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PHYSIOLOGICAL EFFECTS OF CHILLING STRESS ON GROWTH AND PHOTOSYNTHETIC CAPACITY OF GERMINATING BROAD BEANS.

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ABSTRACT

Exposure of seven day-old broad bean seedlings to chilling temperature (5°C) daily for 21 days, led to significant decrease in all growth parameters determined (length of radicle, length of plumule, water content, fresh mass and dry mass accumulation) as compared with control seedlings. Significant increases were observed in the amount and in the relative composition of photosynthetic parameters (Chl a, Chl b, Chl a+b, Chl a/b, Cars, total pigments) of the variously treated broad beans in relation to control samples. Photosynthetic activity (photosystem (PS) II activity) of the treated broad beans showed significant decrease as compared with control seedlings throughout the entire period of the experiment. These results are discussed mainly on the basis of the mechanism of action of chilling stress on growth and metabolic changes in broad beans during germination.
Keywords: Chilling stress, germination, growth parameters, photosynthetic pigments, photosystem II activity, *Vicia faba* L.

INTRODUCTION

Faba bean (*Vicia faba* L.) is a major food and feed legume because of the high nutritional value of its seeds, which are rich in protein and starch. Seeds are consumed dry, fresh, frozen or canned. In cool-temperate climates, faba bean is widely grown as a spring crop because of the insufficient winter-hardiness of the current autumn-sown germplasm (Arbaoui *et al.*, 2008). Recent studies have shown the superiority of grain yield in winter beans, as compared to spring beans (Ghaouti, 2007). Therefore, improving winter-hardiness is important to promote faba bean cropping in these climates. However, the irregular occurrence of appropriate natural freezing temperatures and the complexity of winter-hardiness reduce the selection efficiency for this trait. The winter-hardiness of a plant depends mainly on (1) its frost tolerance, (2) its resistance against biotic stress such as snow mould, and (3) its tolerance to adverse abiotic conditions such as levels of saturation of soils with water (Badaruddin and Meyer, 2001).

Chilling injury occurs in the absence of ice nucleation, and involves physical and physiological damage when sensitive plants are exposed to low, but non-freezing temperatures, generally between 15°C and 0°C (Raison and Lyons, 1986). The term chilling applies to the treatment itself, and involves the duration and temperature of the exposure, while the term chilling stress refers to the action of the low temperature that results in chilling injury (Saltveit and Moris, 1990). The symptoms of chilling injury vary, depending on the duration and temperature of the exposure, species, developmental stage, tissue and other factors. Symptoms commonly include cellular changes, altered metabolism, reduced growth, surface lesions, water-soaking of the tissue, accelerated senescence, discoloration, loss of vigor, decay and eventually death (Simonović, 2006).

The development of chilling injury can be considered as a two-stage process: the primary event instantaneously occurs at some threshold temperature, and may include structural or conformational changes of membranes, proteins, and cytoskeleton, which are, in short term, reversible. This is followed by a series of secondary events, which are time and temperature dependent and can include metabolic and ionic imbalances and loss of cellular integrity (Raison and Lyons, 1986). One of the most prominent secondary events is the development of oxidative stress in response to disruption of redox enzyme systems or electron transport chains (Simonović, 2006).

Thus the objective of this study was to investigate the effects of chilling stress on growth and photosynthetic capacity of broad bean seedlings (*Vicia faba* L. c.v. Giza 3) throughout the entire period of the experiment.

MATERIALS AND METHODS

Plant material and experimental design:

Broad bean (*Vicia faba* L. cv. Giza 3) seeds of similar size and appearance were selected. Seeds were sterilized with 2.5% sodium hypochlorite solution for 15 min. and washed with distilled water. These seeds were then germinated in plastic boxes (25 cm in length × 10 cm in width) half filled with acid-washed sand (Hewitt, 1966) moistened by adding 100 cm^3 of dist. water. The germination boxes were incubated at room temperature for 7 days. After seven days, seedlings were transferred to hydroponic culture in plastic containers containing 400 cm^3 of 1/4 full-strength Hoagland solution (Hershy, 1995) with continuous aeration for a period of 14 days with 12 h photoperiod (Photon flux density of 250 µmol m^{-2} s^{-1}). The 1/4 full-strength Hoagland solution was changed every two days to avoid depletion of macronutrients from the solution. The seedlings were divided into two groups one incubated at 5°C and the other left as control to grow at room temperature.

Growth parameters:

Length of root, length of shoot, fresh weight, dry weight and water content were estimated after 7 and 14 days from start of treatment.

Determination of pigments:

Photosynthetic pigments (Chl a, Chl b and carotenoids) were determined using the spectrophotometric method as described by Metzner *et al.* (1965). A known fresh weight of 21 day old broad bean leaves was homogenized in 85% aqueous acetone for 5 min. The homogenate was suction filtered through Whatman No. 1 paper. The filtered extract was made up to volume with 85% aqueous acetone. The extract was measured against a blank of pure 85% aqueous acetone at three wavelengths of 452.5, 644 and 633 nm using a spectrophotometer.

PS II activity assay:

As described by Arnon (1949), leaf discs were used for preparation of chloroplast pellets that were suspended in 1mM Tric-NaOH (pH 7.8), 10 mM NaCl and 10 mM $MgCl_2$ and then kept at 0-4°C until required. PS II activity, as indicated be the rate of 2,6 dichlorophenol indophenol (DCPIP) photoreduction was monitored at 600 nm using a spectrophotometer.

The full data of the different stressed groups of seedlings were statistically analyzed using one-way analysis of variance (ANOVA) and comparison among means was carried out by calculating the Post Hoc L.S.D. with a significant level at $*P < 0.05$. All the analyses were made using the SPSS 13.0 for Windows software package (SPSS Inc., Chicago, IL, USA).

For better quantitative comparison among the different treatments, the percentage of change (increase or decrease) in response to each treatment, in relation to control level, was calculated (Hasaneen *et al.*, 2009) throughout this investigation as follows:

- Percentage change (increase or decrease) immediately after each specific treatment: [(level after treatment – control level) / control level] × 100.

RESULTS AND DISCUSSION

Changes in growth parameters:

The results depicted in table (1) show that there was a steady increase in all growth parameters (length of radicle, length of plumule, fresh mass accumulation, dry mass accumulation and water content) measured in broad bean seedlings exposed to chilling stress in relation to control seedlings germinated at room temperature, throughout the entire period of the experiment.

Exposure of broad bean seedlings to low temperature (5°C) involved significant variable decrease in all growth parameters measured, as compared with those growth parameters measured in control seedlings throughout the entire period of the experiment.

In general the magnitude of decrease (mainly expressed in terms of percentage change) in all growth parameters determined in chilled broad bean seedlings were more pronounced in 14-d chilled broad beans than in 21-d chilled ones (Table 1). In this connection, germination capability and seed vigor are two important characteristics of seed that may be affected by environmental factors such as temperature (Carter *et al.*, 2003), light intensity (Benvenuti *et al.*, 2001), photoperiod (Kurt, 2010), etc.

Farooq *et al.* (2008) reported that germination, early seedling growth, membrane stability, relative water content (RWC), starch metabolism, soluble sugars and antioxidant activities of maize seedlings were significantly affected by chilling stress. Chilling stress increased electrolyte leakage, superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities, and decreased shoot and root length, seedling fresh and dry weights, leaf and root score, RWC, starch metabolism and soluble sugars than at optimal temperature.

Table1 : The effects of chilling temperature (5°C) on growth parameters: length of radicle (cm seedling^{-1}), length of plumule (cm seedling^{-1}), fresh mass (g seedling^{-1}), dry mass (g seedling^{-1}) and water content (g seedling^{-1}) of *Vicia faba* seedlings. * Mean values are significantly different from control at P ≤ 0.05.

Growth stage / Treatments	After 14 days									
	Fresh mass	**% change**	**Dry mass**	**% change**	**Water content**	**% change**	**Length of radicle**	**% change**	**Length of plumule**	**% change**
Control (25 ± 0.1°C)	6.04	-	0.82	-	5.22	-	23.15	-	27.80	-
Chilling (5°C)	3.85*	-36.26	0.78	-4.88	3.07*	-41.19	10.10*	-56.37	10.25*	-63.13
Growth stage / Treatments	**After 21 days**									
	Fresh mass	% change	Dry mass	% change	Water content	% change	Length of radicle	% change	Length of plumule	% change
Control (25 ± 0.1°C)	7.59	-	0.95	-	6.64	-	24.20	-	29.80	-
Chilling (5°C)	5.10*	-32.81	0.83*	-12.63	4.27*	-35.69	12.55*	-48.14	18.00*	-39.60

Decreased growth parameters (length of radicle, length of plumule, fresh mass, dry mass and water content) of 14-d and 21-d old chilled broad bean seedlings, in the present study are likely the result of lower rates of CO_2 assimilation and membrane damage in seedlings germinated under low temperature. Changes in biomass enhanced by chilling temperature which was observed in the broad bean seedlings under investigation may increase their environmental stress tolerance. Changes in plant height often occurs in conjunction with change in stem diameter and self-shading by foliage, which reduces heat load at the base of the seedlings and minimizes cellular damage that occurs at high surface soil treatments (Helgerson, 1990).

The symptoms of chilling injury vary, depending on the duration and temperature of the exposure, species, developmental stage, tissue and other factors. Chilling stress as abiotic stress produced harmful free radicals which cause harmful effects on faba bean growth, including cellular changes, altered metabolism, reduced growth, discoloration, loss of vigor, decay and eventually death (Simonović, 2006).

El-Saht (1998) demonstrated that low temperature treatment induced significant reduction in growth parameters in comparison with controls at all growth stages, the decrease was more pronounced at long-term than short-term chilling.

Changes in photosynthetic capacity:

Exposure of broad bean seedlings to chilling temperature (5°C), resulted in the increase of the synthesis of chloroplast pigments (Chl a, Chl b and carotenoids) in broad beans seedlings. This treatment resulted in an increase in chlorophyll content (table 2). The Chl a/b ratio, Chl a+b and total pigments content of the chilled broad bean seedlings showed variable significant increase above the control levels, throughout the entire period of experiment.

Photosynthetic capacity (PS II) was significantly decreased in broad bean seedlings treated with low temperature (5°C) as compared with control seedlings (Table 2).

Table 2: The effects of chilling temperature on photosynthetic pigments (µg / g fresh weight) and PSII activity (µM DCPIP reduced/ mg Chl /h) of *Vicia faba* seedlings.* Mean values are significantly different from control at P ≤ 0.05.

Growth stage / Treatments	After 21 days											
	Chl a	%change	Chl b	%change	Chl a+b	Chl a/b	Car	% change	Total pigments	%change	PSII	% change
Control (25 ± 0.1°C)	433.96	-	188.24	-	622.20	2.31	178.96	-	801.16	-	0.83	-
Chilling (5°C)	520.75*	+20.00	193.38*	+2.73	714.13*	2.69*	201.30*	+12.48	915.43*	+14.26	0.26*	-68.67

Margulies and Jagendorf (1960) reported that leaves of chilling-sensitive plant bean rapidly lost Hill activity when stored at 0°C. Subsequently, it was shown that the extent of the decrease in Hill activity in chloroplasts isolated from chilled leaves of different species of tomato (Smillie and Nott, 1979) and passionfruit (Smillie, 1979) is related to plant chilling tolerance. Inasmuch as the inhibition develops on the photooxidizing side of photosystem II (PSII) (Smillie and Nott, 1979), which therefore result in a decrease in the yield of chlorophyll (Chl) contents (Smillie and Hetherington, 1983).

Experiments comparing the photosynthetic responses of a chilling-resistant species (*Pisum sativum* L. cv Alaska) and a chilling-sensitive species (*Cucumis sativus* L. cv Ashley) have shown that cucumber photosynthesis is adversely affected by chilling temperatures in the light, while pea photosynthesis is not inhibited by chilling in the light (Peeler and Naylor, 1988). During a whole plant chilling, thylakoids isolated from cucumber plants chilled in the light were uncoupled even when the membranes were isolated at warm temperatures. Pea thylakoids were not uncoupled by the whole-plant chilling treatment. The difference in integrity of thylakoid membrane coupling following chilling in the light demonstrates a fundamental difference in photosynthetic function between these two species that may have some bearing on why pea is a chilling-resistant plant and cucumber is a chilling-sensitive plant (Peeler and Naylor, 1988).

Tsonev *et al.* (2003) reported that for *Phaseolus* plants grown at low temperature (10°C) for 6 d the total Chl content was significantly low. The ratio of Chl a/b did not change significantly as a result of low temperature treatment. Concentrations of total carotenoids (Car) were greater for plants growing at low temperature. A chronic decrease in the efficiency of photosynthetic electron transport through PSII was found in bean plants grown at low temperature. This can be interpreted as an increase in the rate constant for thermal dissipation (Saccardy *et al.*, 1998) and/ or inactivation of reaction centers of PSII (Le Gouallec *et al*., 1991).

Absorbed radiation energy from sunlight surpasses the capacity of chloroplasts to use it in CO_2 fixation, and the glut energy is alternatively used to convert O_2 to reactive oxygen species (ROS) under chilling stress (Apel and Hirt, 2004). Photosynthetic electron transport, stomatal conductance, Ribulose-1,5-Bisphosphate Carboxylate/Oxygenase (Rubisco) activity and CO_2 fixation are the major targets impaired by low-temperature stress in plants (Allen and Ort, 2001).

The present results concerning the increase in Chl a, Chl b, Chl a+b, Chl a/b ratio and in consequence increase in total pigment components of chilled broad bean seedlings compared to non-chilled control, throughout the entire period of he experiment, may be attributed that low temperature stress increases total chlorophyll content of chilled broad bean seedlings by decreasing the activity of chlorophyll degrading enzyme chlorophyllase (Rao and Rao, 1981; El-Saht, 1998), inducing the construction of chloroplast structure and stability of pigment-protein complexes (Singh and Dubey, 1995).

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التـأثيرات الفسـيولوجية للإجهـاد بالتبريـد علـى النمـو و كفـاءة البنـاء الضـوئى لبـذور الفول النـابتة

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أجريت فى هذا البحث تجربـة لدراسـة تـأثير درجـات الحـرارة المنخفضـة (درجـة التبريـد) علـى نمـو و كفـاءة عمليـة البنـاء الضـوئى فـى بـادرات الفـول البلـدى الناميـة علـى محلـول هوجلانـد المغذى. أدى تعريض بادرات الفول النابتة للإجهاد بالتبريد عند درجة حرارة ٥ درجـة مئويـة يوميـا لمدة ٢١ يوما من الإنبات إلى نقص معنوى فى كل دلالات النمو المختلفـة للبـادرات (طـول الجـذير- طول الريشة – المحتوى المائى – الـوزن الطـازج و الـوزن الجـاف) بالمقارنـة بالبـادرات الضـابطة. كذلك لوحظ زيادة معنوية فى المكونات النسبية لدلالات البناء الضوئى (كلوروفيل أ ، كلوروفيل ب ، كلوروفيل أ+ب ، الكاروتينات ، نسبة كلورفيل أ إلى كلوروفيل ب ، و المحتوى الصبغى) لبادرات الفول المجهدة عند مقارنتها بالعينات الضابطة. كذلك أدت معاملة بادرات الفول بالتبريـد إلـى حـدوث زيادة معنوية فى نشاط المسار الضوئى (٢) للبـادرات خـلال فتـرة التجربـة و عنـد مقارنتهـا بالعينـات الضـابطة. و لقد تم تفسير النتائج المتحصل عليها فى ضوء الميكانيكيات المنظمـة لتـأثير نمـو و أيـض البادرات بالإجهاد بالتبريد.

قام بتحكيم البحث

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