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Full Length Research Paper

Genotype-dependent responses of wheat (*Triticum aestivum* L.) seedlings to drought, UV-B radiation and their combined stresses

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Experiments were conducted under controlled conditions to investigate the growth and physiological-biochemical responses of wheat (*Triticum aestivum* L.) seedlings to UV-B, drought, and their combined stresses. Both UV-B and drought treatments retarded seedling growth with UV-B having worse impact on wheat plants. Chlorophyll content and relative water content (RWC) in leaves, as stress makers, were significantly affected by UV-B and drought, respectively. The increased rate and amount of H_2O_2 were stress-different and genotype-dependent. Likely, the temporary expression patterns of antioxidant enzymes (superoxide dismutase, SOD, and catalase, CAT) and compounds (proline, and ascobate acid, AsA) exhibited differences under the tested stressful conditions in the two genotypes, indicating that they play significant roles in plant responses to these stresses. Pre-application of either stress reduced the damage caused by subsequent application of the other stress, and this induced defense was greater by UV-B than by drought. Compared to the stress applied separately, the combined application of drought and UV-B at the same time resulted in more adverse effects on the wheat seedlings of the susceptible variety, and more positive effects on the tolerant wheat genotype. These results provide novel insights into understanding the cellular and molecular mechanisms responsible for plant tolerance to various stresses and their interactions.

Key words: Wheat (*Triticum aestivum* L.), UV-B radiation, drought, combined stress, antioxidant system, genotype.

INTRODUCTION

The ongoing depletion of stratosphere ozone layer is resulting in an increase of solar ultraviolet-B (UV-B) radiation (280 to 320 nm) reaching the earth's surface, as measured in many geographic regions (McKenzie et al., 2003). The enhanced levels of UV-B radiation has been shown to have deleterious effects on biological organisms. Plants exhibit different responses to UV-B irradiation with respect to growth, production of dry matter and biochemical changes (Kramer et al., 1991). Some plant species are unaffected by UV-B irradiation and several are apparently stimulated in their growth, but most species are sensitive and damaged (Teramura, 1983).

UV-B irradiation can also influence the physiological responses of plants to the other environmental factors. Elevated UV-B irradiation limited the ability of wheat, rice and soybean to take advantage of the elevated CO_2 in photosynthesis (Teramura et al., 1990), but however, enhanced heat tolerance of cucumber seedlings (Teklemariam and Blake, 2003).

On the other hand, the effect of enhanced UV-B radiation on plants can be modified by other co-occurring stresses or by simply changing environmental factors like atmospheric CO_2 (Bjorn et al., 1997). Temperature affected the extent of growth inhibition of cucumber cotyledons irradiated with UV-B (Takeuchi et al., 1993). Sensitivity of crop plants to UV-B is also influenced by

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Abbreviations: SOD, Superoxide dismutase activity; **CAT,** catalase activity.

water regime, ambient levels of visible radiation and nutrient status (Manetas et al., 1997; Balakumar et al., 1993; Levizou and Manetas, 2001).

Water stress or soil drought is an important restricting factor, which limits the productivity of many crops and affects both quality and quantity of the yield. Drought stress brings about a reduction in growth rate, stem elongation, leaf expansion and stomatal movements (Hsiao, 1973). It also affect various physiological and biochemical processes governing plant growth and productivity (Daie, 1988). Under field conditions plants usually experience several stresses simultaneously. The stresses may cause a variety of plant responses which can be additive, synergistic or antagonistic.

In many cases, UV-B irradiation appears companying with drought stress during crop plant growth seasons. Elucidation of the interaction between drought and UV-B stresses and their effects on plant growth and development would help in understanding the mechanism responsible for plant adaptation to changing environmental conditions and developing agronomic systems for crop productions. Although the responses of plants to UV-B or drought have been intensively investigated, evidence of study in interaction between UV-B exposure and drought stress in plants had just emerged in the latest years. The mechanisms of sensitivity or tolerance of crop plants, either in growth and yield, to combined stresses remain unknown. Particularly, genotypic effects on plant responses to the two-stress combination are still unclear.

Wheat (*Triticum aestivum* L.), as one of the crucially important crops in the global food supply, is mainly distributed in the mid-latitude regions in the northern hemisphere (Lantican et al., 2005) where UV-B radiation and drought naturally occur simultaneously or subsequently during wheat growth seasons. Therefore, the purpose of this study was to investigate and compare their effect and interaction on some biochemical stress markers and stress defense enzyme systems in seedlings of winter wheat. We also evaluated the genotypic differences in physiological response of wheat to UV-B radiation and drought combined stress.

MATERIALS AND METHODS

Drought and UV-B treatments

Wheat (*Triticum aestivum* L.) varieties, Jinmai 47(drought-resistant) and Shunmai 1718 (drought-susceptible) were used in this experiment. After surface sterilization, healthy seeds were sown in plastic pots (15 x 15 cm) containing vermiculite, and irrigated with ½ strength Hoagland's solution. Wheat seedlings were grown in a growth chamber (14/10 h photoperiod; 25/20°C day/night). 7 day-old wheat seedlings were subjected to drought stress (+D) by 10% polyethylene glycol (PEG6000), which provoked moderate water stress (-0·5 MPa), to UV-B radiation (+UV), or to a combination (+D + UV), respectively. All stresses were applied throughout 20 days for both genotypes.

As a source of UV-B radiation, a mercury lamp with a characteristic emission in the range of 280 to 320 nm (HPQ 125 W, Phillips, Eindhoven, The Netherlands) was used. The lamp irradiation gave a photon flux density of 64·4 μmol $m^{-2}s^{-1}$ and the UV-B irradiation was about 80% of the total light emission. The seedlings were transferred daily under the HPQ lamp for 2 h in the middle of the day. The distance between the lamp and plants was 25 cm. The plants without UV-B irradiation treatment were kept under a polyester film (0.13 mm Mylar Type D) (Du Pont Co, Newton, CT, USA), which absorbs radiation below 320 nm. For the treatments with UV-B irradiation, a cellulose acetate filter (0.13 mm) was used to cut off the radiation below 280 nm, and plants received 49 $kJm^{-2}d^{-1}$ biologically effective UV-B radiation.

Measurements of wheat seedling growth parameter

All measurements for seedling growth parameter were conducted at the end of each stress treatment. Fresh weight of the above-ground parts was measured, and after that plants were dried at 105°C to constant weight for the determination of dry weight. Relative leaf water content (RWC) was calculated according to the equation of Fletcher et al., (1988). Leaf area was measured according to Tsonev and Sergiev (1993) using a planar scanner and image plot software.

Determination of proline, H_2O_2, chlorophyll and ascorbate

The fresh plant materials collected at various time points were immediately used for the extraction and assay according to the appropriate methods listed here.

Free proline was extracted, derivatized with acid ninhydrin, and absorbance was read according to Bates et al. (1973) method using L-proline as a standard. Content of proline was expressed as μmol/g fresh weight. Total chlorophylls were extracted with 80% acetone and were estimated according to Arnon (1949). Content of the chlorophylls was expressed as mg/g fresh weight. Hydrogen peroxide (H_2O_2) was measured according to Alexieva et al. (2001). H_2O_2 was measured spectrophotometrically after reaction with KI. The reaction mixture consisted 0.5 ml 0.1% trichloroacetic acid (TCA) leaf extract supernatant, 0.5 ml of 100 mM K-phosphate buffer and 2 ml reagent (1 M KI w/v in fresh double-distilled water H_2O). The blank probe consisted of 0.1% TCA in the absence of leaf extract. The reaction was developed for 1 h in darkness, and absorbance was measured at 390 nm. The amount (μmol/g fresh weight) of hydrogen peroxide was determined using a given H_2O_2 standard curve.

Ascorbate content was determined according to the method described by Foyer et al. (1983). Plant materials (0.5 g) were ground in liquid nitrogen and then 2 mL of 2.5 M perchloric acid was added. The crude extract was centrifuged at 4°C for 15 min at 15,000 g, and the supernatant was neutralized with saturated K_2CO_3 using methyl orange as an indicator. Insoluble $KClO_4$ was removed by centrifugation and aliquots of the supernatant were used for measuring ascorbate and dehydroascorbic acid (DHA) contents. The reduced ascorbate (ascorbic acid, AsA) was determined spectrophotometrically at 265 nm in 0.1 M NaH_2PO_4 buffer (pH 5.6), with 0.1 units of ascorbate oxidase. The total ascorbate was determined after incubation in the presence of 30 mM DTT. The standard curve was prepared with AsA. DHA level was obtained as the difference between AsA and total ascorbate. The content was calculated as μmol/g fresh weight.

Enzyme extraction and assay

For enzymatic activities, assays were carried out using the crude extract of the leaves as the enzyme source. Leaves were

Table 1. Effect of drought and UV-B applied alone or in combination on growth parameters of wheat seedlings.

Parameter	Control	UV-B (+U)	Drought (+D)	UV-B-drought (preU+D)	Drought-UV-B (preD+U)	Drought-UV-B (U+D)
Resistant genotype						
Plant height (cm)	16.6±1.5	14.3±0.9**	16.1±1.3	15.5±1.2 *	15.1±0.8*	15.9±1.4*
Fresh weight (mg)	102.2±3.5	89.1±5.2 **	99.1±3.4	96.3±3.9*	93.4±4.5*	97.2±3.6*
Dry weight (mg)	17.1±1.4	14.8±0.9**	16.6±0.5	16.1±0.6*	15.6±0.8*	16.5±0.5*
Leaf area (cm^2)	6.94±0.53	5.98±0.31**	6.59±0.48	6.46±0.65	6.26±0.40*	6.50±0.37
RWC (%)	95.08±1.85	93.70±2.03	74.25±3.46**	85.70±1.57*	78.69±2.82*	84.03±2.91*
Chlorophyll content (mg/g fresh weight)	2.49±0.09	2.02±0.13**	2.40±0.10	2.20±0.11	2.43±0.15	2.11±0.15*
Susceptible genotype						
Plant height (cm)	17.9±1.3	14.8±1.1**	17.6±1.1	16.3±1.3*	15.6±1.0**	13.6±1.2**
Fresh weight (mg)	113.4±3.9	93.4±5.7**	109.6±3.5*	100.2±4.4 *	98.5±4.9**	87.3±4.2**
Dry weight (mg)	18.3±1.5	15.2±1.1**	17.6±0.5*	16.4±0.7*	15.8±0.8**	14.6±0.6**
Leaf area (cm^2)	7.28±0.46	5.24±0.12**	5.79±0.28*	5.68±0.39	5.44±0.21**	5.03±0.10**
RWC (%)	96.04±1.76	94.09±2.12	70.10±3.77**	80.30±1.62*	73.88±3.11**	78.20±2.89*
Chlorophyll content (mg/g fresh weight)	3.07±0.21	2.69±0.20*	2.97±0.19	2.74±0.26	2.99±0.24	2.82±0.23

Values in brackets are percentage of control. The data are mean±SE (n=20). * and **, indicate significant difference between control and UV-B radiation, Drought or their combined stress at $P<0.01$ or $P<0.05$, according to T-test

homogenized at 4°C in 100 mM K-phosphate buffer (pH 7.8), 10 mM $MgCl_2$, and 0.2 mM EDTA. The homogenate was centrifuged at 17000 *g* for 30 min to yield a crude enzyme extract.

Catalase activity (CAT) was determined according to Brennan and Frenkel (1977). The reaction mixture contained 25 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2 and prepared enzyme extract in a final volume of 3 ml. The reaction was initiated by the addition of 100 µl of the enzyme extract, and activity was determined by measuring the initial rate of disappearance of H_2O_2 at 240 nm for 1 min (E=39.4/ (mM cm).

Superoxide dismutase activity (SOD) was detected with the method described by Beyer and Fridovich (1987). One unit of SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm. The reaction mixture with a total volume of 3 ml including the prepared enzyme extract, 50 mM potassium phosphate buffer (pH 7.8), 13 mM L-methionine (Met), 75 mMNBT, 10 mM EDTA and 2.0 mM riboflavin.

Total soluble protein content was determined according to the method of Bradford (1976) with BSA as a calibration standard.

Statistics analysis

Data presented are the averages of at least six replicates, obtained from independent experiments. One-way ANOVA was followed by Dunnett post t-test. In the results presented asterisks are used to identify the levels of significance: *$p < 0{\cdot}05$ and **$p < 0{\cdot}01$. Bars in the figures show standard errors (S.E.) of the means.

RESULTS

Effects of drought, UV-B and combination of stresses on growth of wheat seedlings

The effects of the stresses on growth parameters of wheat seedlings measured for both genotypes are documented in Table 1. In general, the growth of wheat seedlings was retarded by all the stresses tested. However, the inhibited effect was different for different stresses and genotypes. For example, the fresh weight of wheat seedlings was 102.2 mg per plant without any stress (control) and drought. UV-B as well as the combination of the stresses inhibited growth by 3.0, 12.8 and 4.9% for the drought-tolerant genotype (Jinmai 47), respectively, while the growth reduction was 3.4, 17.6 and 23.0% for the drought-susceptible genotype (Shunmai, 1718), respectively. Compared to the plants treated by UV-B or drought alone, the pretreated plants by either stress showed less damage caused by subsequent treatment of the other stress. The tolerance of the UV-B pretreated plants to drought was stronger than the tolerance of drought-pretreated plants against UV-B radiation.

In addition to the fresh weight, UV-B and drought influenced other growth parameters such as plant height, dry weight, leaf area, WRC and chlorophyll content in the two wheat genotypes. Notably, UV-B radiation significantly affected chlorophyll content while drought had a marked effect on RWC especially. Like the effects on fresh weight, in comparison with the injurious effects of a single stress factor, the negative influences of UV-B on chlorophyll and drought on RWC were reduced when UV-B irradiation and drought stress were applied simultaneously or successively. However, a difference in response between the two genotypic wheat seedlings could be noted in double-stress applications. In Shunmai 1718 (drought-susceptible genotype), the RWC was significantly lower than the control value, whereas the

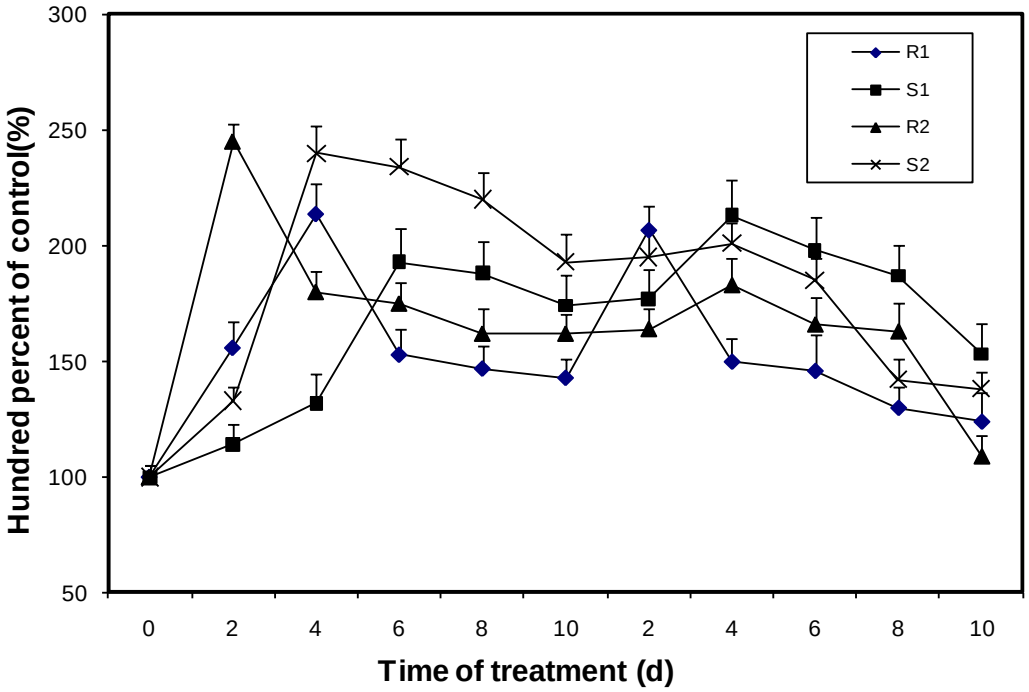

Figure 1. The level of H_2O_2 in wheat seedlings under the successive stress (pred+u, preu+d) of UV-B and drought. 100 percent (%) corresponds to the H_2O_2 level in the control plants without any stress. Samples were collected at 0 d, 2 d, 4 d, 6 d, 8 d and 10 d after the first stress (preD or preU) and then the second stress (+U or +D), respectively. R1, drought-resistant genotype treated by preD+U stresses; S1, drought-susceptible genotype treated by preD+U stresses; R2, drought-resistant genotype treated by preU+D stresses; S2, drought-susceptible genotype treated by preU+D stresses. Resistant genotype (R) and susceptible genotype (S) were indicated. Vertical bars are standard errors (S.E.) of means.

reduction of chlorophyll content were considerable in comparison with the control in Jinmai 47 (drought-tolerant genotype). These data indicated that the stress pretreatment induced plant defense response which subsequently alleviated the negative effects of the other stress. Moreover, plant responses to drought, UV-B and the combined stress were genotype-dependent.

Time course of H_2O_2 content in wheat seedlings under the stresses

H_2O_2 concentrations were maintained around 1.1 to 1.3 μmol g^{-1} FW in the control leaves at the beginning and end of the experiment. However, the content of H_2O_2 increased significantly under stressful conditions following seedling growth (Figure 1 and 2) compared to the control plants without any stress. Moreover, the peak value and time course of H_2O_2 level were different for different stresses and genotypes.

In the pretreatments, UV-B induced H_2O_2 up to its peak level at days 2 and 4 for Jinmai 47 and Shunmai 1718, respectively; two days earlier than drought stress. The maximum amount of H_2O_2 under UV-B was 31 and 47% higher than that under drought stress for the two genotypes, respectively. After the peak time, H_2O_2 content declined till the end (day 10) of the pretreatments, at which H_2O_2 level was higher in the UV-B-stressed plants than the drought-stressed plants for both varieties. In the following second treatments, H_2O_2 was induced to elevate again, and then reduced. The enhancement in the drought-pretreated seedlings by UV-B was greater than the UV-B-pretreated seedlings by drought. At the end of the second stress, all the plants showed a lower H_2O_2 level ($P>0.01$) than at the end of the pretreatments. Furthermore, H_2O_2 level in the tolerant genotype was significantly lower ($P>0.01$) than in the susceptible one. Particularly, the pre-UV-B and following drought treatments led to lower H_2O_2 compared to the pre-drought and consequent UV-B stresses for both varieties.

Under the combined stress of UV-B and drought applied simultaneously (Figure 2), H_2O_2 level quickly increased to its maximum and then reduced through to the end of the treatments. Comparison between the two genotypes showed that the peak level and time were 37% lower and were 2 days earlier in the tolerant genotype than in the susceptible one. At the end of the combined stress, H_2O_2 level was 62% higher in the susceptible variety than in the tolerant variety. Overall, UV-B radiation caused more effects on H_2O_2 than the drought treatment. Pretreatment of either stress reduced the impact of subsequent application of the other stress.

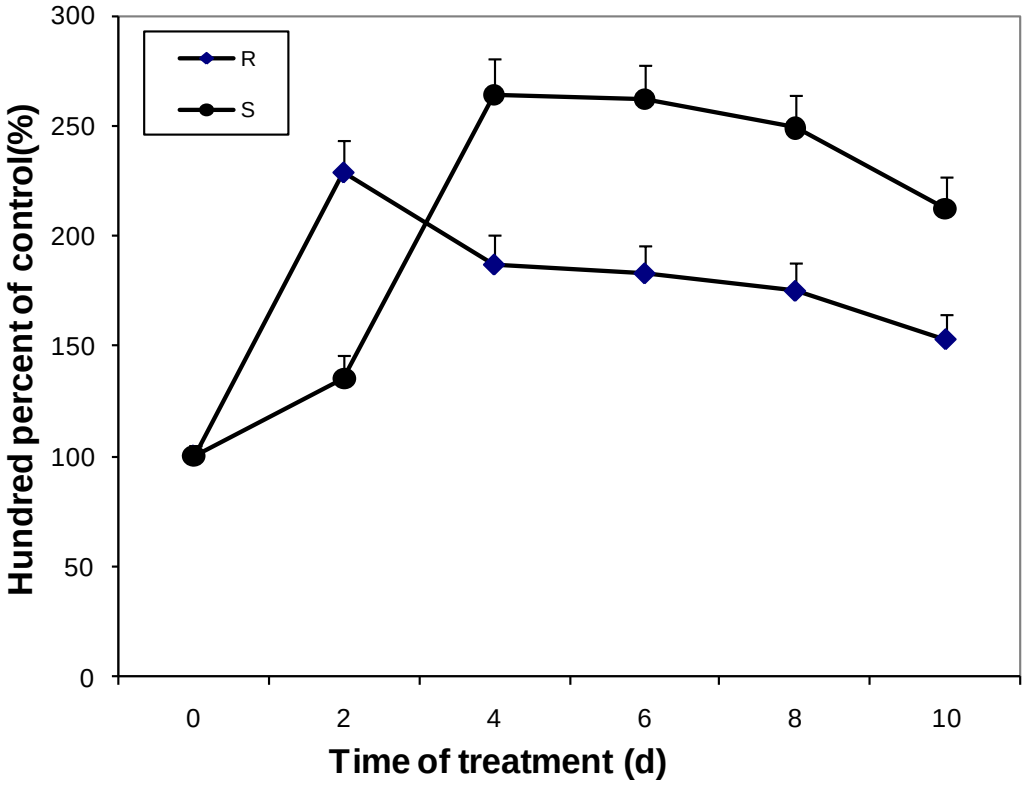

Figure 2. The level of H_2O_2 in wheat seedlings under the combined stress (U+D) of UV-B and drought. 100% corresponds to the H_2O_2 level in the control plants without any stress. Samples were collected at 0, 2, 4, 6, 8 and 10 days after the combined stress. R,Resistant genotype; S, Susceptible genotype. Vertical bars are standard errors (S.E.) of means.

For susceptible genotype, the combined stress produced more H_2O_2 than the drought or UV-B treatment alone, while the H_2O_2 level induced by the combined stress was higher than by the drought and lower than by UV-B stress in the tolerant variety.

Temporary expression of antioxidative enzymatic activity induced by the stresses

The activity of SOD and CAT experienced a slight change in the control plants during the 20 days of growth, and this variation was not significant. However, the three enzymes displayed distinct patterns of activity expression under the stressful conditions.

Following the pretreatment of drought (preD) and subsequent UV-B stress (+U), SOD activity (Figure 3A) increased to its first peak on day 2 and day 4 post preD in Jinmai 47 and Shunmai 1718, respectively. The second higher peak occurred on day 2 post +U for both genotypes. After the second peak, SOD level slowly increased till the end of the treatment. However, only one peak of SOD activity was induced on day 2 and day 4 post pretreatment of UV-B (preU) in Jinmai 47 and Shunmai 1718, respectively. Unlike in the preD+U treatments, the subsequent drought stress (+D) did not significantly enhance the additional SOD activity to the end of the preU+D treatments. As shown in Figure 1A, SOD levels at the peak and the end of the preU+D treatments were both higher in the susceptible variety (Shunmai, 1718) than in the tolerant one (Jinmai 47).

CAT activity (Figure 3B) was also changed greatly following the treatments. The first peak of CAT activity occurred at day 2 or day 4 after the pretreatment of either UV-B or drought. The subsequent stress of the other induced more enhancement of CAT activity and resulted in the second peak at different time for different stresses and genotypes. In contrast to SOD, the increased CAT level was much higher in the tolerant variety than in the susceptible one, particularly at the peak time and the end of the treatments. Moreover, CAT activity post the peak was reduced rapidly in the susceptible variety while the activity kept stable or just slightly declined in the tolerant genotype. Again, UV-B radiation exhibited more effects on CAT activity than drought stress.

The activity expression of SOD and CAT under the combine stresses (Figure 4) was mostly like the pattern under UV-B radiation alone. For tolerant genotype, there were a higher level of CAT and a lower level of SOD in the double-stress treatments.

Conversely, higher SOD and lower CAT were detected in the double stresses for the susceptible variety.

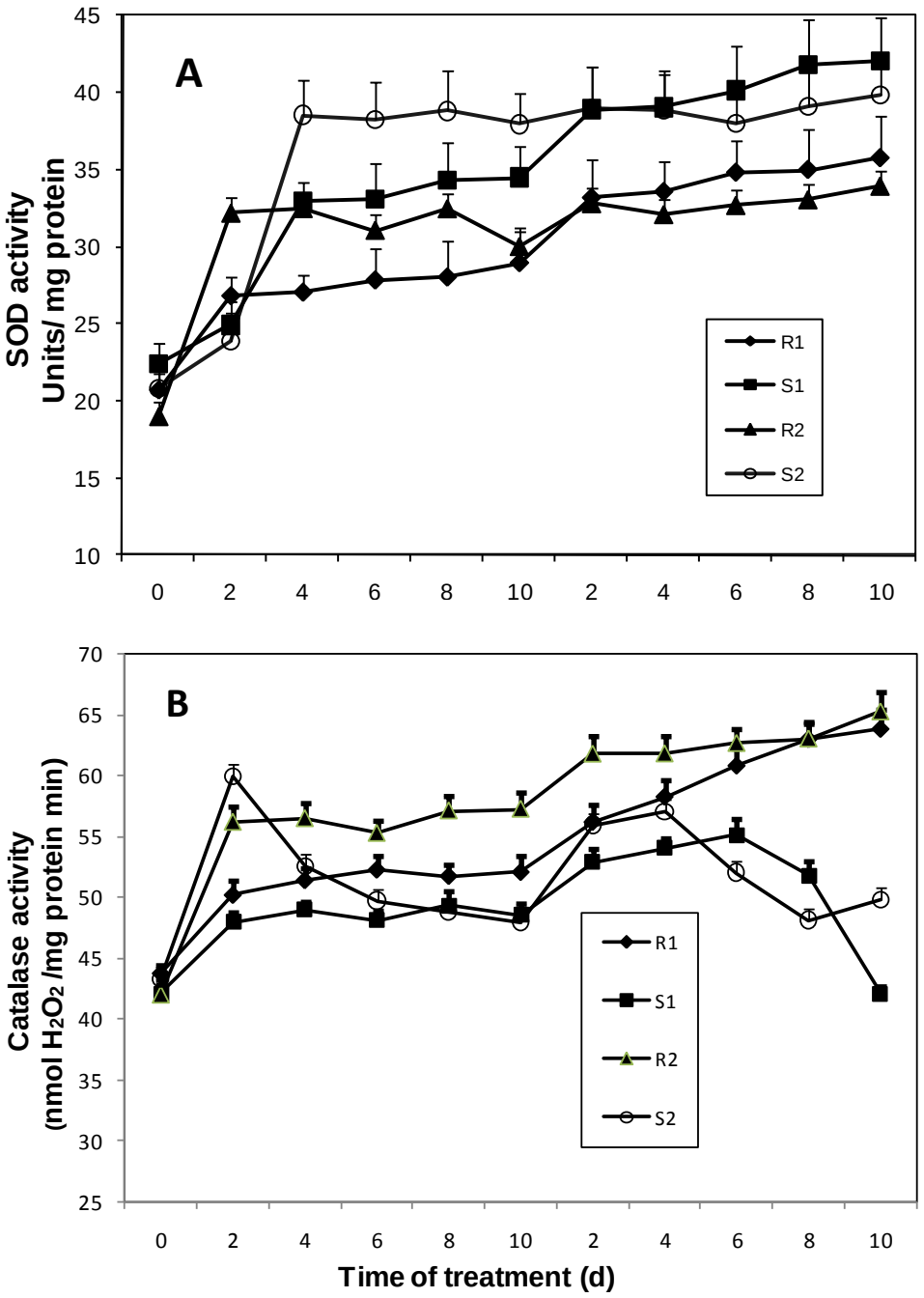

Figure 3. Changes in activities of antioxidant enzymes under the successive stresses (preD+U, preU+D) of UV-B and drought. Samples were collected at 0, 2, 4, 6, 8 and 10 days after the first stress (preD or preU) and then the second stress (+U or +D), respectively. R1, drought-resistant genotype treated by preD+U stresses; S1, drought-susceptible genotype treated by preD+U stresses; R2, drought-resistant genotype treated by preU+D stresses. S2: drought-susceptible genotype treated by preU+D stresses. Resistant genotype (R) and susceptible genotype (S) were indicated. Vertical bars are standard errors (S.E.) of means.

Changes in contents of antioxidative metabolites under the stresses

Free proline in the drought-pretreated plants were observed to increase by 1.16 and 0.5 folds greater than that in the control at the end of the pretreatment for tolerant and susceptible genotype, respectively (Figure 5). However, pretreatment of UV-B just enhanced proline by a smaller amount, and there was no significant difference between the tolerant and susceptible genotypes. Sub-

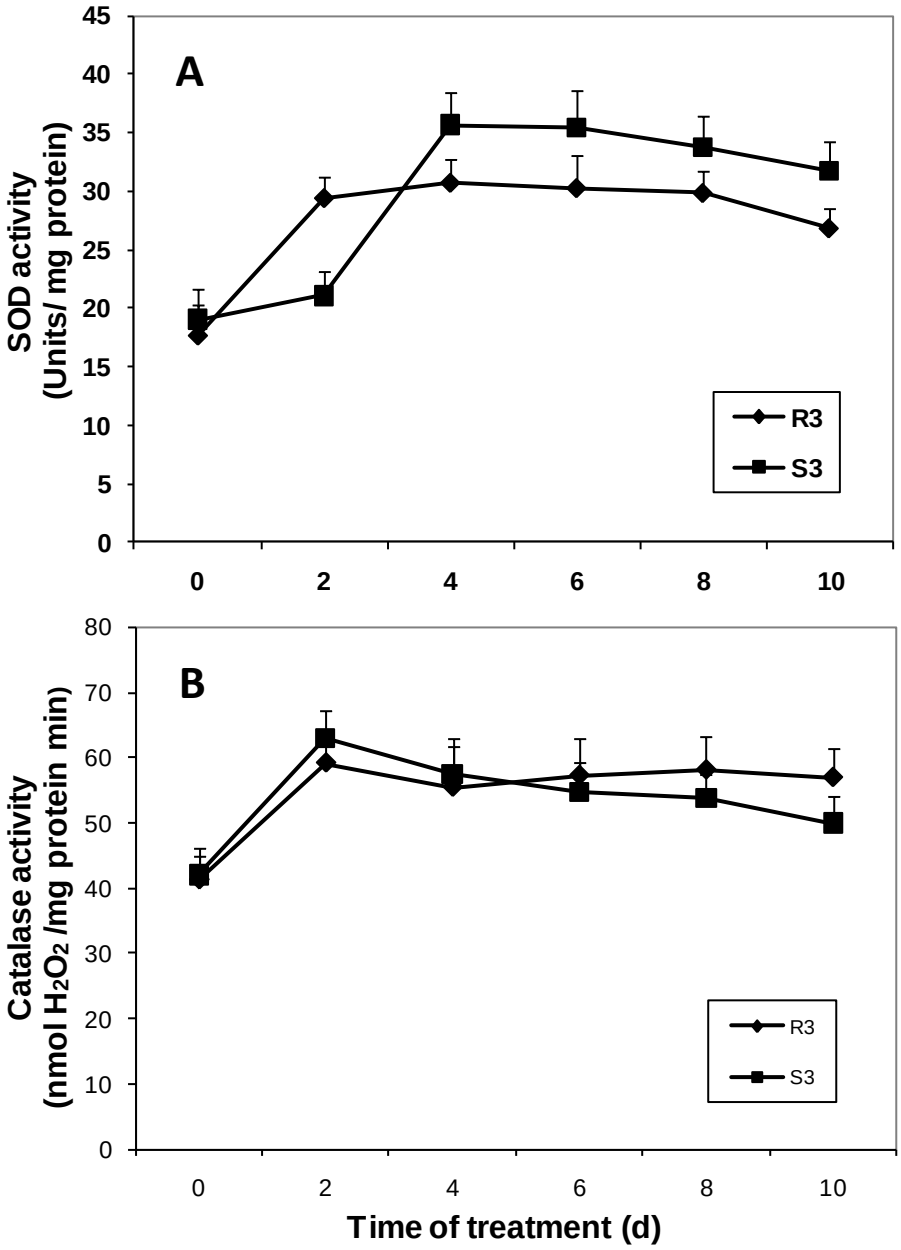

Figure 4. Changes in activities of antioxidant enzymes under the combined stress of UV-B and drought. Samples were collected at 0, 2, 4, 6, 8 and 10 days after the combined stress. R3, drought-resistant genotype treated by the combined stresses; S3, drought-susceptible genotype treated by the combine stresses. Vertical bars are standard errors (S.E.) of means.

sequent UV-B stress only induced proline level which additionally accumulated a little in the drought-pretreated plants, while subsequent drought stress resulted in a larger enhancement of proline content in the UV-B-pretreated plants. The combination of both stresses led to a slightly lower proline content in comparison with the drought application alone, and this depressed effect on proline content was greater in the susceptible genotype than in the tolerant one (Figure 5). The data showed that drought stress had a much stronger impact than UV-B stress in relation to proline accumulation regardless applied together, separately, and successively.

All stresses increased ascorbic acid (AsA) concentration, and did not cause much change in dehydroascorbic acid (DHA) content compared to the control (data not shown). As a result, a higher ASA/DHA ratio was observed following the treatments (Figure 6). No significant difference was detected between the two genotypes. The percent increment of ASA/DHA ratio was lower in the pretreatment of either UV-B (36%) or drought

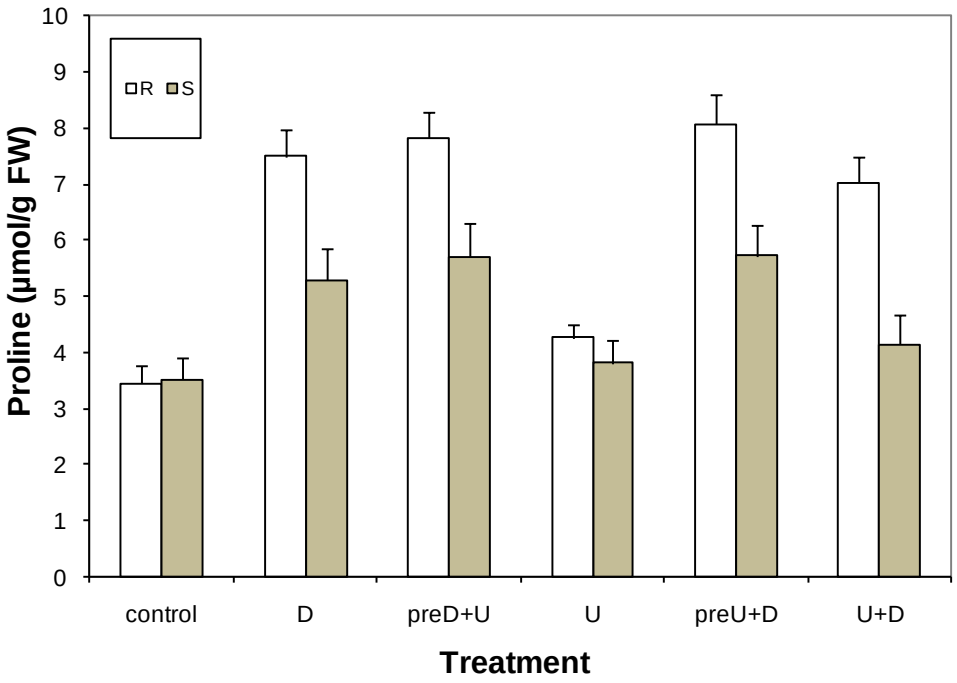

Figure 5. Free proline content in winter wheat seedlings under UV-B, drought, and the combined stresses. Samples were collected at the end of each treatment. D, Drought treatment alone; U, UV-B treatment alone; U+D, UV-B and drought treatments applied simultaneously; preD+U, drought treatment applied firstly and then UV-B treatment was applied; preU+D, UV-B treatment was applied firstly and then drought treatment was applied. Values are means of six replicates and standard errors (S.E.).

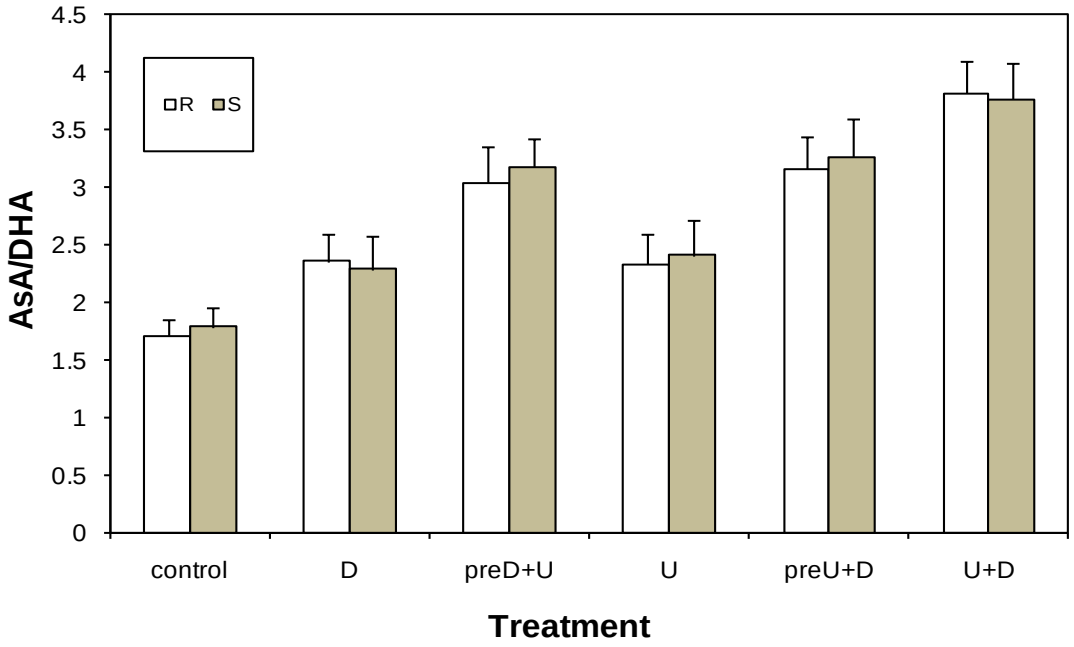

Figure 6. The reduced-to-oxidized ascorbate ratio (AsA/DHA) in winter wheat seedlings under UV-B, drought, and the combined stresses. Samples were collected at the end of each treatment. D, Drought treatment alone; U, UV-B treatment alone; U+D, UV-B and drought treatments were applied simultaneously; preD+U, Drought treatment was applied firstly and then UV-B treatment was applied; preU+D, UV-B treatment was applied firstly and then drought treatment was applied. Values are means of six replicates and standard errors (S.E.).

(38%) than in the subsequent application of drought (81%) or UV-B (77%) stress. This enhancement was more pronounced under the combined stresses (Figure 6) so that ASA/DHA ratio at the end of the treatment was higher in the plants (3.82) by the combined stresses than those when UV-B or drought stress was applied alone (2.33 and 2.36) and successively (3.16 and 3.14), indicating that the combined stress exceeded the effect of a single stress factor on ASA concentration.

DISCUSSION

With increasing depletion of stratospheric ozone and accelerating global warming, UV-B irradiation and drought stresses are becoming much more detrimental to ecological systems and crop production in the world. The mechanisms of crop plants to the combined stress of these two factors remain unknown although the interaction between UV-B and drought stress in plants has emerged these years. Therefore, the present study was conducted to investigate physiological responses of different genotypes of winter wheat to the combined stresses of UV-B and drought applied simultaneously and successively.

Our data (Table 1) of wheat growth parameters measured under the stresses tested displayed that pretreatment of either UV-B or drought reduced the damage caused by subsequent application of the other stress, conforming the viewpoint that exposure of plants to a moderate stress can induce a tolerance to a more severe stress and such treatment also can improve tolerance to other stresses (Wang et al., 2003). Moreover, the present study showed that the tolerant variety exhibited better performance than the susceptible one in this aspect, and the UV-B-induced wheat plant protection against drought was stronger than the drought-induced tolerance to UV-B radiation. This result indicated that the expression of the so-called cross-acclimation to stresses was related to the plant genotypes and stress factors.

In comparison to the inhibited effects on plant growth of UV-B or drought stress alone, the effect of the combination of the two stress factors could be additive (more damage) (Tian and Lei, 2007) or antagonistic (reduced damage) (Sullivan and Teramura, 1990; Alexieva et al., 2001). Our data further indicated that this combined effect was genotype-dependent. For the tolerant variety, the combined stress of UV-B and drought resulted in the moderated injury to wheat seedlings, which was less than the injury caused by the stresses applied individually. However, the combined stress caused more severe damage to wheat seedlings than stress factors applied separately for the susceptible variety. Thus, the combined application of drought and UV-B had more strong adverse effects on wheat seedlings of the susceptible genotype, but more positive effects on the tolerant wheat genotype.

UV-B and drought stresses seemed to act in two different ways, as it could be expected, drought signifi-cantly influenced the RWC, and UV-B largely affected chlorophyll of the treated leaves (Table 1). This finding further support that chlorophyll content and RWC were proposed as a typical maker for plant response to UV-B and drought stresses, respectively (Teramura et al., 1991; Correia et al., 1999; Santos et al. 2004). When UV-B irradiation and drought stresses were applied simultaneously or successively, the specific effects of UV-B on chlorophyll and drought on RWC were slightly reduced compared to the single stress, again indicating that interaction between UV-B and drought alleviated, to some extent, the negative effect of UV-B or drought alone.

Generation of reactive oxygen species (ROS) including H_2O_2 is recognized as one of the early effects of various biotic and abiotic stresses on plants (Bowler and Fluhr, 2000), and high accumulation of ROS can result in oxidative damage in the absence of effective protective mechanism (Alexieva et al., 2001). In our case, an increase in H_2O_2 was observed in all the treated plants compared to the control. However, the increase was more evident in the UV-B treated plants than in the drought-stressed plants (Figure 1), and thus UV-B caused a more severe damage than the drought stress on wheat seedlings measured as there was more obvious reduction in growth (Table 1). Comparatively, the interaction of UV-B and drought led to less damage on wheat seedlings, which was evidenced by a lower level of the peak value of H_2O_2 in the plants measured at the end of the combined stresses applied simultaneously or successively (Figure 2).

On the other hand, H_2O_2 received much attention as a signal molecule in response to different stresses (Prasad et al., 1994; Gong et al. 2001; Aroca et al., 2003). H_2O_2 mediated the regulation of transcription in response to UV-B exposure as an important early upstream signal (Brosche and Strid, 2003). Moreover, H_2O_2 was also implicated in the gene expression related to cold acclimation to resist freezing stress (Foyer et al. 1997). Activation of endogenous protective mechanisms can in turn tolerate or delete excess ROS burst. We found that the enhanced H_2O_2 level under the stresses was followed by the up-regulation of the enzyme activities (Figure 3). This suggests that H_2O_2 may act more as a signal molecule than directly inducing oxidative damage. In other words, the increased H_2O_2 concentration by UV-B or drought may trigger the cross-acclimation to tolerant the subsequent stresses in winter wheat through stimulation of the antioxidant defense systems.

SOD, the first antioxidant enzyme for scavenging ROS, catalyzes the dismutation of superoxide into oxygen and H_2O_2, while CAT reacts with H_2O_2 to produce water and oxygen. In this study, it was observed that activities of the two key antioxidant enzymes increased following all the treatments compared to the control plants, but their expression patterns were different for different genotypes

and stress conditions. For example, under the successive stress conditions of first UV-B radiation and then drought, SOD expression was “rapid up to the peak and then kept stable” in both genotypes, whereas the peak level was higher and occurred two days later in the susceptible genotype than in the tolerant one (Figure 3A). Consequently, SOD level was greater in the susceptible genotype than in the tolerant one at the end of the treatment. CAT expression pattern, however, was “rapid up to peak, sustaining roughly stable, and then increasing again” for the tolerant genotype or “decreasing” for the susceptible one (Figure 3B). As a result, the CAT level was higher in tolerant genotype than in the susceptible one at the end of the treatments. Similarly, SOD and CAT activities also increased following the successive stresses of first drought and then UV-B except that the enhancement rate was lower in the first stress than in the second stress. Therefore, different levels of H_2O_2 under all stresses, particularly higher H_2O_2 content in the susceptible genotype than in the other may be due to the differential expressions of activities of those enzymes following the stress. Our inference is in agreement with the report that cellular H_2O_2 concentration is the result of the balance between its production and utilization (Bowler et al., 1992).

Under the combined stresses of UV-B and drought applied simultaneously, the two enzyme activities (Figure 4) were expressed like in the case under UV-B stress alone, but SOD activity was lower and CAT was higher than that under drought and UV-B stress alone for the tolerant variety. Again, our data of the two enzyme activities indicated that the interaction of UV-B and drought stresses alleviated the negative effect of the stress factors on the tolerant genotype, but the combined stress led to more damage on the susceptible one. Notably, many contradictory results about antioxidant enzyme response to different stresses have emerged due to the fact that the levels of enzyme responses depend on the plant species, the developmental stage, the organs, as well as on the duration and severity of the stress (Wilson et al., 1993; Caldwell et al., 1983; Chappell et al., 1994). In addition, the presence of different enzyme isoforms expressed to a different extent in different seedling growth conditions (stressed and unstressed), and with different substrate affinity could be not excluded.

In addition to the activation of antioxidant enzymes, the enhancement of antioxidant metabolites such as proline and ascorbate acid (AsA) in cells is also another defense mechanism against the ROS burst caused by various stresses (Monk et al., 1989). In many plants, free proline accumulates in response to biotic and abiotic stresses, including water stress (Day et al. 1993), extreme temperatures (Strid et al., 1992), heavy metal toxicity (Long et al., 1983), and UV-B irradiation (Schreiber et al., 1986). Our data showed that drought stress had a much stronger impact than UV-B stress in relation to proline accumulation regardless applied together, separately, and successively (Figure 5), confirming that proline is a typical marker for osmotic stress, which is well-described in water and salt stresses (Heuer, 1994; Fedina et al., 2006). However, proline may exert a protective action on UV-B stress because drought-treated wheat seedlings accumulated higher level of proline, and appeared less damaged by UV-B radiation compared to the control plants (Table1). Consistence with our results, a positive effect of proline accumulation on the reduction of the UV-B induced damage is proposed in other reports (Kurkdjian and Guern, 1989; Alexieva et al., 2001).

Ascorbate is a major primary antioxidant, reacting directly with hydroxyl radicals, superoxide and singlet oxygen, and is also a powerful secondary antioxidant, reducing the oxidized form of a-tocopherol (Agrawal and Rathore, 2007). In our study, foliar ascorbic acid concentration was found to increase in all the stressful conditions. Moreover, the rate of increase was lower in the pretreatments of ether UV-B or drought than in the subsequent stresses (Figure 6), suggesting that enhancement of ASA content was not a rapid response to the single stress of UV-B or drought. However, under the combined stress of the two factors applied together, ASA increase was faster and much higher (Figure 6), indicating that the interaction between UV-B and drought stresses generated a protective effect on plants. Unlike the other parameters measured here, the time course ASA level induced by the stress showed no significant difference between the two genotypes.

In conclusion, pretreatment of either UV-B or drought can increase wheat seedling tolerance to the subsequent stress, and moreover, UV-B induced tolerance against drought stress was stronger than the drought-induced defense to UV-B stress evaluated as changes in plant growth parameters and in amounts of stress markers. The combined application of drought and UV-B together brought out strong adverse effects on wheat seedlings of susceptible variety, but more positive effects on the tolerant wheat genotype. By scavenging excessive ROS, antioxidant enzymes and compounds functioned importantly in the plant responses to UV-B radiation, drought, and their combined stresses. The expression patterns of the enzymes and temporary changes of the antioxidants tested following the treatments displayed differences to some extent for different stress conditions and genotypes, leading us to infer that the expression of plant defense strategy to stress was differently regulated by a complicate system consisting of plant genetic background, physiological status, stress factors and their interactions although the presence of a basic common response in both genotypes under the stress conditions was observed.

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