعينات الضابطة . بالاضافة الى نقص معنوي في كمية الكربوهيدرات الذاتية و ذلك في حالة الاجهاد الحراري و الاشعاعي بينما كانت هناك زيادة في حالة الاجهاد الملحي و ايضا بتغيرات في نشاط الانزيمات المصاحبة لايض الكربوهيدرات مثل انزيمات الانفرتيز و الاميليز و ذلك خلال فترة التجربة.

قام بتحكيم البحث

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Table 1: Effects of heat, UV- irradiation and salinity on growth parameters (root length, shoot length, fresh mass, dry mass and water content) of broad bean plants, at different stages of growth and development.

Treatments		Root length (cm)	Shoot length (cm)	Whole plant length (cm)	Fresh Mass (g/plant)	Dry Mass (g/plant)	Water conten (g/plant)t
Vegetative stage	Control	16.2	30.6	46.8	15.2	3.1	12.1
	20 °C	13.6	23.0	36.6	13.6	2.8	10.8
	40 °C	10.2	21.9	32.1	12.8	2.41	10.39
LSD		0.5	0.5	0.5	0.8	0.8	1.0
Flowering stage	Control	16.6	32.4	49.0	15.9	3.25	12.7
	20 °C	15.7	24.2	39.9	14.2	2.9	11.3
	40 °C	---	---	---	---	---	---
LSD		0.4	0.5	0.6	0.6	0.3	0.5
Fruiting stage	Control	16.8	33.2	50.0	16.2	3.3	12.9
	20 °C	16.6	26.7	43.3	14.9	3.2	11.7
	40 °C	---	---	---	---	---	---
LSD		0.2	0.5	0.4	1.0	0.3	0.7
Treatments		Root length (cm)	Shoot length (cm)	Whole plant length (cm)	Fresh mass (g/plant)	Dry mass (g/plant)	Water content (g/plant)
Vegetative stage	Control	16.2	30.0	46.2	15.2	3.05	12.5
	UV- A	14.0	25.0	39.0	12.4	2.5	9.9
	UV- C	13.2	21.0	34.2	11.0	2.2	8.8
LSD		1.0	1.2	1.3	1.2	0.3	0.6
Flowering stage	Control	16.6	32.4	49.0	15.9	3.20	12.7
	UV- A	14.7	26.7	41.4	12.7	2.6	10.1
	UV- C	13.9	25.6	39.5	13.4	2.3	9.2
LSD		1.1	1.0	1.3	0.7	0.2	0.6
Fruiting stage	Control	16.8	33.2	50.0	16.2	3.29	12.9
	UV- A	14.9	27.8	42.7	13.2	2.7	10.5
	UV- C	14.4	25.8	40.2	12.7	2.4	10.3
LSD		0.6	1.1	1.3	1.3	0.14	0.6
Treatments		Root length (cm)	Shoot length (cm)	Whole plant length (cm)	Fresh mass (g/plant)	Dry mass (g/plant)	Water content (g/plant)
Vegetative stage	Control	12.5	32.8	45.3	17.0	3.8	13.2
	6 mmhos NaCl	12.9	32.9	45.8	16.5	3.7	12.8
	12 mmhos NaCl	10.2	25.0	35.2	13.4	2.5	11.0
LSD		0.8	1.4	1.3	1.7	1.7	0.8
Flowering stage	Control	13.1	38.5	51.7	20.4	4.3	20.1
	6 mmhos NaCl	14.9	39.7	54.6	20.1	4.0	16.1
	12 mmhos NaCl	10.9	26.7	37.6	14.0	3.0	11.0
LSD		1.7	1.1	1.1	0.9	0.8	0.9
Fruiting stage	Control	13.2	49.8	63.1	23.4	4.5	23.4
	6 mmhos NaCl	15.2	46.8	62.0	23.4	4.2	19.2
	12 mmhos NaCl	11.3	26.8	38.1	15.6	3.1	11.9
LSD		1.1	1.3	1.2	0.6	0.7	0.6

---: plants exposed to 40 °c died and samples were not available.

Table 2: Effects of heat, UV-irradiation and salinity on carbohydrate content of broad bean plants, at different stages of growth and development. All values given are expressed as mg glucose equivalent 100 g⁻¹ dry

Treatments		Glucose	Fructose	Sucrose	Total soluble saccharides
Vegetative stage	Control	162.1	70.1	103.0	335.2
	20 °C	160.0	62.1	95.6	317.7
	40 °C	149.2	50.6	81.2	296.4
	LSD	3.8	6.1	5.6	16.3
Flowering stage	Control	179.4	79.2	92.2	350.8
	20 °C	162.1	70.2	82.4	314.7
	40 °C	---	---	---	---
	LSD	5.0	3.5	3.5	8.5
Fruiting stage	Control	190.6	82.1	86.3	359.0
	20 °C	169.0	79.1	76.1	324.2
	40 °C	---	---	---	---
	LSD	5.3	3.1	3.2	18.1

Treatments		Glucose	Fructose	Sucrose	Total soluble saccharides
Vegetative stage	Control	162.1	70.1	103.1	335.3
	UV- A	150.0	61.0	93.1	304.1
	UV- C	181.1	90.2	123.1	394.4
	LSD	4.0	4.2	5.2	14.4
Flowering stage	Control	179.4	79.2	92.2	350.8
	UV- A	156.2	64.2	81.0	301.4
	UV- C	196.7	101.2	117.0	414.9
	LSD	7.2	5.4	8.0	14.7
Fruiting stage	Control	190.6	82.1	86.3	359.0
	UV- A	164.3	69.1	73.0	306.4
	UV- C	211.7	109.7	109.2	430.6
	LSD	6.6	9.2	3.9	17.7

Treatments		Glucose	Fructose	Sucrose	Total soluble saccharides
Vegetative stage	Control	143.0	102.0	92.6	337.6
	6 mmhos NaCl	154.2	112.1	119.4	385.7
	12 mmhos NaCl	172.1	126.4	128.0	426.5
	LSD	5.2	4.9	5.5	15.0
Flowering stage	Control	151.0	112.6	99.2	362.8
	6 mmhos NaCl	162.0	121.0	126.0	409.0
	12 mmhos NaCl	181.6	137.0	136.0	454.6
	LSD	6.2	5.5	6.0	15.7
Fruiting stage	Control	154.1	116.7	158.0	428.8
	6 mmhos NaCl	166.0	129.2	170.0	465.2
	12 mmhos NaCl	189.2	141.2	193.0	523.4
	LSD	7.7	7.7	8.5	19.9

--- : Plants exposed to 40 °C died and samples were not available.

Table 3: Effects of heat, UV-irradiation and salinity on invertase and α - amylase enzyme activities; expressed as Units (100 cm³)⁻¹ enzyme preparation of broad bean shoots, at different stages of growth and development.

Treatments		Invertase	α - amylase
Vegetative stage	Control	112.6	24.0
	20 °C	132.1	29.6
	40 °C	63.4	18.6
LSD		3.1	3.1
Flowering stage	Control	114.8	25.6
	20 °C	136.7	36.3
	40 °C	---	---
LSD		2.9	3.1
Fruiting stage	Control	115.2	26.4
	20 °C	139.3	38.0
	40 °C	---	---
LSD		3.5	2.8
Treatments		Invertase	α - amylase
Vegetative stage	Control	112.4	24.1
	UV- A	114.6	25.9
	UV- C	116.9	27.7
LSD		4.6	3.6
Flowering stage	Control	114.8	25.7
	UV- A	118.3	27.2
	UV- C	122.1	29.9
LSD		5.5	4.8
Fruiting stage	Control	115.2	26.5
	UV- A	119.3	28.8
	UV- C	124.6	31.3
LSD		6.7	4.8
Treatments		Invertase	α - amylase
Vegetative stage	Control	66.3	11.9
	6 mmhos NaCl	69.2	13.9
	12 mmhos NaCl	60.2	10.0
LSD		7.5	2.4
Flowering stage	Control	69.4	13.8
	6 mmhos NaCl	76.6	15.9
	12 mmhos NaCl	62.3	11.6
LSD		4.6	1.8
Fruiting stage	Control	72.2	16.2
	6 mmhos NaCl	79.3	19.2
	12 mmhos NaCl	69.1	13.9
LSD		5.3	2.3

--- : Plants exposed to 40 °C died and samples were not available.

