Biophysical And Interaction Effect Of UV-B Radiation And Water Stress On *Cucurbita Pepo* L.

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Abstract. UV-B radiation substantially reduced the growth criteria of 22- days old squash (Cucrbita pepo L) seedlings. This reduction increased by the exposure time to UV-B radiation. The inhibition of shoot growth was accompanied by reduction in levels of carbohydrate and phosphorus fractions, as well as in nitrate reductase activity. The activities of alkaline and acid phosphates slightly increased. The sensitivity of squash to UV-B irradiation decreased by subsequent exposure to visible light. Moreover, irradiation of water - stressed plants for short time with UV-B followed by exposure to visible light induced a great recovery in squash growth and all of measured metabolic parameters as compared with the drastic effects induced by prolonged exposure to UV-B radiation. These results emphasize the usefulness of photo reactivation by realistic level of visible light that can minimize the harmful effect of prolonged UV-B irradiation time.

Key words: Solar UV-B radiation, Water stress, *Cucurbita pepo* L. photo reactivation.

Introduction

Plants grown under natural conditions are usually subjected to different types of stresses that often interact with each other and affect plant growth and development. Solar ultraviolet-B (UV-B, between 280 and 313 nm) radiation has been recognized as an environmental stress. This radiation is of particular interest to photobiologists and biophysist since it can be intensified by partial depletion of the stratospheric ozone layer (Blumthaler and Ambach, 1990; Kerr and McElroy, 1993). Moreover, the increase in the level of UV-B radiation constitutes a significant stress on the productivity of many terrestrial plants.

Numerous studies were shown that the higher levels of UV-B radiation restrict the normal growth of many economically important crop plants (Tevini and Teramura, 1989; Dai, *et al.*, 1992; Ballare *et al*, 1995, Al-Ayed;1998). Chloroplast is a major site of damage induced by UV-B irradiance which leads to impairment of photosynthetic capacity and causes a decline in enzyme activity and protein levels (Boniman, 1989; Liu, *et al.*, 1995; Mackerness *et al*, 1997). On the other hand, water stress is one of the most common limiting factors to plant productivity; as it causes changes in plant growth and many physiological and biochemical processes (Chavan and Karadge, 1986; Kameli and Losel, 1993; Al-Ayed, 1998).

The interaction between UV-B radiation and water stress has received little attention and showed an additive inhibitory effects on plant growth, pigmentation, net photosynthesis and protein levels as compared with plants exposed to either stress singly (Sullivan and Teramura, 1990; Balakumar *et al.*, 1993).

Photoreactivation by simultaneous or subsequent exposure of plants to sunlight, visible light (which is part of sunlight) or high photosynthetic photon flux densities (PPFD) during or after UV-B radiation has been reported to be an effective mechanism in repairing the effect of UV-B singly (Mirecki and Teramura, 1984; Beggs *et al.*, 1986; Brift, 1995) and in combination with water stress (Al-Ayed, 1998). Therefore, the purpose of the present study is to further examine the interactive effects of UV-B radiation and mild water stress on the growth and some metabolic changes, including carbohydrate and phosphorus levels, and some related enzyme activity in squash seedlings. Moreover, the photoreactivation response of squash seedlings by subsequent exposure to natural visible light is also examined.

Materials and Methods

Squash seeds (*Cucurbita pepo* L.) were surface sterilized with 0.1% HgCl for 5 min., thoroughly washed and soaked in distilled water for two hours. Presorted seeds were germinated for 4 days. Germination was carried out in germinating trays lined with two sheets of filter paper and wetted with distilled water for 4 days. Fifty seedlings of uniform size were transplanted into perforated plastic sheets covering plastic plates (30 x 20 x 7 cm) containing $1/10^{th}$ strength of Hoagland nutrient solution (Hoagland and Arnon, 1950). Other seedlings were subjected to mild stress induced by NaCl, to give an osmotic pressure of -4 bars. The nutrient solution was renewed every 4 days. All culture plates were kept under natural conditions in a glasshouse. The photoperiod was approximately 14 hours and the day/night temperature was about 27/18°C±2. The seedlings received UV-B radiation according to the following time - course treatments:

Treatment 1 (T_1): Seedlings irradiated for 18 days. Treatment 2 (T_2): Water -stressed seedlings irradiated for 18 days. Treatment 3 (T_3): Seedlings irradiated for 8 days. Treatment 4 (T_4): Water-stressed seedlings irradiated for 8 days.

The plants of the last two treatments were left under natural daylight for another 10 days up to the time of harvest. A completely randomized experimental design was adopted with six replications for each irradiation treatment, as well as non-irradiated control and water-stressed treatments.

UV-B radiation was artificially supplied by fluorescent lamps (Phillips; 40W, type TL/33) suspended above the plants, at 40 cm distance. The daily irradiation was carried out by placing the culture plates of each treatment under the UV-B lamps for 1, 2 or 3 hours, centered at solar noon.

All seedlings were harvested after 22 days from the start of germination time. Fresh and dry weight of both roots and shoots as well as their water content were determined. For the biochemical analysis, samples of dried shoots were used to determine sugar and phosphorus. The reducing values of soluble and hydrolyzed insoluble carbohydrates were assayed by Nelson test (Clark and Switzer, 1977) and phosphorus fractions were determined by Fiske-Subbarow procedure as outlined by Clark and Switzer (1977). In the fresh shoot samples, nitrate reductase activity was assayed by the method recommended by Harper and Hageman (1972), whereas the activities of acid and alkaline phosphates were assayed according to Bergmeyer (1974). The data were statistically analyzed on the basis of analysis of variance by the two-way ANOVA procedure (Campbell, 1996).

Results and Discussion

The growth of 22-days old squash seedlings was sensitive to increasing the time of exposure to UV-B radiation. Plants exposed to prolonged UV-B radiation showed a significant reduction in root and shoot fresh and dry weights as well as their water content relative to non-irradiated control plants (Fig.1). Such reduction was less pronounced in plants irradiated for short period followed by subsequent exposure to visible light (T_3). The deleterious effect of UV-B radiation on squash growth was also demonstrated in many other plant

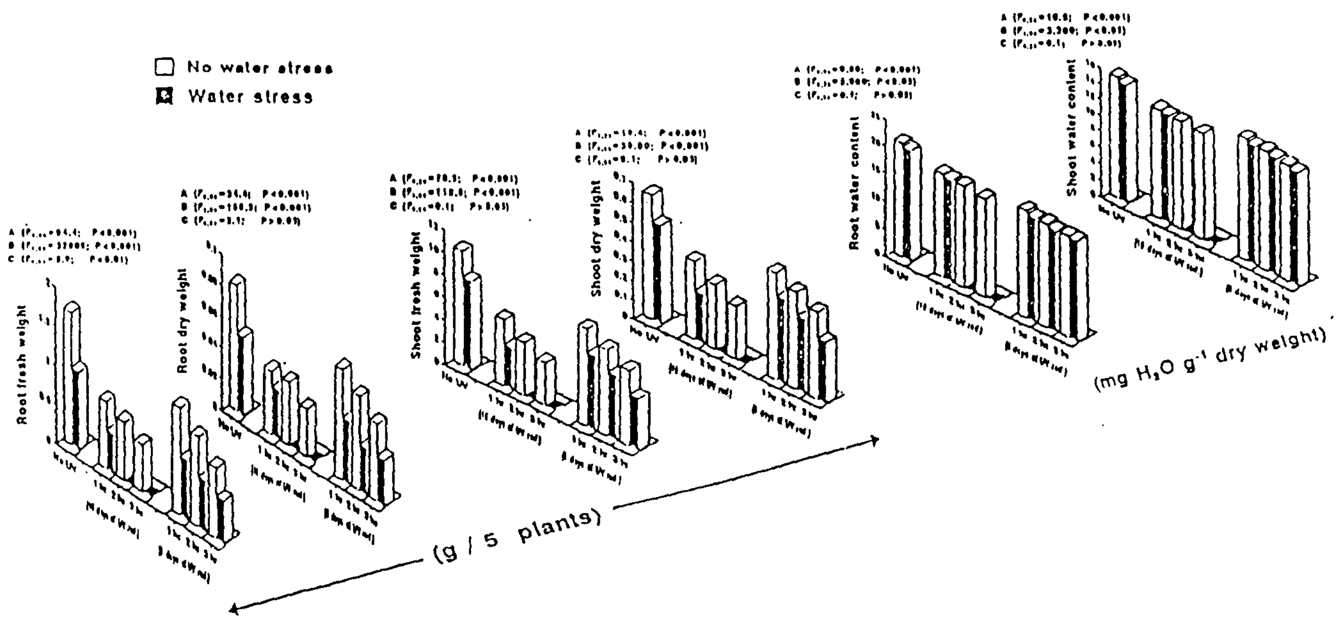


Fig. 1. Effects of UV-B radiation on fresh, dry weight and water contents of root and shoot of *Cucurbita pepo* L. grown under normal and water stress conditions. (A) UV-B treatments, (B) water stress treatments, (C) interaction between UV-B and water stress.

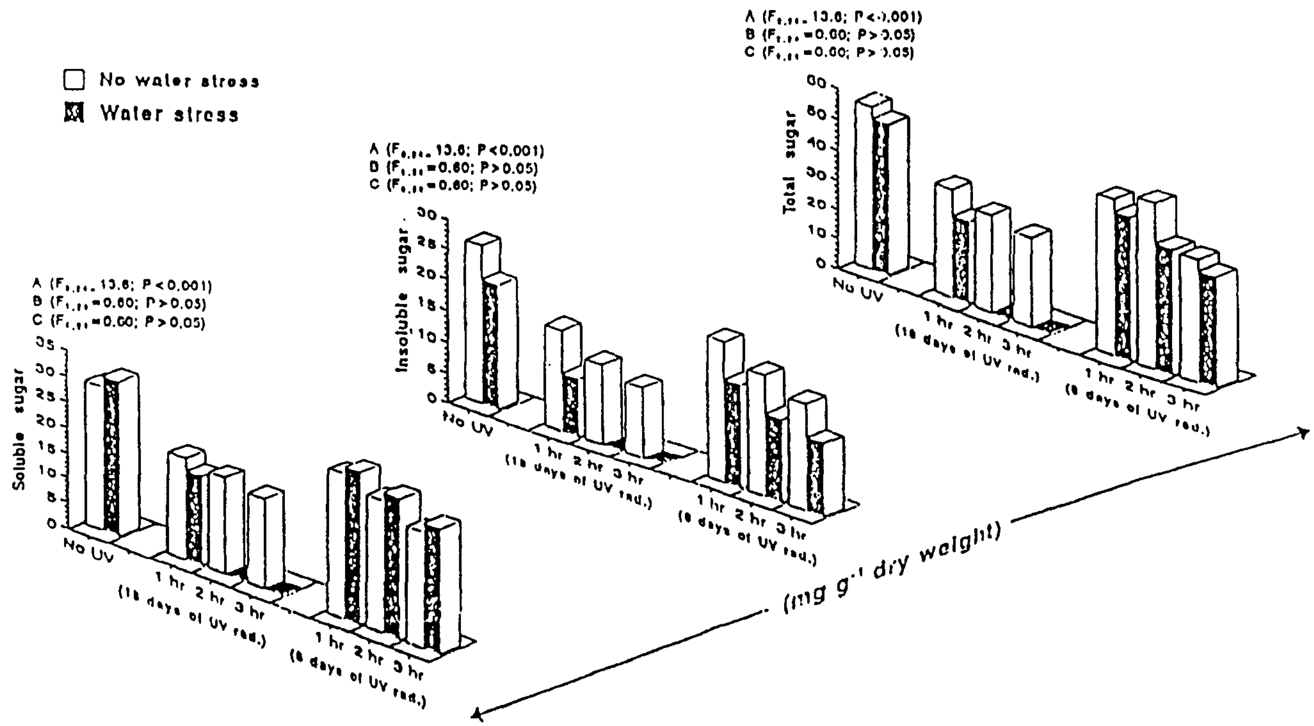


Fig. 2. Effects of UV-B radiation on the sugar contents of the shoots of *Cucurbita pepo* L. grown under normal and water stress conditions. (A) UV-B treatments, (B) water stress treatment, (C) interaction between UV-B and water stress.

species at different irradiation conditions (Dai *et al.*, 1992; Liu *et al.*, 1995; Singh, 1996). The amelioration of squash growth under treatment 3 as compared with treatment 1 indicates the importance of photoreactivation by visible light to repair the harmful effect of UV-B radiation. These results are in accordance with that obtained by Mirecki and Teramura (1984), Beggs *et al.* (1986) and Britt (1995). They reported that the harmful effect of UV-B radiation could be photorepaired by simultaneous or subsequent exposure to visible light, sunlight or high PPFD.

Under mild water stress, the growth of squash seedlings was significantly inhibited as compared with that under unstressed condition. The reduction in root growth was more pronounced than shoot growth, whereas the water content of both organs was slightly affected (Fig.1). A similar reduction in the growth of different plant species grown under saline conditions has been reported by Vyas et al. (1985), Chavan and Karadge (1986), and Hamed and Al-Wakeel (1994).

The combination of water stress with prolonged period of UV-B radiation (T_2) induced an additive injurious effect on seedling growth relative to unstressed seedlings irradiated for long period (T_1). Such combination produced a seek and poor growth when irradiation was one-hour daily, whereas those irradiated for two or three hours daily revealed an eventual plant death. Irradiation of water-stressed plants for a short period with UV-B followed by subsequent exposure to visible light (T4) induced a great recovery in root and shoot fresh and dry weights as well as their water content as compared with the damaging effect observed under treatment T_2 (Fig.1). These observations emphasize the usefulness of exposing the water stressed plants to realistic level of visible light after UV-B irradiation that can minimize the harmful effect induced by prolonged UV-B treatment. The additive inhibitory effect on squash growth resulting when prolonged UV-B radiation was combined with water stress was previously demonstrated on other plant species by Tevini *et al.* (1983), Sullivan and Teramura (1990) and Balauumar *et al.* (1993). Such effect was attributed to cell membrane damage and tissue deterioration induced by using higher levels of UV-B irradiation (Dai *et al.* 1992). Moreover, photoreactivation by subsequent exposure to visible light can repair the epidermis and inner cell membranes of leaves and cell organelles that contain DNA including the nucleus, chloroplast and mitochondria (Britt, 1995; Stapleton *et al.*, 1997).

The variations in seedling growth parameters under the different treatments may reflect an integration of changes in physiological and biochemical processes occurring during the earlier growth stages. The present results showed that the inhibition of shoot growth was associated with a significant reduction in the total sugar content when squash seedlings were exposed to UV-B radiation and water stress either singly or in combination (Fig.2). The magnitude of reduction increased by the exposure time. Moreover, the decline in the level of insoluble carbohydrate was more pronounced than in the soluble fraction. UV-B radiation induced a significant reduction in soluble and insoluble carbohydrate in plants irradiated for a long time (T1) compared with those irradiated for a shorter period (T3) where the reduction increased as the daily exposure time was increased. In this connection, UV-B radiation has been shown to reduce photosynthetic capacity in leguminous plants both in field and in growth chamber studies (Sullivan and Teramura, 1990; Singh, 1996), and reduction could be related to the decline in chlorophyll content and stomatal conductance (Dai *et al.*, 1995; Liu et al., 1995).

The decline in total sugar content of squash shoots by water stress was related to the significant reduction in the insoluble sugar fraction, whereas the soluble fraction increased (Fig.2). This effect is considered a general response to water stress in many plant species (Chavan and Karadge, 1986; Kameli and Losel, 1993, Hamed and Al-Wakeel, 1994). UV-B radiation in combination with water

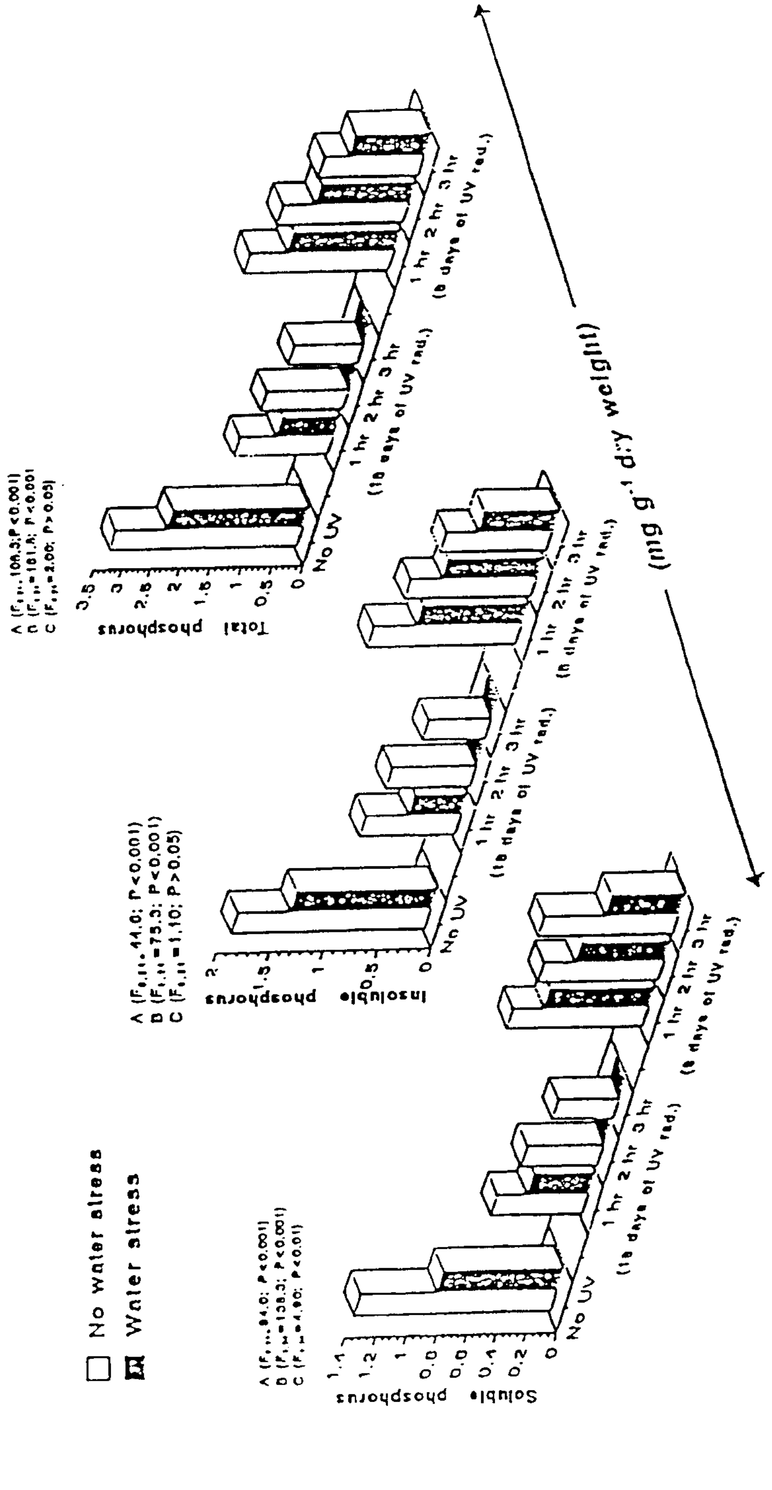
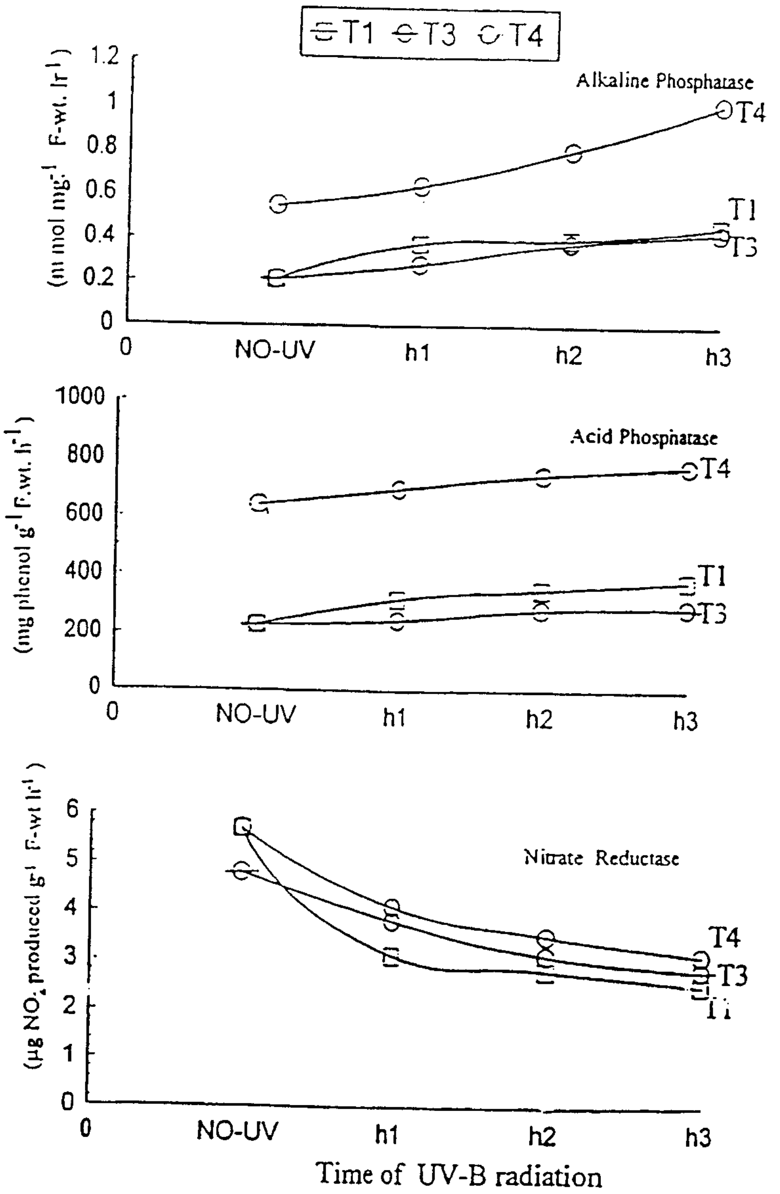


Fig. 3. Effects of UV-B radiation on the phosphorus contents of the shoots of *Cucurbita pepo* L. grown under normal and water stress conditions. (A) UV-B treatments, (B) Water stress treatment, (C) interaction between UV-B and water stress.



T1: Seedling irradiated for 18 days
T3: Seedlings irradiated for 8 days
T4: Water stress seedling irradiated for 8 days

Fig. 4. Effect of UV-B radiation on some enzyme activities of the shoots of *Cucurbita pepo* L. grown under normal and water stress conditions.

stress significantly attenuated the levels of insoluble and total sugar despite of the increase in the soluble fraction in plants exposed to prolonged irradiation time (T2) for one hour daily. A partial recovery in these parameters was induced in plants photoreactivated by visible light after short UV-B irradiation time (T4). In this connection, Teramura *et al* (1984) showed that the combination of water stress and UV-B radiation has an additive inhibitory effects on photosynthetic pigments and net photosynthesis.

UV-B radiation greatly affected the phosphorus content in squash shoot, where the magnitude of reduction increased with the daily exposure time (Fig.3). Prolonged UV-B irradiation) significantly reduced the soluble and insoluble phosphorus contents compared with those irradiated for a short period followed by subsequent exposure to visible light (T3). Salinity also decreased the levels of phosphorus fractions relative to the control treatment (Fig.3). An additive reduction in these parameters was induced when water-stressed plant was exposed to UV-B radiation, particularly for a prolonged time (T2) such reduction was alleviated by the exposure of water-stressed plant to natural light after a short period of UV-B irradiation (T4).

The decline in phosphorus content in squash shoots was accompanied by simultaneous enhancement in the activities of alkaline and acid phosphatases (Fig.4). Such stimulation was significantly higher under water stress than under UV-B radiation. Similar increase in acid phosphates activity has been found in water-stressed cowpea seedlings (Balakumar *et al.*, 1993), which was associated with low phosphorus content in drought-stressed wheat plant (Barrett-Lennard *et al.*, 1982). It has been suggested by Barrett-Lennard and Greenway (1982) that the possible function of increasing phosphates activity during water stress could be due to hydrolysis of phosphate esters at the plasma lemma or in the cell wall to enhance translocation of phosphorus from mature to young leaves.

Moreover, Figure 4 also shows that the NR activity in squash shoots was inhibited by UV-B radiation and progressively declined with increasing the daily irradiation time. This inhibition was less pronounced under short irradiation treatment (T3) than under prolonged one (T1). A similar decline in NR activity by UV-B radiation has been established by Balakumar *et al* (1993). Such effect may be attributed to a reduction in enzyme production due to the disturbance in the rate of protein synthesis and degradation by UV-B irradiation (Dai *et al.*, 1992; Lin and McClure, 1995).

Water stress also reduced the NR activity and induced an additive decline when water-stressed plants were exposed to UV-B radiation (Fig.4). This enzyme is generally known to be highly sensitive to water stress (Vyas *et al.* 1985). Moreover, Balakumar *et al.* (1993) showed a similar effect under water stress, and reported that the combination of water stress and UV-B radiation caused a drastic decline in NR activity which is related to inhibition in protein synthesis, in addition to the inactivation of enzyme protein.

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التاثير البيوفيزيائى المتبادل بين الاشعة فوق البنفسجية والاجهاد المائىعلى نبات القرع

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الملخص: أدى تعرض بادرات القرع للأشعة فوق البنفسجية إلى إنخفاض معنوي على معدلات نمو المجموع الجذري والخضري المختلفة لبادرات ذات ٢٢ يوما من العمر. ولقد زاد هذا الإنخفاض بزيادة وقت التعرض للإشعاع .

كان الإنخفاض فى نمو المجموع الخضري مصحوبا بانخفاض فى المحتوى السكري والفسفورى كذلك فى نشاط أنزيم اختزال النترات ، بينما زاد نشاط كل من أنزيمي الفوسفاتيز الحمضى والقلوى زيادة بسيطة ، وقد إنخفضت حساسية نبات القرع بتعرضه للضوء الطبيعى بعد التعرض لمدة قصيرة من الأشعة فوق البنفسجية .

أظهرت النباتات المجهدة مائيا والمعرضة لوقت قصير للأشعة فوق البنفسجية إلى تحسن ملحوظ فى معدلات النمو وجميع القياسات الأيضية التى تمت دراستها وذلك بالمقارنة للتأثير الضار الناتج عن تعرض البادرات المجهدة مائيا لوقت تعرض طويل للأشعة فوق البنفسجية .

أكدت هذه النتائج فائدة إعادة تنشيط النبات بالتعرض للضوء الطبيعي بعد فترة الإشعاع القصيرة وذلك لتقليل التأثير الضار الذى ينتج من التعرض للأشعة فوق البنفسجية لمدة طويلة وخاصة للنباتات المعرضة للجهد المائي .