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Effects of Ultraviolet-B Radiation on Growth and Photosynthesis in Cucumber Primary Leaves

Hyo-Jin Kim, Tae-Yun Kim and Jung-Hee Hong
Department of Biology, Pusan National University, Busan 609-735, Korea
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In the present study we studied the growth, photosynthetic traits and protective mechanisms against oxidative stress in the primary leaves of cucumber (*Cucumis sativus* L.) seedlings with or without UV-B treatment. Cucumber seedings were irradiated with UV-B for 10 days in environment-controlled growth chambers. The primary leaves irradiated with UV-B showed reduction in leaf length and decreased biomass production. The reduced biomass production seemed to be due to a negative effect of UV-B radiation on the photosynthetic process. Changes in chemical properties of leaf, such as chl a/b ratio affected photosynthesis. UV-B significantly affected chl b content compared with chl a in the light harvesting complex resulting reduced photosynthetic activity. Fv/Fm decreased with an UV-B stress, suggesting that the photosynthetic apparatus, and particularly, PS II was damaged under UV-B stress. Malondialdehyde(MDA) concentration which represents the state of membrane lipid peroxidation increased significantly under UV-B stress confirming an oxidative stress. UV-B exposure with SA solution(0.1-1.0 mM) can partially ameliorated some of the detrimental effects of UV-B stress. Leaf injuries including loss of chlorophyll and decreased ratio of Fv/Fm were reduced with combined application of UV-B and SA. ABA and JA showed similar mode of action in physiological effects on photosynthetic activities though the levels were lower than those from SA treated plants. Chloroplast ultrastructure was also affected by UV-B exposure. The thickness of leaf tissue components decreased and the number of grana and thylakoids was reduced in chloroplast applied UV-B or SA alone. At combined stress granal and stromal thylakoids were less affected. The leaves under combined stress acquired a significant tolerance to oxidative stress. From these results, it can be suggested that SA may have involved a protective role against UV-B induced oxidative damage.

Key Words : Biomass production, Chlorophyll fluorescence, Primary leaf, Cucumber(*Cucumis sativus* L.), Malondialdehyde, Salicylic acid, UV-B

1. Introduction

The anthropogenic depletion of stratospheric ozone caused by industrial emissions of atmospheric pollutions, particularly chlorofluorocarbons(CFCs) has generated significant increase in the amount of incident ultraviolet(UV)-B (280-320 nm) radiation on the earth surface[1]. UV-B radiation can result in deleterious effects on overall plant growth and fundamental physiological processes.

Numerous studies have been conducted to access the effects of enhanced UV-B radiation on higher plants, because it is strongly absorbed by, and causes conformational changes in, many macromolecules, especially proteins and nucleic acids. It is well known that UV-B induces DNA damage by the formation of DNA photoproducts[2]. In addition, it was suggested that harmful free radicals would participate at least partly, in the growth reduction by UV-B[3], although it is only recently that UV-B irradiation has been shown to induce the production of free radicals in plants.

Among the most frequently reported effects of UV-B on leaves are decreased biomass accumulation and photosynthesis, which has been attributed by different authors to a reduction in the accessory pigment and activity of RuBP carboxylase, to decreased activity of photosystem(PS) II photochemistry or to altered stomatal conductance[4]. UV-B radiation can alter sev-

Corresponding Author : Jung-Hee Hong, Department of Biology, Pusan National University, Busan 609-735, Korea
Phone: +82-51-510-2263
E-mail: jhhong@pusan.ac.kr

eral metabolic processes in leaves that ultimately change the levels of many foliar constituents. In general, UV radiation damages lipids, nucleic acids and proteins in leaves of higher plants, specifically targets the PS II reaction center, rubisco and chloroplast ATPase[5]. Olsson et al.[6] have suggested that UV-B induced accumulation of specific UV-absorbing constituents protects the photosynthetic apparatus from the damaging effects of this radiation resource.

Enhanced UV-B radiation can also causes changed in leaf anatomy and these in turn can affect the internal photosynthetically active radiation(PAR, 400-700 nm) environment. Specially, enhanced UV-radiation can increase the backscattered component of PAR and after the depth to which PAR penetrates. Important consequences for photosynthesis may result from such changes. The photosynthetic apparatus is very sensitive to different stresses and one of the primary sites of injury is PS II reaction centre.

To cope with UV radiation damage, plants have evolved a variety of mechanisms including : repair of inflicted damage and screening of the internal tissues against the radiation. Both mechanisms complement each other and both are apparently indispensable. Plants have responded to the stress imposed by UV-B radiation by the development of efficient systems for repair of inflicted damage and by the accumulation of UV-screening substances that prevent the occurrence of damage[7]. Plants are able to protect themselves against the negative effects of UV by efficient photorepair mechanisms, morphological adaptation like leaf thickening and / or by the synthesis and accumulation of UV-aborbing compounds in the epidermal layer, thereby hampering the penetration of UV-B radiation into the leaf[8]. The production of UV-absorbing compounds and the repair mechanism can determine the sensitivity of crops to UV-B radiation. Plants can tolerate or adapt to low levels of UV-B radiation but are impaired by high levels.

In general, plant response to UV-B is highly variable, and is dependent on environmental conditions and plant source, i.e. species, cultivar. It has been already reported that in some species such as cucumber, mung bean and spinach the growth is inhibited by the solar UV-B and in few crops(tomato) growth is stimulated, while in others(cotton and oat) it is unaffected[9]. Moreover, there is considerable inter- and intra- specific variation in the magnitude and type of response to enhanced UV-B radiation. For example, rice cultivars showed that there are intraspecific variations in the sensitivity of rice to UV-B radiation[10]. Differential sensitivities to increased UV-B levels might eventually alter the competitive relationships between plants thereby changing vegetation structure and composition of natural ecosystems.

A wide range of second messengers have been implicated in signaling in response to a variety of stresses[11]. Salicylic acid (SA), abscisic acid (ABA) and jasmonic acid (JA) are all involved in several stress responses. Any or all of these potential second messengers may be involved in pathways switched on in response to UV-B stress. SA is well known as an important component of signaling pathways in response to systemic acquired resistance and the hypersensitive response linked to oxidative responses. It has been shown that SA and ABA frequently show similar biological effects in thermotolerance[12]. There is some evidence that JA may have chemical and physiological similarities to ABA[13].

In the present study, the effects of UV-B radiation on growth, chlorophyll and phtotosynthetic activities were evaluated in seedlings of cucumber. We also investigated the effects of UV-B on plant tolerance to oxidative stress using second messengers, SA, ABA and JA, and discuss their possible role in plant defence response.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of cucumber(*Cucumis satius* L,) were sterilized with 10% NaOCl for 10 min and rinsed thoroughly with sterile distilled water. The seeds were then sown in a mixture of vermiculite, peat moss and perlite in plastic pots (7x11 cm), and were watered with a 1/2-strength Hoagland solution. The seedlings were reared in a growth chamber at a 25 ± 1℃ with 70% relative humidity and a 12 h photoperiod provided by 160 μmol $m^{-2}sec^{-1}$ PAR. The plants were watered every 2 days. At 15 days after sowing, seedlings were transferred to another chamber to be grown under a 20/15 ℃ (light/dark) temperature regimes.

Over the course of the growing period, the plants were exposed to UV-B and SA alone or in combination for 10 days. SA was applied to the 15-d-old plants during the UV-B treatment. Stock of SA(Sigma

Co.) had been prepared in a small volume of ethanol, then diluted to the three treatment concentrations (0.1, 0.5 and 1.0 mM) in a 2 mM sodium phosphate buffer (pH 7.0). The buffer solution alone was applied on control plants.

2.2. Ultraviolet-B radiation treatments

15-d-old seedlings were used for the UV-B treatments. Control plants were grown under visible light only, while treated plants were irradiated with supplementary UV-B during the 12 h light periods. UV-B was provided by two fluorescent UV-lamps(VL-6, Viber lourmat France sun lamp), suspended 53 cm above the plant seedlings. The UV-B fluence rate, at the height of seedlings, was measured to be 6 W $m^{-2} \cdot s^{-1}$ using a UV spectroradiometer (Li-1800, Lycosa).

2.3. Growth measurements

Fresh weight and dry weight of the cucumber primary leaves were determined at daily intervals starting on the 1 day after planting and continuing through the 7 day. Leaf fresh weight was determined by means of a electrobalance (Model H 51, Saritorius GmbH, Germany), and leaf dry weight was obtained by drying sample for 72 h at 80℃ in a drying oven and reweighing.

2.4. Chlorophyll measurements

Fully expanded leaves from the plants were frozon, then transferred to N,N-dimethylformamide and stored in the dark at 4℃ until they were analyzed. The chlorophyll contents were measured spectrophotometrically using specific absorption coefficients of 647 and 664 nm. The concentrations of chl a, chl b and total chl were calculated according to Inskeep and Bloom[14].

2.5. Chlorophyll fluorescence measurements

Fully expanded leaves were selected and chlorophyll fluorescence measurements were perfomed using a pulse-amplified modulation fluorometer (PAM 2100, Walz, Effeltrich, Germany) at the adaxial leaf surface of fresh plant material.

The leaves were dark-adapted for 10 min prior to measurement at room temperature (20℃). The measuring light of the PAM fluorometer was used to determine the initial fluorescence level, Fo. The maximum fluorescence was obtained using a saturating a pulse (3000 μmol $m^{-2}s^{-1}$ from a halogen light source). Fluorescence data were collected during a 5-min continuous illumination period using the LED of the PAM as the actinic light source (63 μmol $m^{-2}s^{-1}$). Saturating pulses were given every 20 s and the quencing coefficients and photochemical quantum yield were calculated[15]. The maximal photochemical efficiency of PSⅡ photochemistry was evaluated as Fv/Fm = (Fm-Fo) / Fm.

2.6. Lipid peroxidation determination

Lipid peroxidation was measured by the amount of malondialdehyde(MDA), a product of unsaturated fatty acid peroxidation. MDA concentration was estimated by the method of Zhao et al.[16]. Fresh leaves (0.5 g) were homogenized in 10 ml 5% (w/v) trichloroacetic acid. Homogenates were then centifuged at 4,000 g for 10 min. The supernatant(2 ml) was mixed with 1 ml of 0.67% (w/v) thiobarbituric acid, and then boiled at 100℃ for 20 min and cooled immediately in an ice bath. After centrifugation at 12,000 g for 10 min, the absorbance of the supernatant at 532 and 620 nm was determined using a spectrophotometer. The MDA content was calculated using the extinction coefficient, 155 $mM^{-1}cm^{-1}$.

2.7. Transmission electron microscopy

Leaves were sectioned and fixed with 3% glutaraldehyde in phosphate buffer (pH 7.4) at 4℃ for 12 h. After rinsing in buffer, the leaf samples were post-fixed in 2% OsO_4 for 12 h, again rinsed in phosphate buffer, dehydrated through a graded ethanol series, and embedded in Spurr's epoxy resin. Thin sections were cut on ultramicrotome (Reichert, Austria) at 50 nm, stained with 2.5% uranyl acetate followed by lead citrate solution according to Reynolds[17]. The specimens were observed on a transmission electron microscope (JEOL 1200 EX, Japan). The ultrastructure was assayed for 20 mesophyll chloroplast sections.

2.8. Statistical analysis

Data were statistically evaluated by the standard deviation and T-test methods. The results presented are combined from at least 3 replicated experiments for survival data, or at least 2 replicated experiments for biochemical measurements.

3. Results and discussion

3.1. Effect of UV-B on growth

Under carefully controlled growth chamber conditions UV-B radiation affected the leaf characteristics.

Although surviving plants commonly showed signs of UV-damage and leaf chlorophyll loss, they still had growing, green shoots. Those symptoms are likely to have been mediated by the generation of free radicals during oxidative UV-stress[4]. Under UV-B exposure, the elongation of primary leaves lagged significantly behind that of controls during growing period. The final leaf length on day 7 showed 18% decrease compared with controls (Fig. 1). UV-B radiation resulted in reduction of fresh weight and dry matter production, but these differences were not statistically significant (Table 1).

Growth characteristics are altered in plants showing UV-B sensitivity. Cucumber is reported to be sensitive to UV-B radiation. UV-B stress to plants exercised a considerable effects on the growth of the seedlings, such as delayed leaf emergence, slow expansion of blades and narrow and shorter mature blades. Probably the most significance among the morphological changes is the reduction of leaf expansion. Supplementation of UV-A radiation has promoted the overall growth of the black gram plants than the control plants, while UV-B radiation has inhibited the growth of the plants[18]. Reduction in biomass accumulation with increased UV-B radiation have been observed in a range of agronomic species including wheat, barley, tomato and lettuce.

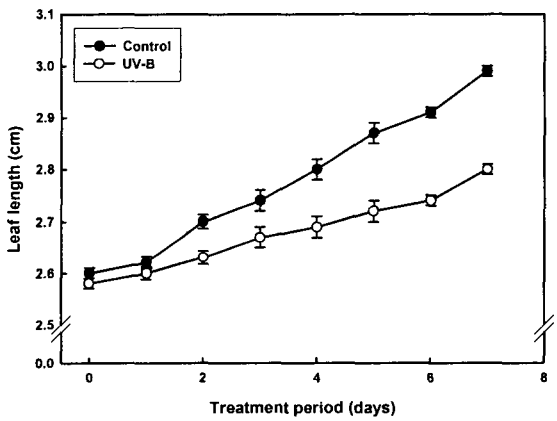

Fig. 1. Effect of UV-B radiation on elongation of cucumber primary leaves.

3.2. Effect of UV-B on photosynthetic responses

UV-B stress also affected plant physiological function. Depending on the particular crop species, chlorophyll content can either increase or decrease in response to enhanced UV-B radiation[19]. Total chlorophyll content decreased marginally in UV-B(36.7%) exposed plants on 20th day of growth(Table 2) The data indicate that UV-B radiation during growth showed a significant reduction in the chlorophyll concentration. Reduction in chlorophyll content might be due to inhibition of biosynthesis or increased degradation of chlorophyll or breakdown of pigments or their precursors by the UV irradiation[20]. Decreased amount of total chlorophyll have been associated with the inhibition of aminolevulinic acid synthesis or a reduction in protochlorophyllides[21]. It was found that UV-B radiation influences the genetic regulation of the chlorophyll-binding protein, thereby leading to chlorophyll destruction[22].

UV-B exposure has also been linked to rubisco damage either by direct mechanisms or, indirectly, through the formation of reactive oxygen species within the cell. Furthermore, plants exposed to high levels of PAR and UV-B have been reported to be especially susceptible to formation of active oxygen species and precede down-regulation of photosynthesis[1]. In our study, the chlorophyll a/b ratio decreased when plants were UV irradiated. This may have been a result of faster breakdown or decreased synthesis of chl b compared with chl a.

As mentioned above, changes in chemical properties of leaf, such as chl a/b ratio can affect photosynthesis. UV-B significance affect chl b content, whereas it decreased the amount of chl b by 90.3% and therefore increased the ratio of chl a/b by 16.9-fold.. Compared with chl a, in the light harvesting complex, chl b is located further from the reaction center and transfer the absorbed energy to chl a, which is lower in energy[23]. In our study, the lower

Table 1. Effect of UV-B radiation on fresh weight and dry weight of cucumber primary leaves

Treatment	Fresh weight (mg)		Dry weight (mg)	
	1 d	7 d	1 d	7 d
Control	168±6.4	242±8.1	150±2.2	210±5.4
UV-B	165±2.4	230±3.4	148±5.4	199±5.1

Table 2. Effect of UV-B radiation on chlorophyll content in cucumber primary leaves

Treatment	Chl a	Chl b	Chl a/b	Total Chl
	(mg / g FW)			
Control	82±0.1	155±0.3	0.53±0.0	237±0.5
UV-B	135±0.2	15±0.2	9.0±0.4	150±0.2

amount of chl b produced under UV-B radiation most likely lowered the energy gradient and resulted in some changes to funneling of energy to the reaction center and, in turn, to reduced photosynthetic activity. Many report exist on the target site of UV-B radiation in PS II activity of the photosynthetic system of higher plants, suggesting that the decrease in PS II activity might be due to the damage in the PS II complex. This is in agreement with the reports of many authors.

3.3. Effect of UV-B on chlorophyll fluorescence

The overall changes in photosynthetic activities in cucumber leaves grown under UV-B were followed using chlorophyll fluorescence measuring the Fv/Fm ratio. UV-B irradiation caused gradual decrease in Fv/Fm ratio for the first 5 d(Fig. 2). However, the Fv/Fm ratio decreased by 21.4% compared to the control after 7 d, and was kept to be lower than the control level.

Fv/Fm decreased with an increase in UV-B stress, suggesting that photosynthetic apparatus and particularly PS II was damaged more under UV-B exposure than in the controls[24]. Therefore, our observation the reduction in chlorophyll fluorescence led to a large decline in photosynthetic efficiency further supports that these processes inhibit photosynthesis and growth.

At the end of UV-B exposure there was a decrease of Fv/Fm indicating that the efficiency in the energy conversion of the PS II was altered and the rate of non-cyclic electron flux through PS II was affected[25]. The limitation of photosynthesis appears to be correlated with stomatal closure and a decrease in mesophyll activity as demonstrated by alteration of chlorophyll fluorescence parameters.

3.4. Tolerance of primary leaves to oxidative stress

UV-B stress also affects many important physiological processes involved in oxidative stresses, e.g. lipid peroxidation and the antioxidant enzyme defence system. Malondialdehyde (MDA), a product of lipid peroxidation, was greatly increased under UV-B stress. MDA concentration which represents the state of membrane lipid peroxidation was higher in UV-B exposed cucumber primary leaves compared to the control, confirming an oxidative stress(Fig. 3). UV-B exposure to the leaves induced a slow rise in MDA level up to 24 h, followed by rapid increase in the MDA level. The UV-induced increase in MDA content in the leaf was nearly 3-fold in comparison to the untreated controls.

We examined whether SA, ABA and JA treatments

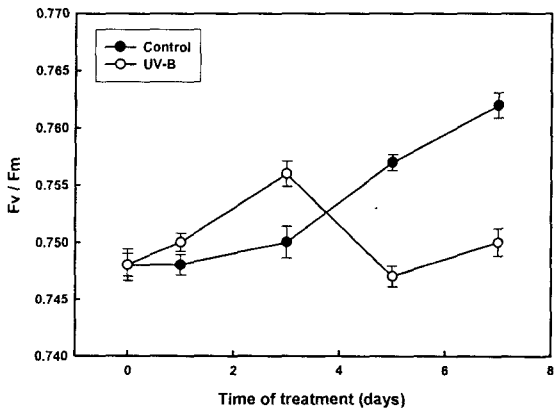

Fig. 2. Effect of UV-B radation on the chlorophyll fluorescence of cucumber primary leaves.

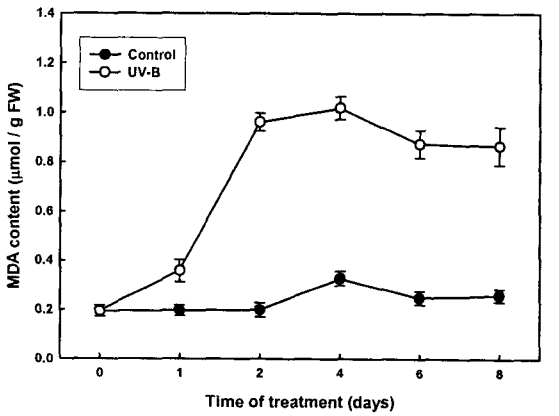

Fig. 3. Effect of UV-B radiation on the MDA content in cucumber primary leaves.

could protect cucumber seedlings against UV-B induced oxidative stress. The plants were treated with or without SA, JA and ABA (0.1, 0.5 and 1.0 mM) under a UV-B induced stress. Recovery from UV-oxidative effect of chlorophyll degradation was observed in the SA, ABA and JA-treated leaves(Fig. 4). The incidence of leaf injuries, e.g. losses of chlorophyll were reduced in the SA treated plants, particularly at the 1.0 mM level. The plants treated with high concentrations of SA was better able to overcome UV-B toxicity. Compared with the SA-treated plants that were grown under -UV-B, there was slightly, but significant reduction for the Fv/Fm of plants that were grown under +UV-B (Fig. 5). Enhanced tolerance to SA in UV-B treated plants was observed from 1 d after the start of the UV-B irradiation. This enhanced tolerance was maintained even up to 10 d of UV-B treatment. Plants grown on 1.0 mM SA, accelerated recovery from UV-B induced inhibition of leaf photosynthesis, however, the levels were not more tolerant than controls.

In ABA-treated plants, a significant rise in UV-B tolerance was observed in a way similar to the mode of action of SA (Fig. 6). In contrast the plants exposed to JA showed much lower inhibition of UV-B tolerance (Fig. 7). SA treatment was found to be most effective in inducing UV-B tolerance. A wide range of second messengers, such as SA, ABA and JA have been implicated in signaling in response to a variety of stresses[11]. SA may be involved in heat stress and oxidative stress responses. It has also been noted that

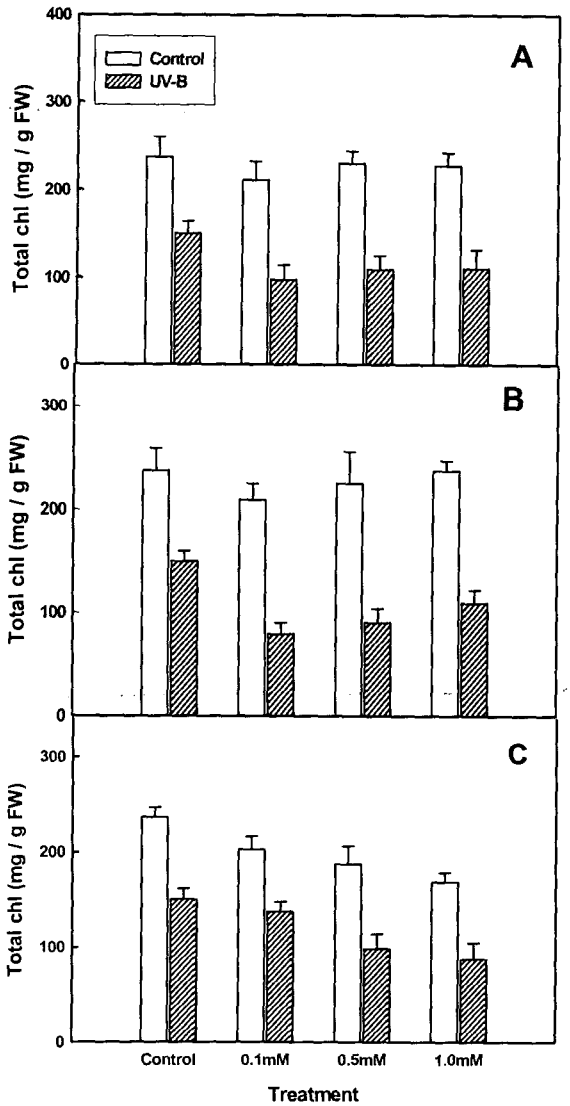

Fig. 4. Effect of SA(A), ABA(B) and JA(C) on the chlorophyll accumulation in cucumber primary leaves with or without UV-B treatment.

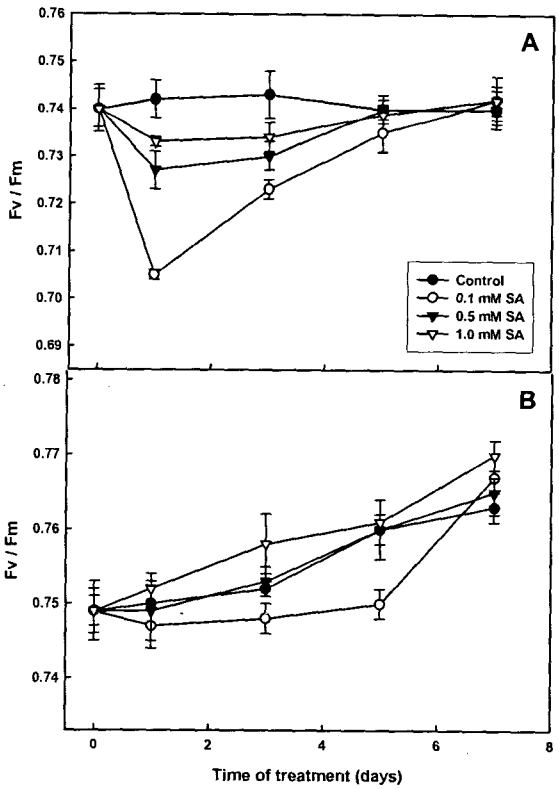

Fig. 5. Changes in chlorophyll fluorescence in cucumber primary leaves by visible light (A) and UV-B (B) treatments in combination with SA.

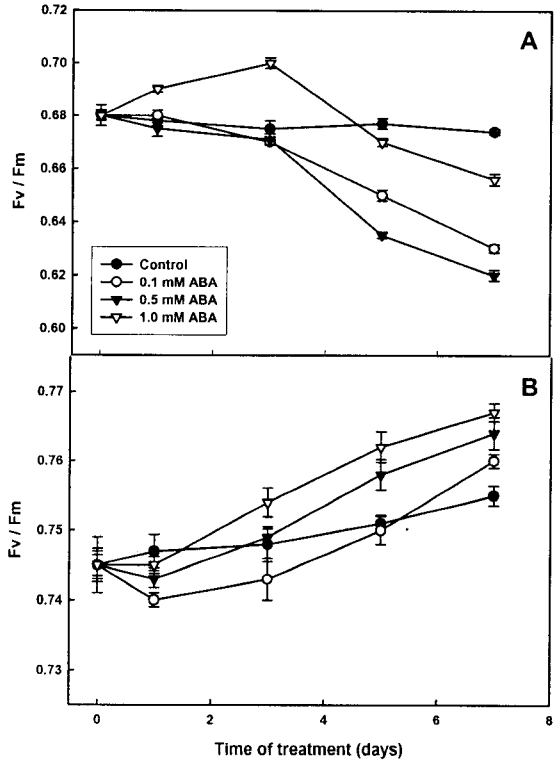

Fig. 6. Changes in chlorophyll fluorescence in cucumber primary leaves by visible light (A) and UV-B (B) treatments in combination with ABA.

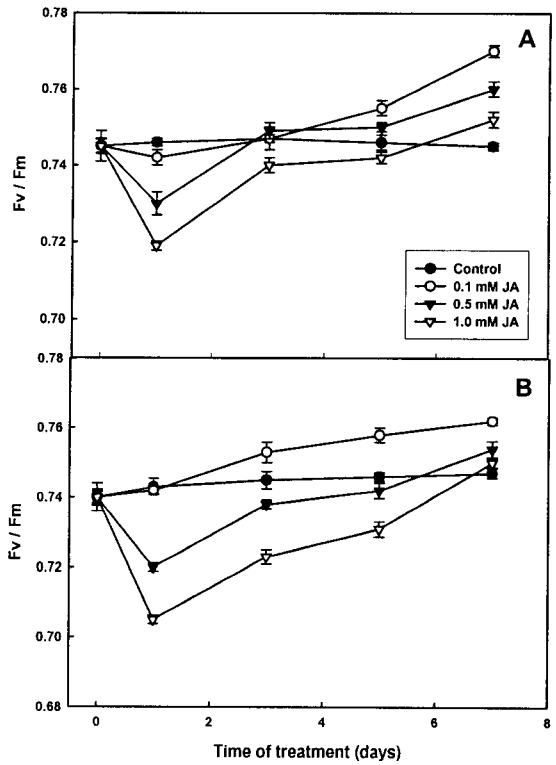

Fig. 7. Changes in chlorophyll fluorescence in cucumber primary leaves by visible light (A) and UV-B (B) treatments in combination with JA.

ABA induces thermotolerance and involves heat stress[12]. Plants given these pretreatments showed reduced oxidative damage in recovery from heating suggesting that they result in prevention of oxidative damage or repair of that damage. It has been shown that SA and ABA frequently show similar biological effects in thermotolerance. Some evidence support that SA and ABA may mediate protection against, or repair of oxidative stress.

There are some evidences that jasmonic acid (JA) have been regarded to be putative regulator of plant growth and development involved in inhibition of growth, wound response, senescence and chilling tolerance[13]. JA have chemical and physiological similarities to ABA. From the above results it is suggested that JA and ABA induce the same abundant proteins which may be important in acquired stress tolerance.

3.5. Effect of UV-B on the ultrastructure of chloroplasts

The ultrastructural observations were focused particularly on chloroplast, because thylakoids are the membranes which undergo the greatest changes during adverse environment conditions. The observed inhibition in the rate of photosynthesis may be related to the changes of the membrane structure of chloroplasts. UV-B markedly affected the plastids. The number of grana and grana stacking was strongly reduced and swollen membranes occurred more frequently than the control (Fig. 8 A and B). In some chloroplasts the envelope was disrupted. Treatment with SA also led to significant changes in the internal structure of plastid, the effect was more pronounced at the higher concentrations (Fig. 8 C).

Electron microphotographs in 1.0 mM SA showed many swollen, dilated thylakoids and undulating membranes. At combined stress (UV-B+SA) the numbers of grana and thylakoids were reduced and chloroplast were largely vacuolated (Fig. 8 D). Similar results have been shown in ultrastructure of chloroplasts exposed to NO_2, high temperature and water stress[26,27]. These changes can probably be explained by altered osmotic conditions in the stroma and permeability

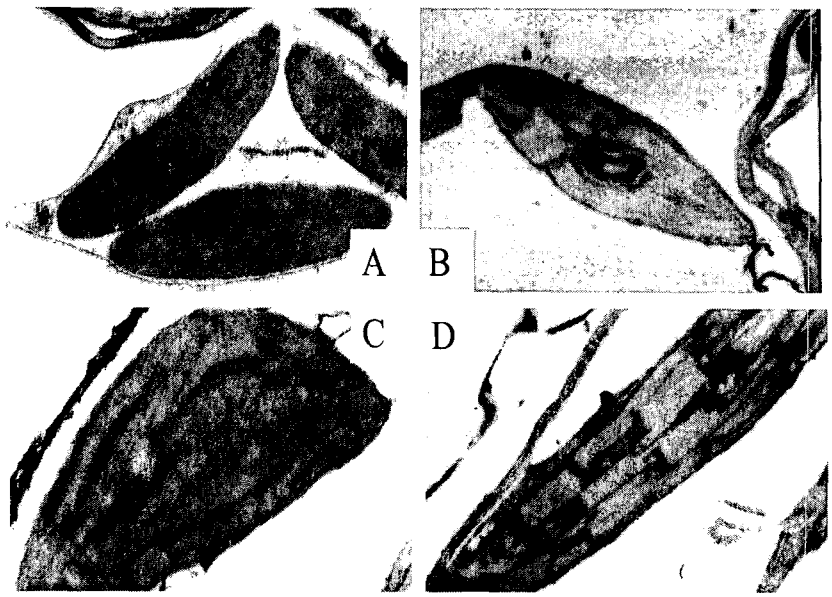

Fig. 8. Transmission electron micrographs of chloroplasts from leaves of cucumber plants. A, control ; B, UV-B; C, 1.0 mM SA ; D, UV-B+1.0 mM SA.

changes in these membranes. We observed that the destructive processes were less expressed at simultaneous action of UV-B and SA than at separation application. Many of the observed plant reponses to SA may have adaptive significance because they conserve water. According to the current results, the apparent role of SA seems to be potentiating the stress response during the osmotic or oxidative stress.

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