RESEARCH ARTICLE

# Stimulation of antioxidant system and lipid peroxidation by abiotic stresses in leaves of *Momordica charantia*

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The purpose of the present work was to evaluate both the antioxidant response system and oxidative stress in leaves from bitter gourd (*Momordica charantia* L) subjected to NaCl, UV-B and water stresses at three different stages of plant growth: pre-flowering, flowering and post-flowering. Except for peroxidase (POX), all enzyme activities including superoxide dismutase (SOD), catalase (CAT), polyphenol oxidase (PPO), glutathione reductase (GR), as well as concentrations of ascorbate (ASA), hydrogen peroxide ($H_2O_2$) and thiobarbituric acid reactive substances (TBARS) exhibited maximum values at the flowering stage under all three stresses. All the enzyme activities, SOD, CAT, POX, PPO, GR and the concentrations of ASA, $H_2O_2$ and TBARS were elevated under NaCl and UV-B stresses at all growth stages with the exception of $H_2O_2$ concentration at the post-flowering stage under UV-B radiation. Greater quantities of the inorganic ions $Na^+$ and $Cl^-$ were accumulated at all growth stages under salt stress. Drought led to decreases in the concentrations of $H_2O_2$, ASA and activities of PPO and GR; conversely, it led to elevated concentrations of TBARS and activities of SOD, CAT, POX at all three stages in comparison with control values. The POX activity at the third stage was, however, reduced. The photosynthetic pigments decreased at all stages under all stresses. The chlorophyll stability index decreased under NaCl stress, accelerated only at the post-flowering stage under UV-B radiation and significantly increased at pre- and post-flowering stages under water stress. The protein concentration under NaCl stress decreased except at the pre-flowering stage and under UV-B and water stresses the protein concentration was higher than that of the control and NaCl treatment. It is suggested that *M. charantia* exhibits a protection mechanism against oxidative damage by maintaining a highly induced antioxidant system under all three stresses.

**Key words**: antioxidative enzymes, environmental stresses, $H_2O_2$, ion concentration, thiobarbituric acid-reactive substances

**Estímulo do sistema antioxidativo e peroxidação de lipídios causados por estresses abióticos em folhas de *Momordica charantia*:** O objetivo deste trabalho foi avaliar as respostas do sistema antioxidativo e o estresse oxidativo em folhas de plantas de *Momordica charantia* L. submetidas a NaCl, radiação UV-B e déficit hídrico, em três estádios de crescimento: pré-floração, floração e pós-floração. Com exceção da peroxidase (POX), as atividades das enzimas dismutase do superóxido (SOD), catalase (CAT), oxidase de polifenóis (PPO) e redutase da glutationa (GR), assim como as concentrações de ascorbato (ASA), $H_2O_2$ e de substâncias reativas ao ácido tiobarbitúrico (TBARS) foram máximas no estádio de floração, independentemente do tipo de estresse aplicado. As atividades das enzimas e dos metabólitos supramencionados forma maiores sob estresses associados ao NaCl e à radiação UV-B em todos os estádios de crescimento, exceto a concentração de $H_2O_2$, que foi maior no estádio de pré-floração sob radiação UV-B. Maiores quantidades de íons $Na^+$ e $Cl^-$ acumularam-se em todos os estádios de crescimento, sob estresse salino. O déficit hídrico acarretou decréscimos nas concentrações de $H_2O_2$ e ASA, e menores atividades de PPO e GR; de modo oposto, a seca acarretou aumento na concentração de TBARS e maiores atividades de SOD, CAT e POX em todos os três estádios, em comparação com os respectivos controles. A atividade da POX no estádio de pós-floração foi, contudo, reduzida. As

concentrações de pigmentos fotossintéticos foram reduzidas em todos os estádios de crescimento para os três estresses estudados. O índice de estabilidade de clorofilas decresceu sob estresse salino, aumentou somente no estádio de pós-floração sob radiação UV-B, aumentando também nos estádios de pré- e pós-floração sob déficit hídrico. A concentração de proteínas sob estresse salino foi menor que nas plantas-controle, exceto no estádio de pré-floração; sob estresses salino e de radiação UV-B, as concentrações protéicas foram maiores tanto em relação às dos respectivos controles como às das plantas tratadas com NaCl. Sugere-se que *M. charantia* exibe um mecanismo de proteção contra danos oxidativos via manutenção de um sistema antioxidante altamente induzido, em resposta aos três estresses aplicados.

**Palavras-chave**: concentração de íons, estresses ambientes, $H_2O_2$, sistema antioxidativo, susbstâncias reativas ao ácido tiobarbitúrico

## INTRODUCTION

Oxidative stress is a central factor in abiotic and biotic stress phenomena that occurs when there is a serious imbalance in any compartment between the production of reactive oxygen species (ROS) and antioxidant defense, leading to dramatic physiological challenges (Foyer and Noctor, 2003). Reactive oxygen species have been considered mainly as dangerous molecules, whose concentrations need to be maintained as low as possible, but this concept has changed because of the multiple functions of activated oxygen (Gratão et al., 2005). Thus, it is important for cells to control the concentration of ROS tightly, but not to eliminate them completely (Schützendübel and Polle, 2002). Reactive oxygen species can be viewed as cellular indicators of stress and secondary messengers involved in all aspects of plant biology from gene expression and translation to enzyme chemistry (Foyer and Noctor, 2003). Environmental stresses that cause oxidative stress include drought, salt stress, extreme temperatures, air pollution, oxidant-forming herbicides, heavy metals, wounding, UV light, and high intensity light conditions that stimulate photoinhibition of photosynthesis. Special attention has been given to plants which face these extreme conditions in their natural environment, because most of their tolerance mechanisms are not yet well understood, and once they become well understood they could prove to be important tools for genetically engineered stress tolerant crop plants (Bohnert and Jensen, 1996). Many reports have indicated that the negative effect of environmental stresses may be partially due to the generation of ROS and/or inhibition of the system which defends against them.

During the reduction of $O_2$ to $H_2O$, a transfer of one, two or three electrons to $O_2$ can occur to form superoxide ($O_2^{\cdot -}$), hydroxyl radicals ($^{\cdot}OH$), hydrogen peroxide ($H_2O_2$) and singlet oxygen ($^1O_2$). The superoxide radical is produced at the membrane level in most plant cell organelles and hydrogen peroxide is the product of superoxide dismutase and of several oxidases of the peroxisomes. These reactive molecules, especially $^{\cdot}OH$, are highly destructive to lipids, nucleic acids, and proteins. Nevertheless, ROS such as $O_2^{\cdot -}$ and $H_2O_2$ are required for lignification and function as signals in the defense response to pathogen infection (Gratão et al., 2005).

Contamination of soils and water with metals has created a major environmental problem, leading to considerable losses in plant productivity and hazardous health effects. Exposure to toxic metals can intensify the production of ROS, which are continuously produced in both unstressed and stressed plant cells. Some of the ROS species are highly toxic and must be detoxified by cellular stress responses, if the plant is to survive and grow (Gratão et al., 2005).

Recent genetic evidence suggests that in plants purely physicochemical damage may be much more limited than previously thought. The most potentially deleterious effect of ROS under most conditions is that at high concentrations they trigger genetically programmed cell suicide events. Moreover, plants use ROS as secondary messengers in signal transduction cascades in processes as diverse as mitosis, tropisms and cell death, so their accumulation is crucial to plant development as well as defense (Noctor, 2005). Direct ROS signal transduction will only proceed if ROS escape destruction by antioxidants or consumption in a ROS cascade. Plants protect themselves by scavenging and

disposing of these reactive molecules by use of an enzymic and non-enzymic antioxidant system present in several subcellular compartments. When these defenses fail to halt the self-propagating autooxidation with ROS, cell death ultimately results. The primary scavenger is superoxide dismutase (SOD; EC 1.15.1.1), which converts $O2^{\cdot-}$ to $H_2O_2$ which is eliminated by ascorbate peroxidase (APOX; EC 1.11.1.11) in association with dehydro-ascorbate reductase (DHAR; EC 1.8.5.1) and glutathione reductase (GR; EC 1.6.4.2). The latter two enzymes help in regeneration of ascorbic acid (ASA). Hydrogen peroxide is also scavenged by catalase (CAT; EC 1.11.1.6), though the enzyme is less efficient than the APOX-GR system.

Low molecular weight antioxidants, such as ASA, glutathione and tocopherol are information-rich redox buffers that interact with numerous cellular components. In addition to crucial roles in defense as enzyme cofactors, cellular antioxidants influence plant growth and development by modulating processes from mitosis and cell elongation to senescence and death. Plants maintain most cytoplasmic thiols in the reduced state (-SH) and high concentrations of ASA that provide robust protection against oxidative challenge. Plants also make tocopherols that act as important liposoluble redox buffers. The ability of the ASA, glutathione and tocopherol pools to act as redox buffers in plant cells is one of their most important attributes (Foyer and Noctor, 2005).

The plant *Momordica charantia* (bitter gourd) is a medicinal plant used in the cure of diabetes. It belongs to the family Cucurbitaceae and is chiefly an African genus of forty species. Although its native country is uncertain, the regions of eastern India and southern China have been suggested as possible centres of domestication. There has been little research to date on physiological and biochemical aspects of *M. charantia*. In some of the previous studies, attempts have been made to evaluate physiological and biochemical changes such as effect of environmental stresses on carbohydrate and nitrogen metabolism at different stages of plant growth. To study the effect of salinity stress (75 mmol $L^{-1}$ NaCl) on different stages of plant growth, various components including carbohydrates, α and β-amylases, chlorophylls, carotenoids, chlorophyll stability index (CSI), nitrate reductase, glutamine oxaloacetate transaminase, glutamine pyruvate transaminase, and glutamine dehydrogenase were assayed (Agarwal and Shaheen, 2005a,b). Salinity, UV-B and water stress-induced changes in activities of the enzymic and non-enzymic antioxidant system have been reported by various workers (Hernandez et al., 2000; Sairam et al., 2002; Agarwal and Pandey, 2003 a,b, 2004; Agarwal 2007). However, little is known about the effect of abiotic stresses in ROS metabolism in *M. charantia.* Thus the objective of the present investigation was to study the effect of abiotic stresses (salinity, UV-B and water stresses) on ionic content, lipid peroxidation, photosynthetic pigments and various antioxidant enzymes and metabolites in order to evaluate the relative significance of these antioxidant systems in conferring tolerance to these stresses.

## MATERIAL AND METHODS

*Plant material and experiment design:* The seeds of *Momordica charantia* L. were surface sterilized by treatment with 0.1 % mercuric chloride solution for 5 min and then thoroughly washed with double-distilled water. Seeds were sown in 12" earthenware pots filled with a mixture of garden soil and compost in the ratio of 3:1. Six seedlings were maintained in each pot. Plants were watered as and when required. Several experiments were conducted to establish the concentrations of NaCl, times of radiation and water stress to evaluate plant tolerance to these stresses. The salt treatment was begun 20 d after sowing (DAS). Three concentrations of NaCl were applied: 0 (control), 25 mmol $L^{-1}$ (non-saline) and 75 mmol $L^{-1}$ NaCl (saline). Each treatment of 500 mL was applied at 3-d intervals for 9 d at pre-flowering (30 d), and at 3-d intervals for 12 d at the flowering (45 d) and post-flowering (60 d) stages. Pots given water throughout the experiment served as control. Top terminal leaves served for sampling of plant material. For UV-B treatment, the uppermost leaves from plants treated with water during the entire life of the plant were subjected to UV-B irradiation with a UV-B lamp (radiation 290-320 nm) in a chamber. The UV-B radiation was supplied by a Philips green safe lamp (25 W lamp covered with eight layers of green cellophane) mounted in mobile frames whose distance to the plants could be adjusted to provide 2.66 W $m^{-2}$ $s^{-1}$ for 30 and 45 min. Normal leaves not exposed to UV-B radiation served as control. Before starting the water stress treatment pots were saturated with water and water was withheld for 1 d and 3 d and thereafter the

plants were irrigated. The first water stress treatment was started at the pre-flowering stage (30 d) and subsequently at the flowering (45 d) and post-flowering stages (60 d).

*Ascorbate (ASA) conten*t: Ascorbic acid was estimated as described by Mukherjee and Choudhuri (1983). The absorbance was recorded at 530 nm.

*Antioxidative enzyme activities*: Enzyme extracts for determination of SOD, peroxidase (POX, EC 1.11.1.7) and polyphenol oxidase (PPO, EC 1.10. 3.1) activities were prepared by grinding 0.2 g of the uppermost leaves taken at various stages with 5 mL of chilled phosphate buffer, while the extract for CAT was prepared by grinding 0.2 g leaf material with 10 mL of chilled phosphate buffer. For SOD the extraction medium was 0.1M phosphate buffer at pH 7.8 and for CAT, POX and PPO 0.1 M phosphate buffer at pH 6.8 was used. The brei was filtered through cheesecloth and the filtrate centrifuged in a refrigerated centrifuge at 10,000 *g* for 20 min. The supernatant served as enzyme extract. All operations were carried out at 4ºC. Superoxide dismutase activity was estimated according to the method of Beauchamp and Fridovich (1971) as modified by Giannopolitis and Ries (1977). Absorbance was recorded at 560 nm. One unit 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 nmol $mg^{-1}$ protein. Activities of CAT, POX, and PPO were assayed according to Chance and Maehly (1955) with modifications. One unit of CAT activity is defined as 1 µmol of $H_2O_2$ consumed $g^{-1}$ FW $min^{-1}$. For POX the amount of purpyrogallin formed was determined by taking the absorbance at 420 nm. One enzyme unit is defined as mmol $H_2O_2$ consumed $g^{-1}$ FW $min^{-1}$. The reaction mixture for PPO was the same as that for POX except that $H_2O_2$ was not added. Increase in absorbance at 420 nm was recorded for 1 min. One PPO unit is defined as µmol pyrogallol oxidized $g^{-1}$ FW $min^{-1}$. For GR, its activity was assayed by recording the increase in absorbance in the presence of oxidized glutathione and DTNB (5,5-dithiobis-2-nitrobenzoic acid) according to Smith et al. (1988). The increase in absorbance at 412 nm was recorded at 25ºC over a period of 5 min.

*Thiobarbituric acid-reactive substances*: The level of lipid peroxidation was measured as the amount of thiobarbituric acid-reactive substances (TBARS) determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). The leaf material (0.2g) was homogenized in 10 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15000 *g* for 5 min. To 1.0 mL aliquot of the supernatant 4.0 mL of 0.5% TBA in 20% TCA was added. The mixture was heated at 95ºC for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 *g* for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The TBARS content was calculated using its absorption coefficient of 155 $mM^{-1}$ $cm^{-1}$ and expressed as mmol TBARS $g^{-1}$ FW.

*Hydrogen peroxide content:* Hydrogen peroxide was estimated with titanium reagent as described by Teranishi et al. (1974). One gram of titanium dioxide and 10 g of potassium sulphate were mixed and digested with 150 mL of concentrated sulphuric acid for 2 h on a hot plate. The digested mixture was cooled and diluted to 1.5 L with distilled water and used as titanium reagent. Sample preparation for $H_2O_2$ estimation was carried out as described by Mukherjee and Choudhuri (1983). Leaf material (0.2 g) was homogenized in 10 mL of cold acetone. The homogenate was filtered through Whatman No.10 filter paper. To the whole extract 4 mL of titanium reagent were added followed by 5 mL of concentrated ammonium solution to precipitate the peroxide-titanium complex. After centrifugation for 5 min at 10,000 *g*, the supernatant was discarded and the precipitate dissolved in 10 mL of 1 M $H_2SO_4$. It was centrifuged again to remove the undissolved material and absorbance recorded at 415 nm against a blank. The concentration of $H_2O_2$ was determined using a standard curve plotted with known concentrations of $H_2O_2$ using catechol (0.1 mg $mL^{-1}$ distilled water).

*Other assays:* Total soluble protein content was determined according to Bradford (1976) using BSA as a standard. Chlorophylls and carotenoids were estimated by extracting the leaves in 80% acetone. Absorbance was recorded at 665, 645 and 470 nm. Chlorophylls (*a* + *b*) were calculated according to Arnon (1949) and carotenoid content according to Lichtenthaler and Wellburn (1983). Chlorophyll stability index (CSI) was calculated

according to the method of Murty and Majumdar (1962). Sodium concentrations were determined as described by Allen et al. (1976). Chloride concentration was estimated argentometrically.

*Statistics*: Each data point is the mean of three replicates. The analysis was carried out using the statistical package MINITAB version 13.1. All data were subjected to a one-way analysis of variance (ANOVA) and the significant differences ($P < 0.05$) between control and each treatment was analyzed at 30, 45 and 60 DAS, using Tukey's test.

## RESULTS

Treatment of NaCl accelerated the activity of SOD (Figure 1A). The SOD activity which was elevated at the pre-flowering stage was further enhanced at the flowering stage but decreased at the post-flowering stage. However, an increase over control values was maintained at all stages. Under water stress for 1 d ($WS_1$) the activity of SOD was almost similar to that of 75 mmol $L^{-1}$ NaCl but with water stress for 3 d ($WS_2$) the activity was suppressed but still above the control value. Under UV-B 45 treatment the SOD activity was elevated as compared to UV-B 30 but was nevertheless much lower than for the other stresses.

Under salt stress the CAT activity was elevated significantly but under UV-B and water stresses there was almost a 50% reduction (Figure 1B). However under all three stresses maximum activity was observed at the flowering stage.

The activities of POX and PPO were elevated at 75 mmol $L^{-1}$ NaCl (Figures 2A, 3A). The POX activity was depressed under UV-B and water stresses as compared to 75 mmol $L^{-1}$ NaCl. The activity of PPO under UV-B stress decreased at pre- and post-flowering stages and at flowering it was similar to that of NaCl stress. Under water stress the PPO activity was however depressed in comparison to NaCl and UV-B stresses. The POX activity was maximum at the flowering stage while the PPO activity was maximum at the pre-flowering stage.

Activity of GR increased under NaCl stress and the maximum activity was evident at the flowering stage (Figure 2B). Under UV-B stress the activity at pre-flowering was marginally higher than the NaCl value; apart from this, at other stages and under both exposures (30 and 45 min to UV-B) the GR activity was lower than that obtained under salt stress. However, the activity was greater than the control values. Under water stress the GR activity was reduced below control values.

Ascorbate concentration increased at the first two stages under NaCl stress as compared to control values (Figure 3B). The ASA concentration under UV-B exposures was lower than values for the 75 mmol $L^{-1}$ NaCl treatment at the first two stages but at the post-flowering stage it was slightly higher. Under water stress *M. charantia* plants responded negatively in comparison to the control, NaCl stress and UV-B exposure except at the pre-flowering stage of $WS_1$ where the ASA concentration was higher than that of UV-B 45 min exposure.

The $H_2O_2$ concentration was higher than control values for 75 mmol $L^{-1}$ NaCl at all three stages of growth (Figure 4A). Under UV-B exposures the $H_2O_2$ concentration was higher than the 75 mmol $L^{-1}$ NaCl values both at pre-flowering and flowering but at post-flowering there was a marked decline as compared to the NaCl value. The $H_2O_2$ concentration at the flowering stage of UV-B 45 min exposure was, however, marginally lower than the NaCl value. Under water stress at the first two stages the concentration was lower than in control plants, 75 mmol $L^{-1}$ NaCl and UV-B exposures but at post-flowering the concentration was elevated above the UV-B radiation value.

An increasing trend of TBARS concentration was observed in *M. charantia* for the 75 mmol $L^{-1}$ NaCl treatment (Figure 4B). Under UV-B radiation the TBARS concentration was elevated at pre-flowering, almost similar at flowering and decreased at post-flowering as compared to NaCl values. Under water stress the TBARS concentration was higher than the NaCl and UV-B values at all three stages of plant growth.

The $Na^+$ and $Cl^-$ concentrations at different stages of growth were measured (Table 1). Under salt stress, *M. charantia* accumulated more of the inorganic ions $Na^+$ and $Cl^-$ in the leaves, shoot and root. The $Na^+$ and $Cl^-$ concentration was significantly higher than in control plants at all stages and in all plant parts at 75 mmol $L^{-1}$ NaCl whereas at 25 mmol NaCl $L^{-1}$ an opposite response was found. The leaves under all treatments showed the highest $Na^+$ and $Cl^-$ concentration while the shoot showed the lowest $Na^+$ and $Cl^-$ concentration.

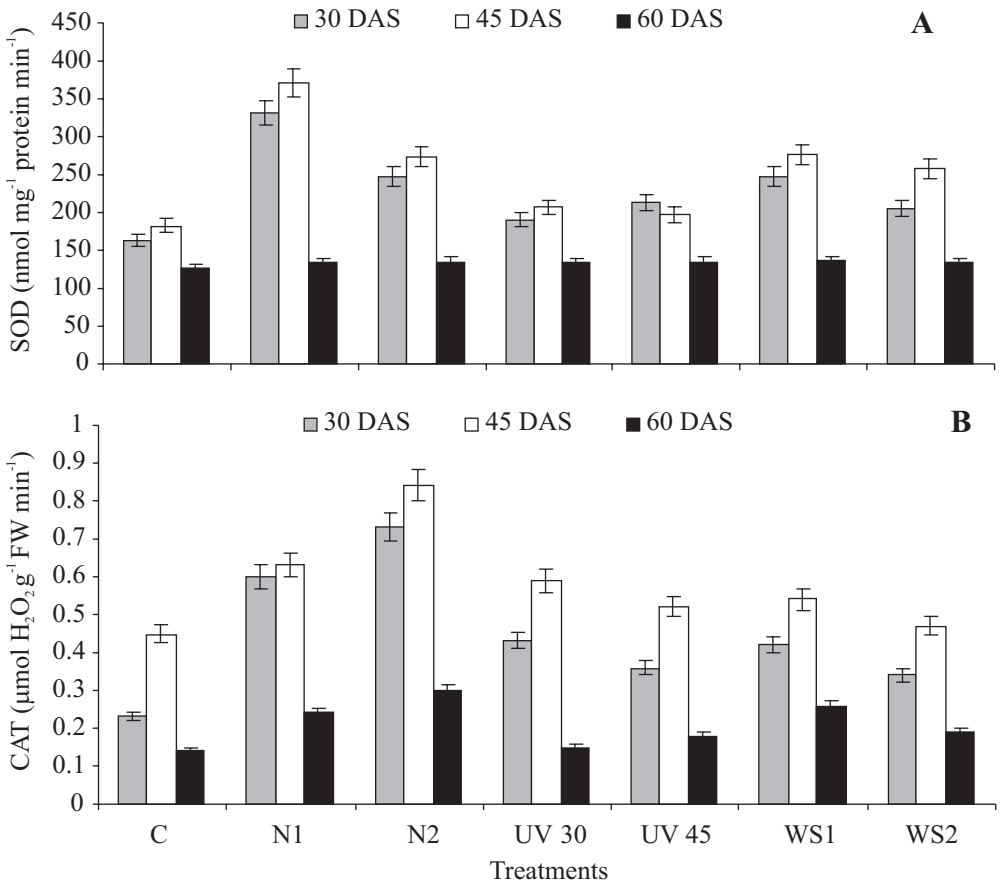


**Figure 1.** Effect of abiotic stresses on the activities of superoxide dismutase, SOD (**A**) and catalase, CAT (**B**) in leaves of *Momordica charantia* at pre-flowering, flowering, and post-flowering growth stages (respectively, 30, 45, and 60 days after sowing, DAS). C = control; N1 = NaCl 25 mmol $L^{-1}$, N2 = 75 mmol $L^{-1}$; UV 30 = UV-B 30 min, UV 45 = UV-B 45 min; WS1 = water stress for 1 d, WS2= water stress for 3 d. Vertical bars indicate ± SD values of three replicates.

Photosynthetic pigments were reduced significantly under all stresses at all stages of plant growth except N2 at the second stage for carotenoids (Figures 5 A, B). The CSI was reduced under salinity stress (75 mmol $L^{-1}$). Under UV-B exposures it was elevated over control and NaCl values and almost similar to the water stress value at the post-flowering stage. Under water stress the CSI was marginally lower than the control value at the first stage, significantly higher than the control, 75 mmol $L^{-1}$ NaCl and UV-B values at the second stage and significantly higher than the control and NaCl values at the third stage (Figure 6A).

The protein concentration increased at the flowering stage under NaCl stress but subsequently decreased at the last two stages. Protein concentration under UV-B radiations was significantly higher than the control and 75 mmol NaCl $L^{-1}$ values at the first two stages but declined at the post-flowering stage. Under water stress the protein concentration was significantly higher than controls and other stress values at all stages (Figure 6B).

## DISCUSSION

Superoxide dismutase is responsible for the dismutation of superoxide into $H_2O_2$ and is considered to be the first line of defense against ROS. Increased SOD activity under NaCl stress was reported in plants (Agarwal and Pandey, 2004; Madan Pal et al., 2004). Santos et al. (1999) have emphasized that UV-B radiation interferes with SOD in a similar manner as with other stresses and also affects the isoenzymes of SOD differently. There are several reports concerning the isoenzymes of SOD and APX (e.g., Bowler et al., 1992;

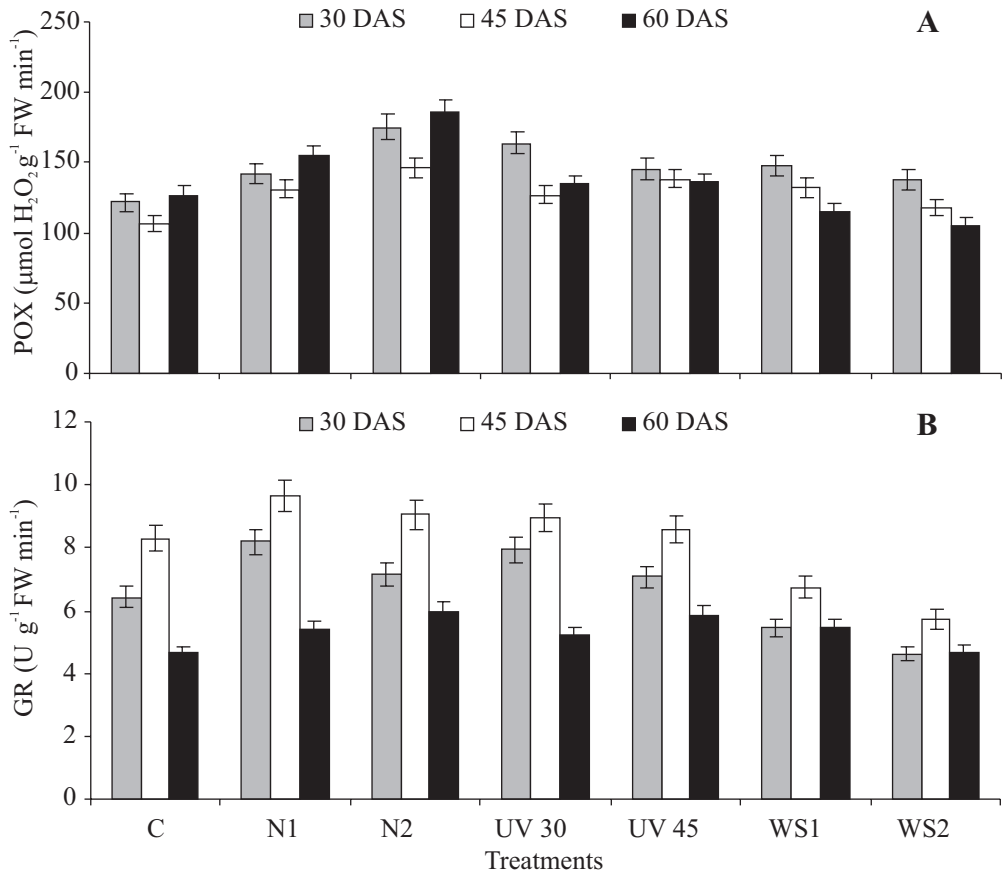


**Figure 2.** Effect of abiotic stresses on the activities of peroxidases, POX (**A**) and glutathione reductase, GR (**B**) in leaves of *Momordica charantia* at pre-flowering, flowering, and post-flowering growth stages (respectively, 30, 45, and 60 days after sowing, DAS). See additional details in legend to Figure 1.

Hernandez et al., 2000; Gomez et al., 2004). Possibly, results obtained with the isoenzymes of antioxidants reported here may help reveal the mechanism underlying the tolerance of *M. charantia* plants to abiotic stresses. The activity of SOD under NaCl and $WS_1$ were very similar at all three stages. The tolerance of *M. charantia* leaves to UV-B and water stresses may be at least partially associated with increased SOD activity and other antioxidative enzymes which is consistent with the results of Santos et al. (1999, 2004), Agarwal and Pandey (2003b), Jain et al. (2004), Da et al. (2005), Zhang et al. (2005), Agarwal (2007), and Kataria et al. (2007).

Catalase scavenges $H_2O_2$ by breaking it down directly to form water and oxygen and an increase in its activity is related to an increase in stress tolerance. The CAT activity was elevated significantly under all the stresses at the flowering stage and maximum activity was registered under NaCl stress. This is in conformity with the data of Agarwal and Pandey (2004), Mishra and Das (2004) and Agarwal and Pandey (2003b) who found maximum activity of CAT at the flowering stage for *Cassia angustifolia.* Increased CAT activity under water stress has been reported by Agarwal and Pandey (2003a) and Da et al. (2005). The activity of CAT under UV-B stress was increased in other plant species (Ervin et al., 2004; Santos et al., 2004; Agarwal, 2007). The higher CAT activity under these stresses may be associated with tolerance of the plant to NaCl, UV-B and water stress.

Peroxidases decompose $H_2O_2$ by the oxidation of phenolic compounds. The activities of POX and PPO were triggered at non-saline and saline concentrations of NaCl. Increase in POX and PPO activities under NaCl stress was reported by Agarwal and Pandey (2004). Analysis of isoforms by native-PAGE and activity staining under UV-B exposure intensified the activity of POX, GR and SOD (Yannarelli et al., 2005; Kataria et al.,

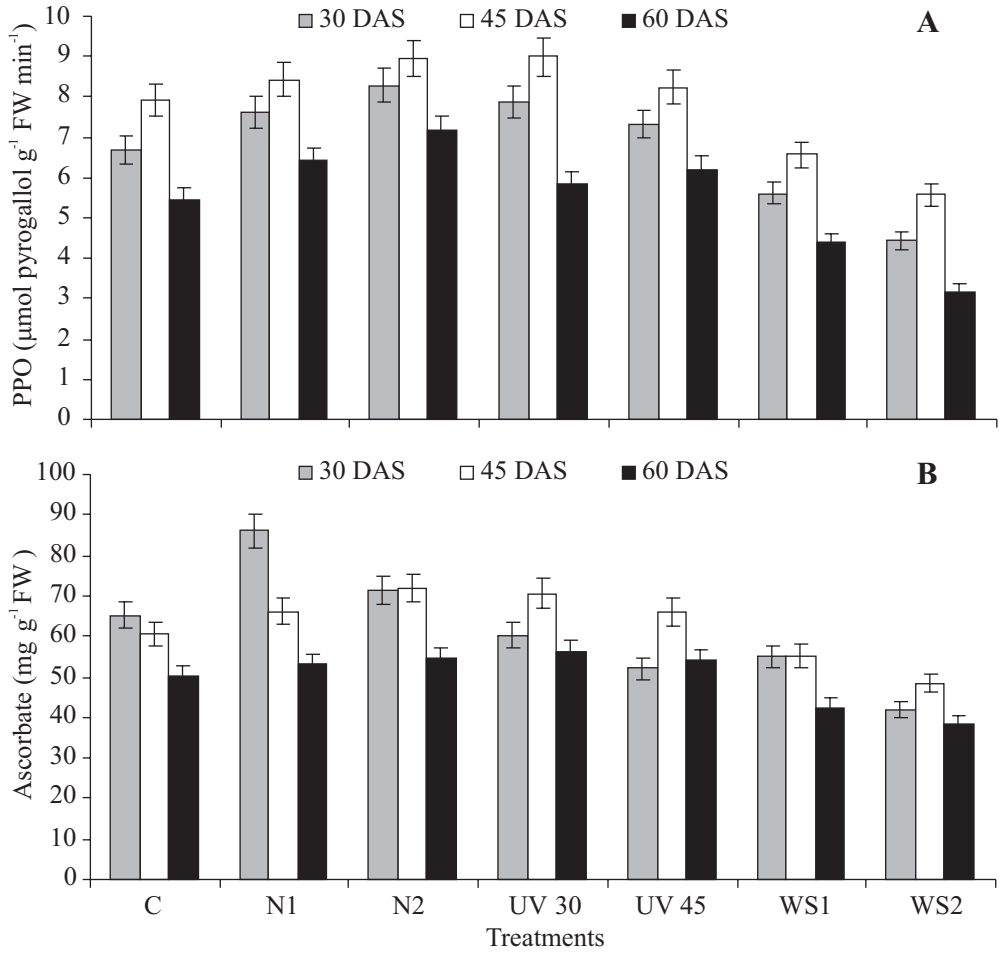


**Figure 3.** Effect of abiotic stresses on the activity of polyphenol oxidase, PPO (**A**) and concentration of ascorbate (**B**) in leaves of *Momordica charantia* at pre-flowering, flowering, and post-flowering growth stages (respectively, 30, 45, and 60 days after sowing, DAS). See additional details in legend to Figure 1.

2007). The different isoforms of POX that function as oxygen species scavengers could play a role in polyphenol metabolism. It seems possible that the oxyreductases POX and PPO may play an important role as a defense against salt stress. Elevated activity under UV-B radiation has been reported in *C. auriculata* by Agarwal (2007). Increased activities of POX and PPO under water stress were observed in *Cassia* seedlings (Agarwal and Pandey, 2003a).

Glutathione reductase plays an important role in the control of endogenous $H_2O_2$ through an oxyreduction cycle involving glutathione and ASA. Activity of GR was increased under NaCl and UV-B stresses and the maximum activity was evident at the flowering stage. Similar observations were made by Hernandez et al. (1999) and Parida et al. (2003, 2004). Increase in GR activity in plants resulted in accumulation of GSH levels and ultimately confers tolerance of the plants. This is well correlated in the present study. Under water stress the activity was reduced at every stage except the post-flowering stage of $WS_1$. In contrast, Bartoli et al. (1999) reported increased GR activity after exposure to drought.

Ascorbate can scavenge superoxide and $H_2O_2$ radicals nonenzymatically. Increase in ASA concentration due to salinity was reported earlier (Panda and Upadhyay, 2004; Parida et al., 2004) but in *Cassia* seedlings a decrease was found (Agarwal and Pandey, 2004). At flowering and post-flowering stages UV-B produced almost the same results as those of NaCl. Agarwal (2007) reported a similar finding of increased ASA concentration in *C. auriculata* seedlings. Ascorbate may thus play a central role in non-enzymatic scavenging of superoxide and $H_2O_2$, and thus

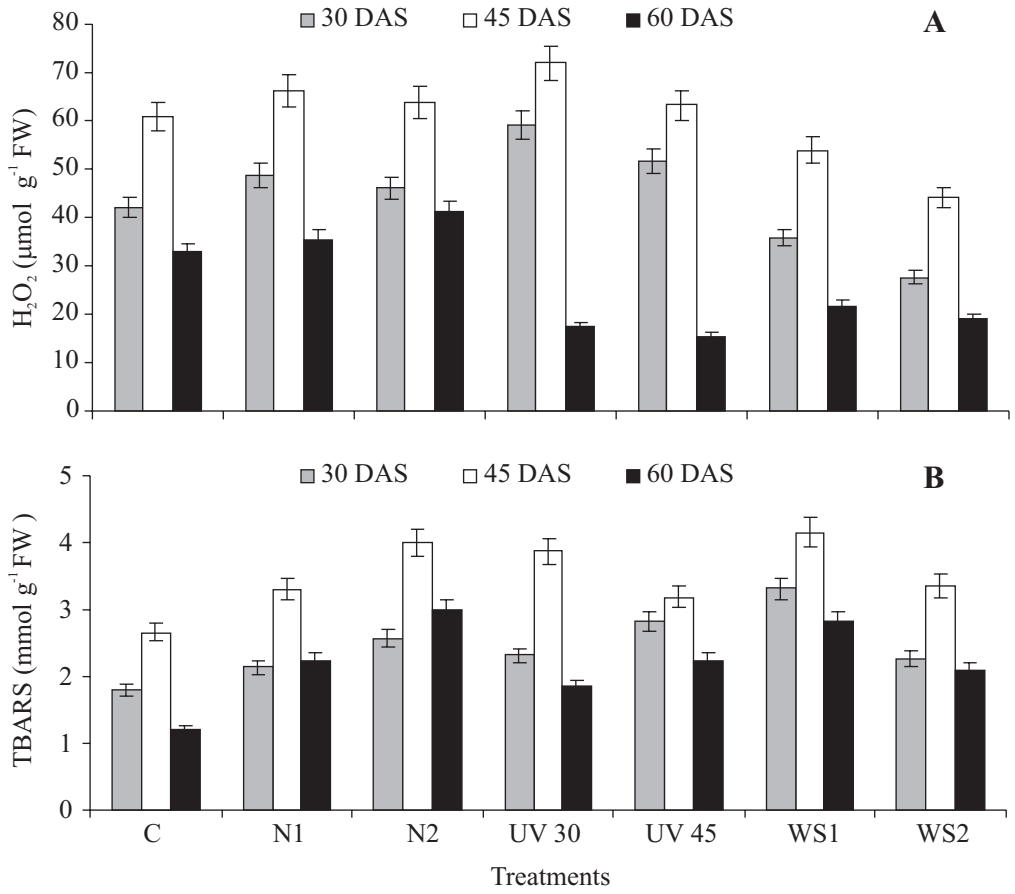


**Figure 4.** Effect of abiotic stresses on the concentrations of $H_2O_2$ (**A**) and thiobarbituric acid-reactive substances, TBARS (**B**) in leaves of *Momordica charantia* at pre-flowering, flowering, and post-flowering growth stages (respectively, 30, 45, and 60 days after sowing, DAS). See additional details in legend to Figure 1.

**Table 1.** Accumulation of $Na^+$ and $Cl^-$ in different parts of *Momordica* plants subjected to salt treatments at pre-flowering, flowering, and post-flowering growth stages (respectively, 30, 45, and 60 days after sowing, DAS). Means followed by different letter in columns differ significantly (Tukey's test, $P< 0.05$); $n = 3$.

| Treatment | DAS | $Na^+$ Concentration (mg $g^{-1}$ DM) | | | $Cl^-$ Concentration (mg $g^{-1}$ DM) | | |
|---|---|---|---|---|---|---|---|
| | | Leaves | Shoot | Root | Leaves | Shoot | Root |
| Control | 30 d | 15.8 b | 6.6 b | 8.9 b | 9.8 b | 2.6 b | 7.6 b |
| | 45 d | 18.8 b | 10.1 b | 11.9 b | 12.6 b | 4.9 b | 10.6 b |
| | 60 d | 24.3 b | 13.9 b | 16.2 b | 18.1 b | 7.9 b | 14.4 b |
| NaCl 25 mmol $L^{-1}$ | 30 d | 12.3 a | 4.3 a | 7.4 a | 8.5 a | 1.3 a | 5.2 a |
| (Non-saline) | 45 d | 13.8 a | 6.4 a | 10.2 a | 11.2 a | 2.6 a | 7.9 a |
| | 60 d | 18.9 a | 8.6 a | 12.4 a | 14.0 a | 4.1 a | 10.5 a |
| NaCl 75 mmol $L^{-1}$ | 30 d | 20.4 a | 8.2 a | 12.2 a | 15.3 a | 6.9 a | 11.2 a |
| (Saline) | 45 d | 26.4 a | 11.6 a | 17.1 a | 18.6 a | 8.6 a | 14.9 a |
| | 60 d | 29.8 a | 15.3 a | 21.2 a | 27.9 a | 12.7 a | 22.1 a |

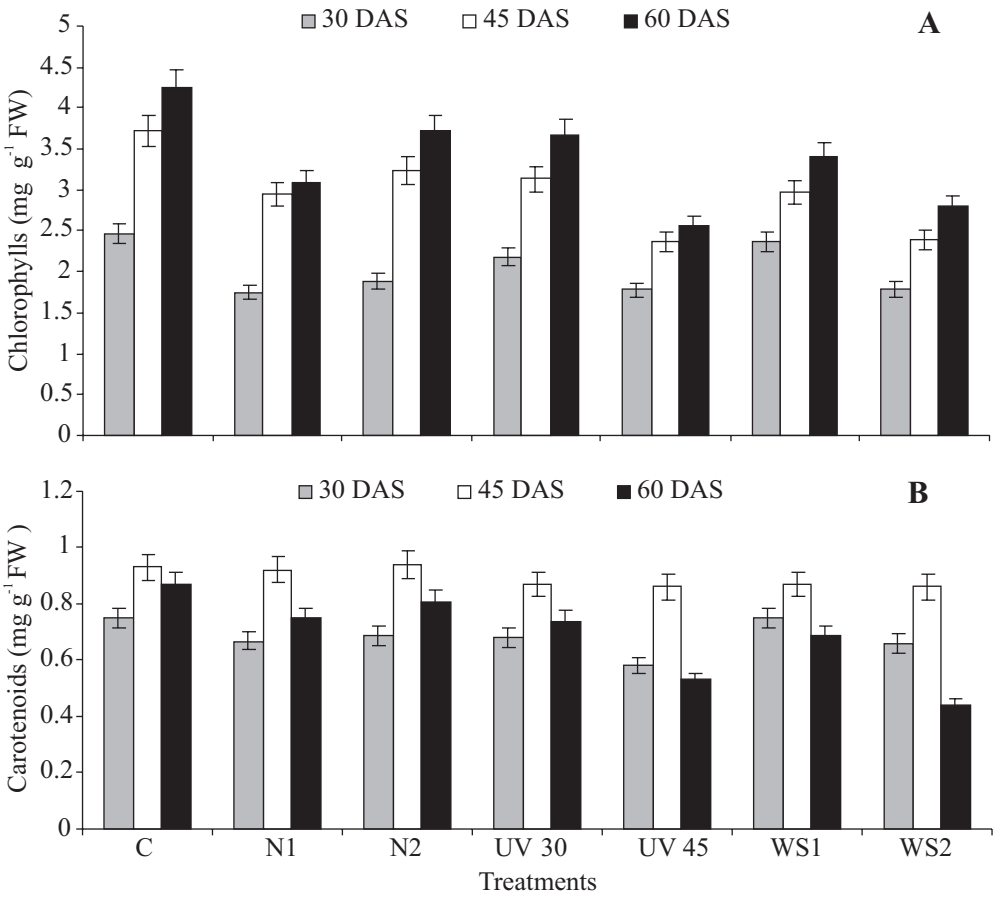


**Figure 5.** Effect of abiotic stresses on chlorophyll (*a* + *b* ) (**A**) and carotenoid (**B**) concentrations in leaves of *Momordica charantia* at pre-flowering, flowering, and post-flowering growth stages (respectively, 30, 45, and 60 days after sowing, DAS). See additional details in legend to Figure 1.

protect *M. charantia* from the ROS.

An increasing trend of TBARS concentration, a measure of lipid peroxidation, together with $H_2O_2$ was observed in *M. charantia* with the 25 mmol $L^{-1}$ and 75 mmol $L^{-1}$ NaCl treatments (Figure 4). Similar results have been reported by Singh et al. (2005) for mustard, Panda and Upadhyay (2004) for roots of *Lemna minor*; and Agarwal and Pandey (2004) for *C. angustifolia* and mulberry plants (Sharma et al., 2003). Under UV-B exposures, TBARS and $H_2O_2$ concentrations were higher than control values at all growth stages. These results are in good agreement with those of Jain et al. (2004) for cucumber cotyledons, Agarwal and Pandey (2003b) for *C. angustifolia* and Agarwal (2007) for *C. auriculata* seedlings. Under water stress TBARS concentration increased but $H_2O_2$ concentration declined at all stages. Water stress increased TBARS concentration in tea (Jeyramraja et al., 2005), summer maize (Da et al., 2005). The high TBARS and $H_2O_2$ concentrations establish oxidative damage but this is presumably suppressed by the strong antioxidant system prevailing in *M. charantia* plants.

Under salt stress (75 mmol $L^{-1}$) the highest $Na^+$ and $Cl^-$ concentrations were detected in the leaves at all growth stages while the lowest was detected in shoots. The $Na^+$ and $Cl^-$ concentration at 25 mmol $L^{-1}$ (non-saline) were significantly lower than control values. Similar results were reported for *Cassia* seedlings (Agarwal and Pandey, 2004) and sugarbeet cultivars (Ghoulam et al., 2002). This accumulation of $Na^+$ and $Cl^-$ ions might be involved in osmotic adjustment. Overall, *M. charantia* is a moderately salt-tolerant crop whose response to salinity is associated with maintenance of high $Na^+$ and $Cl^-$ ratios in both leaves and roots.

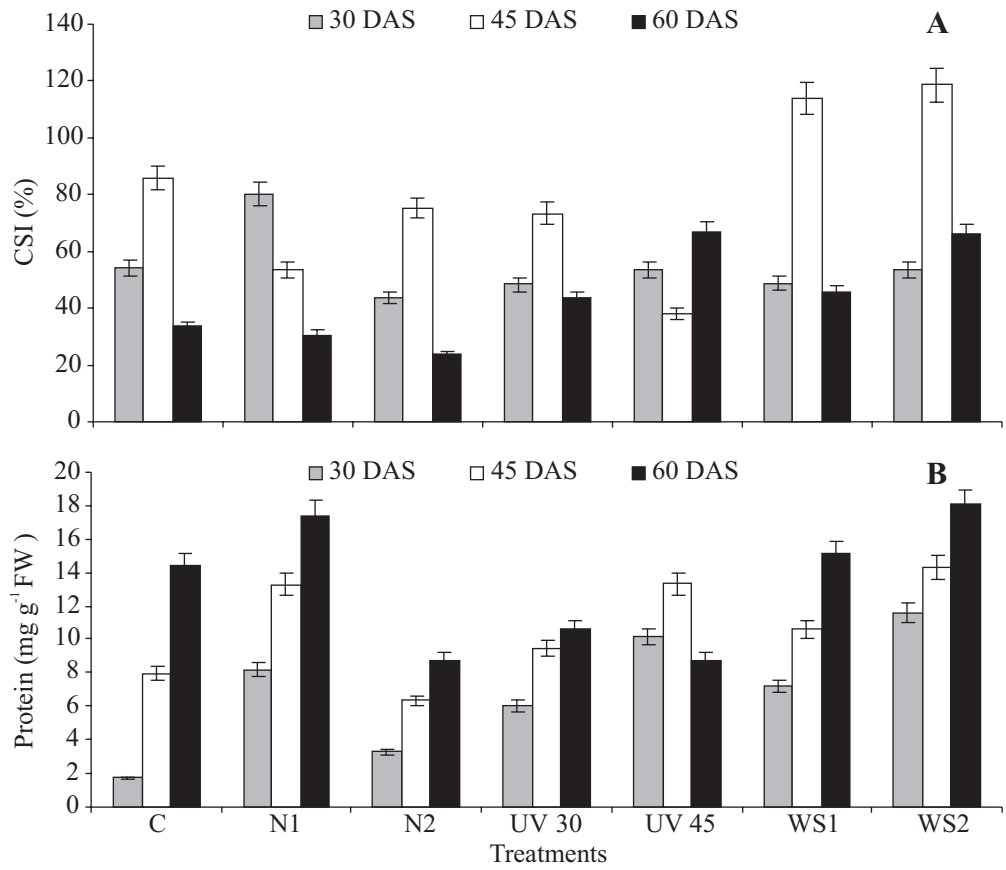


**Figure 6.** Effect of abiotic stresses on the chlorophyll stability index, CSI (**A**) and protein content (**B**) in leaves of *Momordica charantia* at pre-flowering, flowering, and post-flowering growth stages (respectively, 30, 45, and 60 days after sowing, DAS). See additional details in legend to Figure 1.

The photosynthetic pigments were reduced under all three stresses and at all stages of plant growth with minor variations. Decrease in chlorophyll concentration due to salinity and UV-B radiation has already been reported (Sairam et al., 2002; Garg and Singla, 2004; Agarwal, 2007). The reduced chlorophyll concentration may be due to increased chlorophyllase activity (Sudhakar et al., 1997). Reduction in carotenoid concentration under all three stresses in *M. charantia* was recorded. Carotenoids are responsible for quenching of singlet oxygen (Knox and Dodge, 1985) and thus help in overcoming oxidative stress. The CSI is a reliable test of stress tolerance. The CSI was decreased under NaCl stress and has been observed in water-stressed plants while under UV-B exposures the results varied with the growth stage. Under water stress the CSI was triggered significantly which suggests that *M. charantia* is tolerant to water stress.

A decrease in protein concentration would be a typical symptom of oxidative stress and has been observed in plants under drought, but this is not the case here. Soluble proteins increased significantly with age under water stress as compared with the control level. This result is similar to that of Agarwal and Pandey (2003b) for *Cassia angustifolia.*

A close examination of our results suggest that the environmental stresses such as salinity and UV-B radiation induced the antioxidant defenses protecting the plant against deleterious effects of ROS. The damage due to these radicals as indicated by chlorophyll breakdown and significant increase in TBARS and $H_2O_2$ was limited by a combination of characters such as high concentration of ASA and activity of antioxidants SOD, CAT, POX, PPO, GR which have a role in conferring tolerance to *M. charantia* against environmental stresses. Further, the capability for adaptation to drought stress was related to the maintenance of, or increases in,

the ability to detoxify superoxide radicals by SOD which play a key role in protecting plants from oxidative stress by increasing its activity. It is apparent that not only SOD but $H_2O_2$ scavenging systems, as represented by CAT and POX, are equally important in preventing oxidative stress induced by water stress in bitter gourd.

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