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UV-B-Induced Changes in Carbohydrate Content and Antioxidant Activity in Rice Seedling

Jwa-Kyung Sung*, Su-Yeon Lee, So-Hyun Park**, Byoung-Choon Jang*, Sang-Min Lee*, Yong-Hwan Lee*, Du-Hoi Choi*, and Beom-Heon Song***†**

*National Institute of Agricultural and Science Technology, RDA, Suwon, Korea
**Department of Plant Resources and Science, Hankyong National University, Anseong, Korea
***Department of Agronomy, Chungbuk National University, Cheongju, Korea

ABSTRACT : The effects of UV-B radiation on the seedling growth, carbohydrate metabolism and antioxidants activities of rice (*Oryza sativa* L.) were investigated under environmentally controlled chamber. Supplementary UV-B radiation reduced dry matter as well as leaf area, therefore, relative growth rates (RGR) of seedlings were decreased by up to half compared to control. Photosynthetic products such as soluble sugars and starch were rapidly and significantly reduced by within 1 day of enhanced UV-B radiation due to the inhibition and degradation of photosynthetic processes and thylakoid membrane integrity. In our study, nonstructural carbohydrate levels were proved to be a main indicator on UV-B-induced stress. The behavior of SOD, CAT, APX and POD activities was monitored in the leaves of rice seedlings subjected to UV-B radiation. Under UV-B treatments, SOD activity was initially increased, whereas CAT and POD activities were slowly and slightly increased. However, APX activity showed no presumable results with an increase of UV-B dose. In leaves of rice seedlings, supplementary UV-B radiation caused an increase in free putrescine and spermidine, however spermine remained unaltered, although 24-hrs UV-B treatment slightly increased. This result presumes that an excess UV-B dose may induce ethylene biosynthesis (senescence) rather than polyamine biosynthesis (defense).

Keywords: UV-B, rice, carbohydrate, antioxidant enzymes, polyamines

Abbreviation: APX; ascorbate-peroxidase, CAT; catalase, GR; glutathione reductase, PCA; perchloric acid, POD; guaiacol-peroxidase, RGR; relative growth rate, SOD; superoxide dismutase

The depletion of stratospheric ozone levels induces an increase of solar UV-B radiation reaching the Earth's surface (Smirnoff, 1998). Many researches have reported that UV-B irradiation affect reduced photosynthesis, decreased protein synthesis, damaged chloroplast function, impair to DNA, biomass reduction, etc (Jordan, 1996). To escape out of the oxidative damage plants have a complex antioxidant defense system comprising of enzymes, superoxide dismutase, catalase and peroxidase, and non-enzymec constituents, α-tocopherol, ascorbate and reduced glutathione, which scavenge the ROS (Shah *et al.*, 2001). The enzymes of Halliwell-Asada pathway or ascorbate/glutathione cycle such as ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase play a central role in removing H_2O_2 in chloroplasts and in maintaining the redox status of the cell (Foyer *et al.*, 1997). Catalase scavenges H_2O_2 generated during the photorespiration and β-oxidation of fatty acids (Morita *et al.*, 1994). Guaiacol-peroxidases are involved in developmental processes, lignification, ethylene biosynthesis, defense, wound healing, etc (Asada, 1992). Depression of photosynthesis by UV-B has been leaded to down-regulation of photosynthetic genes, modification of thylakoid membranes (Strid *et al.*, 1994), inhibition of photosynthetic enzymes, and disruption of electron transport in photosystem II reaction centers (Murthy & Rajagopal, 1995). Also UV-B can influence photosynthesis indirectly by altering stomatal function, photosynthetic pigments, leaf anatomy, and canopy morphology (Teramura & Sullivan, 1994). In addition, UV-B may change concentrations of structural carbohydrates in leaves (Gehrke *et al.*, 1995), and inhibition of photosynthetic processes caused by enhanced UV-B may also influence soluble carbohydrate metabolism (Yue *et al.*, 1998). Polyamines (putrescine, spermidine and spermine) are ubiquitous aliphatic polycations that have been found as a modulator of plant growth and development, and are also implicated in plant responses to environmental stresses (Bouchereau *et al.*, 1999). Stress provoking expression and activity of arginine decarboxylase is the most common feature of polyamine metabolism. The main aim of this study was to characterize the effects of UV-B radiation on the mobilization and degradation of nonstructural carbohydrate and changes in antioxidants level, and to observe plant

†Corresponding author: (Phone) +82-43-261-2511 (E-mail) bhsong@cbucc chungbuk.ac.kr <Received April 25, 2005>

growth affected concomitantly by an inhibition of carbohydrate and antioxidant metabolism.

MATERIALS AND METHODS

Plant materials and UV-B irradiation

Rice (*Oryza sativa* L., cv. Woonjangbyeo) seeds were surface sterilized with a 2% of NaClO and then thoroughly rinsed with distilled water. Seedlings were grown at 25/20 °C (day and night), with a 12-h photoperiod under fluorescent white light (300 μmol m^{-2} s^{-1}) in a controlled environmental growth chamber. After 25 days of germination, seedlings were subjected to UV-B irradiation with a UV-B lamp (radiation 290-320 nm) at an irradiance of 1.2 W m^{-2} UV light which was filtered through 0.1 mm thick cellulose acetate filters. UV-B doses were adjusted by exposure of plants during from 0 to 24 hr (to analyze antioxidants) and during from 0 to 5 days (to analyze carbohydrate contents). According to Caldwell (1971), the biologically effective UV-B doses were determined. All samples were collected immediately after UV-B exposure and then stored at -70 °C for further analysis.

Photosynthetic pigments

Total chlorophyll and carotenoid were determined with slight modification as reported by Jeffrey & Humphrey (1975) and Strickland & Parsons (1972), respectively. Plant materials were extracted by homogenizing and boiling 0.5 g of fresh weight in 20 ml of 95% ethanol. After centrifugation, the chlorophyll and carotenoid contents were measured spectrophotometrically on the ethanolic supernatant at 470, 648 and 644 nm and expressed as μg g^{-1} FW.

Carbohydrate assay

Soluble sugars (SS) were extracted by heating leaf discs in 80% ethanol, according to Roe method (1955). SS were analyzed by the reaction of 1.0 ml of the alcoholic extract with 2.0 ml fresh 0.2% anthrone in sulfuric acid (w/v) and placed in a boiling water bath for 10 min. After cooling, the absorbance at 630 nm was determined. After the extraction of the soluble fractions, the solid fraction was used for starch analysis. Starch was extracted with 9.3N PCA. The starch concentration was determined by the anthrone method as described above. Glucose was used as standard for both soluble sugars and starch.

Enzyme activity assay

Fresh leaves (1g) were homogenized in 100 mM Na-phosphate buffer (pH 7.8) containing 0.1 mM EDTA, and 1% (w/v) PVP at 4 °C. The homogenates were centrifuged at 12,000 ×g for 20 min, and supernatants were used for enzymes activity and protein content assay. All assays were done at 4 °C. Total soluble protein contents of the enzyme extracts were determined according to Bradford (1976) using BSA as a standard. SOD (E.C. 1.15.1.1) activity assay was performed with a slight modification of Beauchamp & Fridovich method (1971), which measures the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) spectrophotometrically at 560 nm. One unit of enzyme activity was defined as the quantity of SOD required to produce a 50% inhibition of reduction of NBT and the specific enzyme activity was expressed as unit mg^{-1} protein g FW. Catalase (E.C. 1.11.1.6) activity was determined by monitoring the decomposition of H_2O_2 (extinction coefficient 39.4 mM cm^{-1}) at 240 nm following the method of Bergmeyer (1970). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) and a proper amount of plant extract in a 3 ml. The reaction was initiated by adding 10 mM H_2O_2. The enzyme activity was defined as μmol H_2O_2 destroyed min^{-1} mg^{-1} protein g FW. Ascorbate peroxidase (E.C 1.11.1.11) activity was done according to Nakano & Asada (1981). The assay depends on the decrease in absorbance at 290 nm as ascorbate was oxidized (extinction coefficient of 2.8 mM^{-1} cm^{-1}). The reaction mixture contains 50 mM Na-phosphate buffer (pH 7.0), 0.5 mM Ascorbate, 0.1 mM EDTA-Na_2 and 1.2 mM H_2O_2. The enzyme activity is defined as μmol oxidized ascorbate min^{-1} mg^{-1} protein g FW. Peroxidase (E.C. 1.11.1.7) activity was determined by monitoring the formation of guaiacol dehydrogenation product (extinction coefficient 6.39 mM cm^{-1}) at 436 nm followed by the method of Pütter (1974). Reaction mixture (3 ml) contained 100mM potassium phosphate buffer (pH 7.0), 0.3mM guaiacol and plant extract. The reaction was initiated by adding 0.1 mM H_2O_2. The enzyme activity is defined as μmol H_2O_2 destroyed min^{-1} mg^{-1} protein g FW.

Polyamine determination

Leaf discs were frozen in liquid nitrogen and then homogenized with 5% cold PCA (1:5, w/v). The homogenate were kept for 1 h at 4 °C and then centrifuged for 25 min at 9,000 rpm. The benzoyl polyamines (Redmond & Tseng, 1979) were separated on a 5 μm - 25 cm × 4.6 mm (RP-C18) column, using elution gradient from 60 to 45% acetonitrile in the mobile phase. Free polyamines were monitored with an UV detector (254 nm). The peaks were identified with reference to the retention times of PA standards prepared as described above. Quantitative determination was based on external standards.

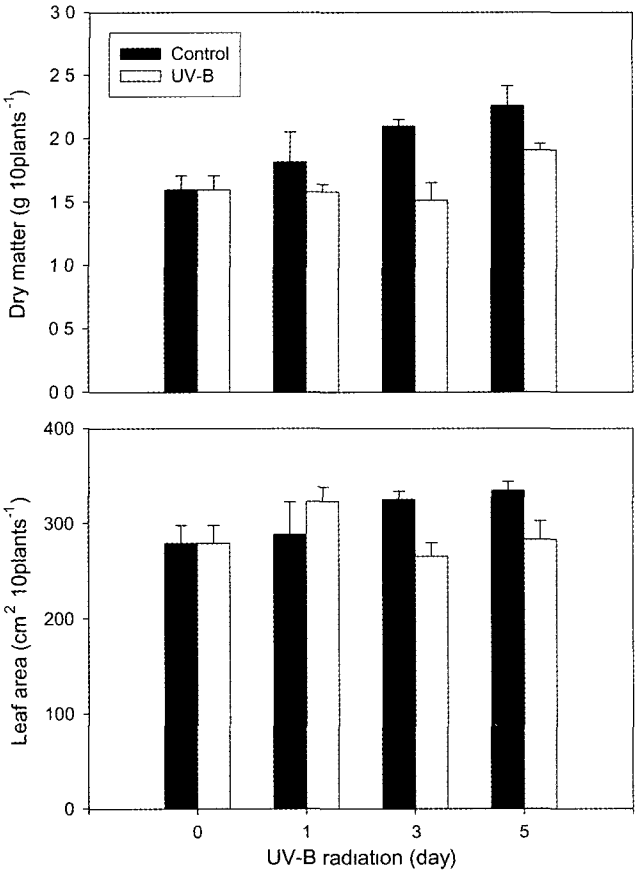

Fig. 1. Growth responses of rice seedlings exposed to UV-B radiation during short-term growth period. Values are the mean ± S. D. (n = 10, p < 0.05).

RESULTS

An external alteration in plants exposed to adverse environmental conditions is an ultimate result to stress factors. Rice seedling growth was much greater in control than in UV-B. During treatment period (5 days), total plant dry weight increased 41.3 % (2.26 ± 0.16) in control and 19.4 % (1.91 ± 0.06 in UV-B-treated seedlings. The relative growth rate in control and UV-B reached 0.17 g g^{-1} day^{-1} and 0.09 g g^{-1} day^{-1}, respectively (data not shown). Also, in comparison with the control seedlings, UV-B exposure significantly reduced leaf area due to the reduction of leaf expansion; 335 ± 10 cm^2 10plants^{-1} for control leaves and 284 ± 19 cm^2 10plants^{-1} for UV-B-treated leaves. There was no statistic difference on total chlorophyll and carotenoid concentrations between control and UV-B exposed leaves (Fig. 2). At zero time, both pigments were 2,326 ± 20 μg g^{-1} fw and 370 ± 13 μg g^{-1} fw, respectively. At the termination of UV-B treatment, chlorophyll and carotenoid were lower in UV-B-treated leaves (1,916 ± 62 μg g^{-1} fw and 255 ± 11 μg g^{-1} fw) than in control leaves (2,074 ± 82 μg g^{-1} fw and 354 ± 20 μg

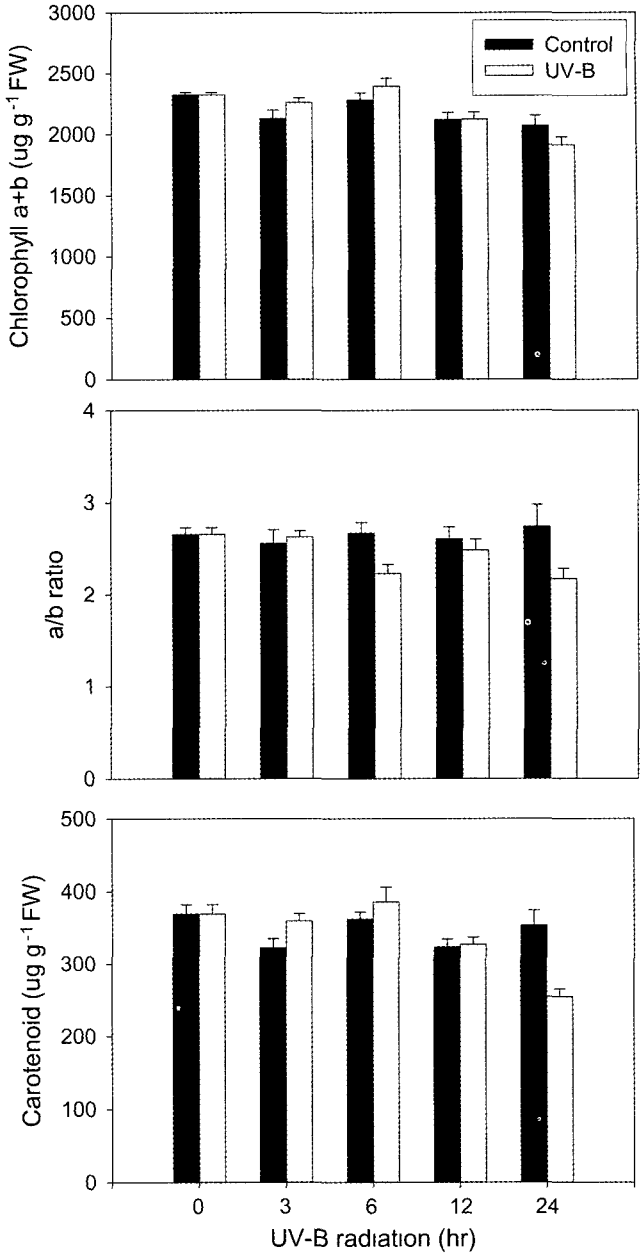

Fig. 2. Effects of UV-B radiation on rice leaf total chlorophyll concentration, chlorophyll a : b ratio and carotenoid concentration. Values are the mean ± S D. (n = 10, p < 0 05).

g^{-1} fw). A decrease of chlorophyll concentration due to successive UV-B irradiation resulted in the reduction of chlorophyll a/b ratio; after 24 hours, 2.74 ± 0.24 in control and 2.17 ± 0.11 in UV-B treatment. Soluble sugars and starch were a dominant leaf nonstructural carbohydrate (Fig. 3). Under normal growth condition, soluble sugars and starch had the highest proportion in leaf blade and leaf sheath, respectively. Both leaf soluble sugar and starch concentrations in each part of rice seedlings significantly decreased

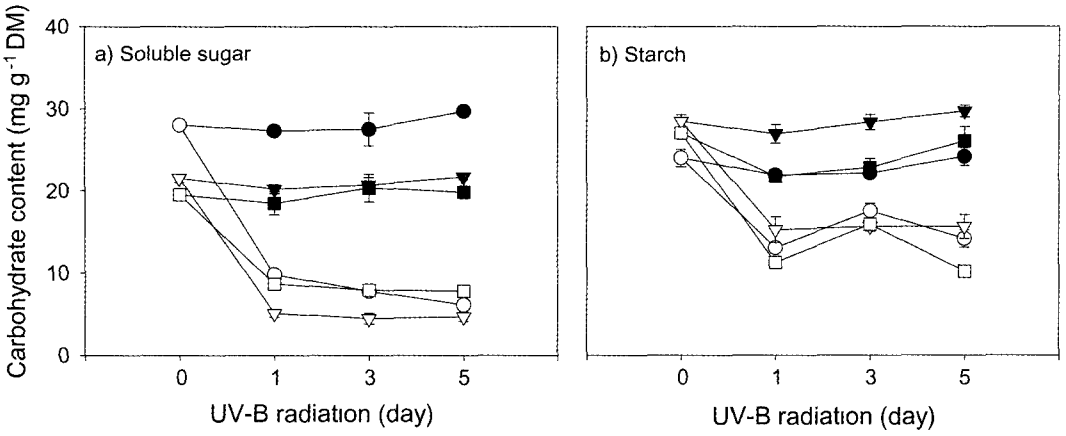

Fig. 3. Carbohydrate contents caused by UV-B irradiation in different shoot parts of rice seedling. Values are the mean ± S. D ($n = 5$, $p < 0.05$). ●, Leaf blade-control; ○, Leaf blade-UV-B; ▼, Leaf sheath-control; ▽, Leaf sheath-UV-B; ■, Young leaf-control; □, Young leaf-UV-B.

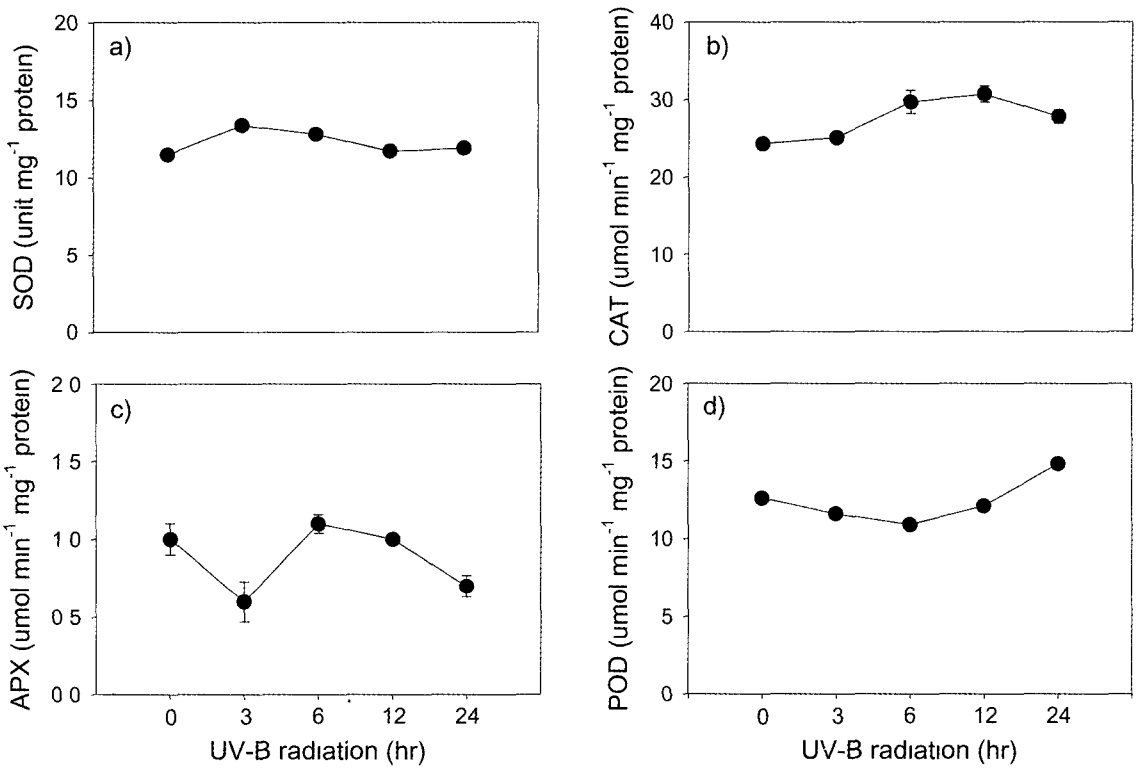

Fig. 4. Responses of SOD, CAT, APX and POD activities of rice leaves irradiated with UV-B. Values are the mean ± S. D ($n = 5$, $p < 0.05$).

within 1 day of enhanced UV-B treatment. Leaf soluble sugar concentration of leaf blade, leaf sheath and young leaf in UV-B radiation showed a decrease of 64, 72 and 79% compared to the control. Exposure to UV-B radiation also linearly decreased leaf starch concentration (leaf blade, 41%; leaf sheath, 44%; young leaf, 48%). Total SOD activity represents the combined action of Cu/Zn-, Mn-, and Fe-SOD, which can be distinguished by their differential sensitivity to cyanide and hydroxide. In comparison to UV-B-untreated seedling, UV-B radiation slightly enhanced total SOD activity (13.4 ± 0.3 unit mg^{-1}protein) within the first 3 hrs of, and then its activity was observed a gradual decrease (Fig. 4a). Catalase sensitively responded to UV-B radiation (Fig. 4b). CAT activity was increased by up to 30.7 ± 1.1 μmol mg^{-1}protein (after 12 hrs of UV-B radiation) compared to non-UV-B radiation (24.3 ± 0.5 μmol mg^{-1}protein). In

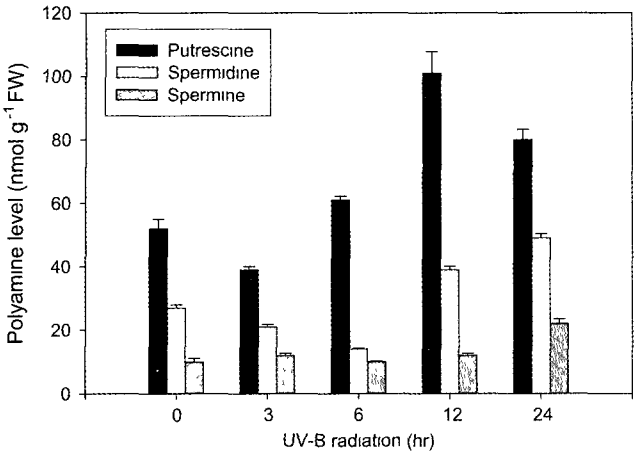

Fig. 5. Changes in free polyamine level in rice seedling leaves exposed to enhanced UV-B irradiance Values are the mean ± S. D. ($n = 5$, $p < 0.05$).

rice seedling, APX activity showed unsystematically with an increase of UV-B radiation (Fig. 4c). Immediately after UV-B radiation, catalase activity steeply declined (near half compared to control), and then immediately recovered up to an initial level. Like catalase, guaiacol-POD activity was momently decreased by UV-B radiation, and then, after 6 hrs of UV-B radiation, ascended stepwise (Fig. 4d). As a result of oxidative stress, free PAs levels in the rice seedling leaves were higher in the order of putrescine, spermidine and spermine (Fig. 5). UV-B-induced stress enhanced putrescine level by up to 2-fold (101 ± 7 nmol g^{-1}fw, at 12 hrs of UV-B radiation), whereas an increase of spermidine and spermine levels was unremarkable compared to putrescine.

DISCUSSION

Our experiments were designed to better understand the distribution of leaf carbohydrate and the responses of antioxidative defense system. Many studies have shown that UV-B radiation may reduce plant biomass, leaf area, plant height and photosynthesis and influence plant development not only at the physiological and biochemical but also at the genetic level (Jordan *et al.*, 1991; He *et al.*, 1993; Strid *et al.*, 1994; Bornman & Sundby-Emanuelsson, 1995; Kumagai *et al.*, 2001; Li *et al.*, 2002). In our materials, it was demonstrated that excess UV-B radiation ultimately resulted in the reduction of leaf expansion, seedling growth and biomass production. Total chlorophyll concentrations and chlorophyll a/b ratio were slightly altered in leaves of UV-B-irradiated rice seedlings (Fig. 2). The decreased chlorophyll concentration is attributed to increased photo-degradation of chlorophylls (Strid & Porra, 1992) and lowered chlorophyll synthesis rates derived from reduced expression of genes encoding chlorophyll-binding protein (Strid *et al.*, 1994). Carotenoid concentrations were observed temporary increase to alleviate UV-B stress, however, excess irradiation resulted preferably in a decrease of carotenoid synthesis. It was previously demonstrated that carotenoids play an important role in the photo-protection of photosynthetic system by dissipating excess excitation energy through the xanthophylls cycle (Demmig-Adams & Adams, 1992). The mechanisms of enhanced UV-B radiation impact on leaf photosynthesis have been reviewed in detail by earlier study (Allen *et al.*, 1998). For investigating photosynthetic products, we monitored the variations of soluble sugar and starch contents after transfer of rice seedlings into the UV-B condition. Non-structural carbohydrate concentrations were initially declined (day 1) and followed by no changes (Fig. 3). Supplemental levels of UV-B induced a decrease of soluble carbohydrate in leaves of *Triticum aestivum* (Yue *et al.*, 1998) and decreased sucrose and starch contents in leaves of the moss *Polytrichum commune* (Barsig *et al.*, 1998). In contrast to our findings, Santos *et al.* (1993) reported that enhanced UV-B exposure in relation to controls accumulated more starch. We suggest that a decrease of soluble sugar and starch contents under elevated UV-B may be due to inhibition and degradation of phothsynthetic processes, transfer into chloroplast to convert starch into sucrose or hexoses, and carbohydrate remobilization. ROS metabolism relies on several functionally interrelated antioxidant enzymes such as SOD, POD, CAT, GR and APX. SODs catalyze the dismutaion reaction of superoxide anion into H_2O_2 and O_2 and can be distinguished into three classes according to their metal co-factor: Cu/Zn-, Mn-, or Fe-SOD. PODs usually occur as multiple molecular forms and have a number of potential roles in plant growth, development and differentiation (Gaspar *et al.*, 1991). PODs require H_2O_2 as a substrate and, therefore, metabolize H_2O_2 to water. Although UV-B irradiation altered the activities of various antioxidant enzymes (Fig. 4), the responses of these antioxidant enzymes differently attained to maximum levels. UV-B exposure initially led to the small increase of total SOD, whereas CAT and POD activities were lately and slightly increased. Similarity to this study, Rao *et al.* (1996) reported that UV-B-induced SOD and POD activities were significantly higher in *Arabidopsis thaliana*. GR and APX play an important role to metabolize H_2O_2 to H_2O through a metabolic cycle widely known as the ascorbate-glutathione cycle. Contradictory to other study (Willekens *et al.*, 1994), in our experment UV-B exposure has been shown to have no effect on APX activity. PAs act as antioxidants by inhibiting lipid peroxidation, and Borrell *et al.* (1997) suggested that inhibition of lipid peroxidation might be one of the mecha-

nisms responsible for the anti-senescence effects of the PAs. Moreover, PAs are shown to play an important role in membrane stabilization by associating with negatively charged phospholipids. It was also demonstrated that an increase of spermidine or spermine levels prevented the loss of chlorophyll, stabilized the molecular composition of the thylakoid membranes and delayed senescence (Borrell *et al*, 1997; Besford *et al*., 1993). The results presented in this paper clearly indicate that elevated UV-B radiation increased putrescine and spermidine rather than spermine (Fig. 5).

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