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Impact of Seed Pretreatment by Static Magnetic Field on Antioxidant Defense of the Maize Seedlings against Ambient Ultraviolet (280 - 400 nm) Radiation

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ABSTRACT: *The impact of static magnetic field (SMF) of 200 mT for 1 h on antioxidant defense against ambient UV (280 - 400 nm) stress was demonstrated in maize (*Zea mays *var. JM 216.) plants. The plants were grown in specially designed UV exclusion chambers, wrapped with filters that excluded UV-B (<315 nm), UV-A/B (<400 nm) or transmitted ambient UV or lacked filters. Exclusion of UV-B and UV-A/B enhanced the leaf area of maize plants. The amount of hydrogen peroxide (H_2O_2) and activity of antioxidant enzymes like superoxide dismutase (SOD), glutathione reductase (GR) and guaiacol peroxidase (GPx) assayed in the leaves of maize seedlings were higher in the plants grown under ambient UV as compared to the exclusion of solar UV. It indicates that ambient UV components exert a significant stress on maize plants. Whereas under ambient UV stress, the plants that emerged from magnetically treated seeds showed higher leaf area along with lower amount of H_2O_2 and lesser activities of antioxidant enzymes as compared to untreated seeds. Thus reduction in the H_2O_2 content and antioxidant enzyme activities after SMF pretreatment and UV exclusion indicated that solar UV components exerted a limitation on the potential growth of maize plants. The levels of UV absorbing substances were also decreased by both exclusion of UV and SMF treatment. Reduction in the production of UAS indicated a changed pattern of metabolism leading to improved primary metabolism. The results indicate that exclusion of solar UV components and SMF pretreatment eliminates the need for defense against the ambient UV stress. Consequently SMF-pretreatment ameliorated UV-B stress; so that the plants do not have to divert their metabolic energy in detoxification of ROS that are generally produced under stress conditions.*

Keywords: *Antioxidant enzymes; Hydrogen peroxide; Static magnetic field; UV-B radiation*

INTRODUCTION

The stratospheric ozone layer is vital to life on Earth because it is the principal agent absorbing the ultraviolet radiation in the Earth's atmosphere. Depletion of stratospheric ozone levels leads to an increase of solar UV-B radiation reaching the Earth's surface [1]. Many studies have shown a deleterious effect of enhanced UV-B such as reduced photosynthesis, biomass reduction, decreased protein synthesis, impaired chloroplast function, damage to DNA, etc. which are extensively reviewed by [2]. Supplementation experiments have revealed that in addition to directly damaging DNA, UV-B causes oxidative stress through the formation of reactive oxygen species (ROS), which in turn cause enhanced lipid and protein oxidation [3-5]. Shine and Guruprasad [6] found that ambient UV-B also leads to the generation of reactive oxygen species (ROS) like superoxide ($O_2^{\bullet-}$) and hydroxyl ($^{\bullet}OH$) and reduced the growth and photosynthesis in maize plants.

To counteract the toxicity of ROS, defense systems that scavenge cellular ROS have been developed in plants to cope with oxidative stress via the non-enzymatic and enzymatic systems [7,8]. The non-enzymatic defense system consists of low molecular weight antioxidants such as ascorbate, glutathione and α-tocopherol and enzymatic defense system consists of several enzymes such as superoxide dismutase (SOD), glutathione reductase (GR), peroxidase (GPx), and ascorbate peroxidase (APX) [9]. UV-B radiation has been shown to increase ROS levels [10-11] in plants. Synthesis of antioxidant enzymes like GPx, APX and SOD have been observed in UV-B treated *Arabidopsis thaliana* seedlings [12]. Very few studies have been conducted on the impacts of ambient UV-B radiation on ROS metabolism under natural environmental conditions [13-15].

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Electron paramagnetic resonance spectroscopy study showed that superoxide radical was reduced after static magnetic field treatment to maize seeds [16]. With decrease in free radical content, antioxidant enzymes like SOD and GPx were also reduced along with the increased growth and PSII efficiency in the maize plants that emerged from magnetically treated seeds [16]. Therefore, the purpose of the present study was to evaluate the effect of static magnetic field on the antioxidant defense of maize seedlings against the ambient UV (280-400 nm) stress. In this study, we used maize (*Zea mays)* as an experimental plant which is sensitive to ambient UV [6] to test the hypothesis that whether magnetopriming of the maize seeds alleviates the oxidative stress caused by the ambient UV stress.

MATERIAL AND METHODS

Plant material and experimental condition

The breeder seeds of *Zea mays* var. JM 216 were obtained from JNKVV, Zonal Agriculture Research Station, Chhindwara, M.P., India. The experiments were conducted on the terrace of the School of Life Sciences, Devi Ahilya University, Indore, India (latitude 22.48?N) during October 2014 to January 2015 under the ambient conditions. The seeds of uniform size and shape were sown in plastic bags (34 cm H x 34 cm B) filled with a mixture of thoroughly sifted soil, sand and farm-yard manure in the proportion of 2:2:1 by volume. Thereafter the bags were immediately placed in metal mesh cages 1.2 m Length x 0.91 m Width x 0.91 m Height covered with Polyester filters (Garware Polyesters Ltd., Mumbai) that cut off UV-B (<315 nm) and UV-A/B (<400 nm) radiations. Filter control (FC) plants were grown under a polyethylene filter which transmits all the ambient solar radiations including the UV-A/B components, or in open field without any filter exposed to ambient solar radiation (open control OC). The transmission characters of these filters are given in Figure 1. Plants were watered daily until the end of experiments.

Absolute solar irradiance with or without UV-B or UV-A/B was measured using a radiometer (Solar light PMA2100, Glenside, PA., U.S.A). The ambient solar irradiance during experimental period at midday was 1450 μmol m^{-2} s^{-1}, under UV-A/B cut-off filters the loss in light intensity at midday was 11.8% (1280 μmol m^{-2} s^{-1}), under UV-B cut-off filters was 12.5% (1270 μmol m^{-2} s^{-1}) and under polythene filter (transmissible to solar UV- filter control) was 4.2% (1390 μmol m^{-2} s^{-1}).

Magnetic field generation and magnetic treatment

An electromagnetic field generator ''Testron EM- 20'' with variable horizontal magnetic field strength (50–500 mT) with a gap of 5 cm between pole pieces was fabricated (Testron Instruments, Delhi, India). Maize seeds were exposed to a static magnetic field of 200 mT (1 h) in a cylindrical shaped sample holder of 42 cm^3 capacity, made from a non-magnetic thin transparent plastic sheet. Hundred visibly sound, mature healthy seeds held in the plastic container were placed between the poles of the electromagnet under a uniform magnetic field for treatment by the procedure described by Vashisth and Nagarajan [17]. The SMF treatments in the experiments were run simultaneously along with untreated controls under similar conditions.

Growth analysis

Growth parameter such as area of third leaf in maize plants was measured using portable laser leaf area meter CID-202 scanning planimeter (CID Inc., USA) at 35 days after emergence (DAE) of the seedlings. Five plants from each replica were randomly selected for recording these parameters.

Biochemical Analysis

All the biochemical analysis in third leaf of the maize seedlings was done on 35 DAE.

Hydrogen peroxide (H_2O_2) was estimated by formation of titanium-hydroperoxide complex [18]. Leaves (0.5 g) of maize were ground in 10 ml cold acetone in a chilled mortar and pestle kept in ice bucket. The homogenate was filtered through Whatman No. 1 filter paper followed by addition of 4 ml of titanium reagent and 5 ml of ammonium hydroxide solution to precipitate the titanium-hydroperoxide complex. The reaction mixture was centrifuged at 15,000 rpm for 15 min. The precipitate was dissolved in 10 ml of 2 M concentrated sulphuric acid and recentrifuged. The supernatant was read at 415 nm against blank and H_2O_2 expressed as μmol H_2O_2 per gram fresh weight.

Accumulation of UAS in leaves of maize was determined spectrophotometrically (Shimadzu-UV 1601) from acidified methanol extract by the method of Mazza et al. [13]. One 0.50 cm diameter leaf disc was placed in 5 mL of 99:1 (methanol: HCl) and allowed to extract for 48 h at (–4°C). Absorbance of the extract was read at 305 nm and the amount of UAS was expressed on the basis of per unit leaf area

(A_{305} cm^{-2}).

Extraction and estimation of antioxidant enzymes

All operations were performed at 4? C. The enzyme extract was prepared by homogenizing 0.5 g leaves of maize with 10% (w/v) polyvinyl polypyrrolidone and 10 ml of 0.1 M phosphate buffer (pH 7.0) for SOD, GR and GPX. The homogenate was filtered through four layers of cheesecloth, centrifuged at 15,000 rpm for 30 min and the supernatant obtained was used to determine the activity of those enzymes described.

Superoxide Dismutase [SOD; EC 1.15.1.1] activity was assayed according to the method of Beauchamp and Fridovich [19] by measuring ability of the enzyme extract to inhibit the photochemical reduction of NBT. The reaction mixture contained 0.24 mM riboflavin, 2.1 mM methionine, 1% triton X 100, 1.72 mM NBT in 50 mM sodium phosphate buffer (pH 7.8) and 200 ìl of enzyme extract in a final volume of 3 ml. Glass test tubes containing the reaction mixture were immersed in a thermostat bath at 25C and illuminated with fluorescent lamp (Phillips-80 W) for 15 min. Non illuminated identical tubes served as blanks. After illumination for 15 min, absorbance was measured at 560 nm. One unit of SOD was defined as the enzyme activity (per mg protein), which inhibited the photo reduction of NBT to blue formazan, by 50%.

Guaiacol Peroxidase [EC 1.11.1.7] was assayed by the method of Maehly [20]. The reaction mixture contained 0.5 ml enzyme extract, 1 ml 20 mM guaiacol and 3 ml 0.02 M phosphate buffer. The reaction was started by the addition of 0.03 ml of H_2O_2 (1 volume). The initial and final absorbance was noted at 470 nm for 2 min. Activity was calculated as change in OD/ min / mg protein.

Glutathione reductase [GR; EC 1.6.4.2] activity was determined at 25?C by following the method of Rao et al. [12]. The 3 ml assay mixture contained 2.5 ml potassium phosphate buffer (50 mM; pH 7.8), 0.1 ml NADPH (1 mM) and 0.1 ml oxidized glutathione (15 mM). The reaction was started by adding 0.3 ml enzyme extract. Decrease in absorbance was recorded at 340 nm for 10 min. Enzyme activity was calculated using extinction coefficient (6.2 $mM^{-1}cm^{-1}$). The GR activity was expressed as ?M NADPH oxidized/ min / mg protein. Protein was estimated by the method of Lowry [21] using BSA as the standard.

Statistical analysis

All the data are presented in triplicates; five plants from each replica were taken for the recording of all parameters studied. The data are expressed as means ± S.E.M.

RESULTS AND DISCUSSION

The results of present study showed that exclusion of solar UV radiation and SMF pre-treatment of 200 mT for 1h significantly enhanced the leaf area as compared to the plants emerged after untreated seeds of maize grown under ambient UV stress (OC and FC). It indicates that ambient UV inhibits the leaf area and growth of the maize plants (Fig. 2A). Response of maize plants to solar UV exclusion and SMF pre-treatment in the present study is similar to that of previous studies [6,17,22].

Flavonoids and other phenolic compounds which are the products of secondary metabolism act as UAS and filter the ambient solar UV [23]. In the present study, UAS was higher in plants grown under ambient UV stress conditions (OC and FC plants) whereas UV excluded plants had lesser amount of UAS. The maximum of 41% and 48% decrease was found in UAS after UV-B and UV-A/B exclusion respectively as compared to OC and FC (Fig. 2B). The plants that emerged after SMF of 200 mT for 1h also showed 20% lesser amount of UAS as compared to untreated seeds grown under ambient UV stress conditions (Fig. 2B). This result indicated that when the plants are grown after excluding UV and magnetopriming there is a signal transduction that alters the metabolism and reduces the synthesis of UAS.

There is very limited information about the effects of UV-B on H_2O_2 content in the higher plants under field conditions. In the present study we have found an higher amount of hydrogen peroxide in leaves of maize plants emerged from untreated seeds grown under ambient UV stress conditions (OC and FC) (Fig. 2C). Whereas, there was a decrease of 35% in the level of H_2O_2 in the plants grown after magnetically treated seeds and 27% decrease was found after UV exclusion as compared to the untreated seeds grown under ambient condition (Fig. 2C).

Spectrophotometeric estimation of antioxidant enzymes like SOD, GR and GPx activities in the leaf extracts of maize plants grown under ambient condition like OC and FC indicates a significantly higher activities (Fig. 3A,B,C) than the SMF pre-treatment and UV exclusion (Fig. 3A,B,C). The reduction in the activity of SOD was up to 66% after the exclusion of solar UV radiation (Fig. 3A). Activity of GPx was reduced by 75% and GR activity was reduced by 53% by exclusion of ambient UV as compared to plants emerged after untreated seeds of

maize grown under ambient control conditions (Fig. 3B). The plants emerged after SMF pre-treated maize seeds showed 53% reduction in the activity of SOD, 43% in GPx and 53% reduction were found in GR activity as compared to untreated seeds grown under ambient UV stress (Fig. 3A,B,C).

The results of the present study revealed that in addition to the increase in H_2O_2 content, the activities of antioxidant enzymes like SOD, GR and GPx were also higher under ambient UV stress. H_2O_2 content and activities of antioxidant enzymes were decreased by SMF pretreatment and also by the exclusion of solar UV in maize leaves. These results indicate that the presence of ambient UV components (i) increases the H_2O_2 content (ii) activates the antioxidant enzymes and (iii) enhances UAS synthesis. The exclusion of solar UV-B and SMF pretreatment to the seeds eliminates the need for the defense against harmful ambient UV stress and leads to enhancement of primary metabolism

Ambient UV (280-400 nm) caused oxidative stress by the production of H_2O_2 and plants responded by activating the antioxidant defenses mainly by increasing the amount of UAS and the activities of SOD, GR and GPx. A parallelism between the two effects SMF pretreatment under ambient UV stress and growing plants under solar UV exclusion was found in the present study. Both treatments enhanced the leaf growth and reduced the amount of UAS, H_2O_2 and activities of SOD, GR and GPx. Thus reduction in secondary metabolism can channelize the carbon to primary metabolism, since secondary and primary metabolic pathways compete for the common pool of carbon. Similar observations were made in previous studies for solar UV exclusion and SMF pre-treatment to the seeds in cotton, maize and soybean [24,25]. The current ambient levels of UV-A and UV-B are high enough to induce accumulation of reactive oxygen species, which trigger antioxidant defense systems in maize. In turn, the growth and development of maize plants were retarded. This increased growth by exclusion of solar UV and SMF pre-treatment appears to be due to the lower level of free radicals in plants that emerged after magnetically treated seeds and solar UVexclusion. Thus magnetopriming of the

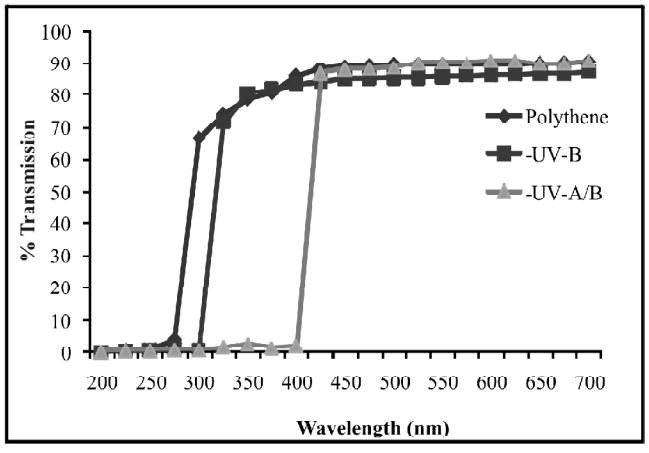

Figure 1: Transmission spectra of UV cut off filters and polyethene filter used for raising maize var. JM-216 plants

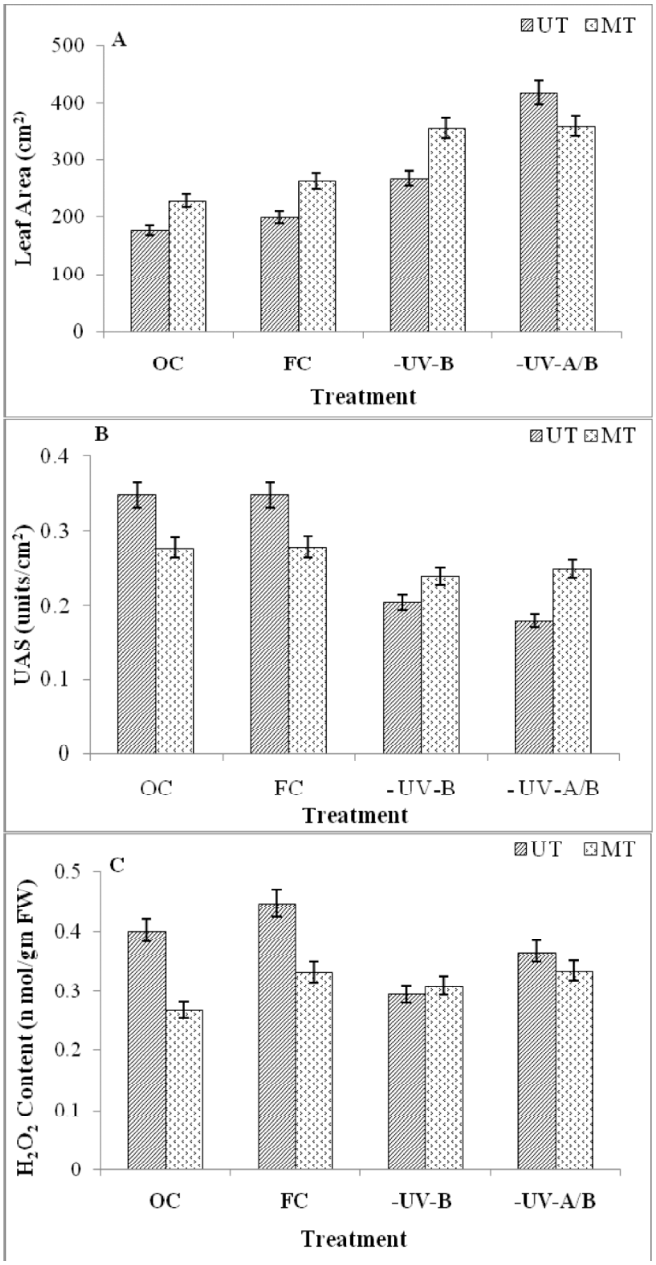

Figure 2: Effect of ambient UV-A/B and SMF pre-treatment (200 mT for 1 h) on (a) leaf area, (b) UV absorbing substances and (c) hydrogen peroxide in the leaves of maize var. JM-216 at 35 days after emergence of the seedlings (DAE). Vertical bar indicates ±SE of mean

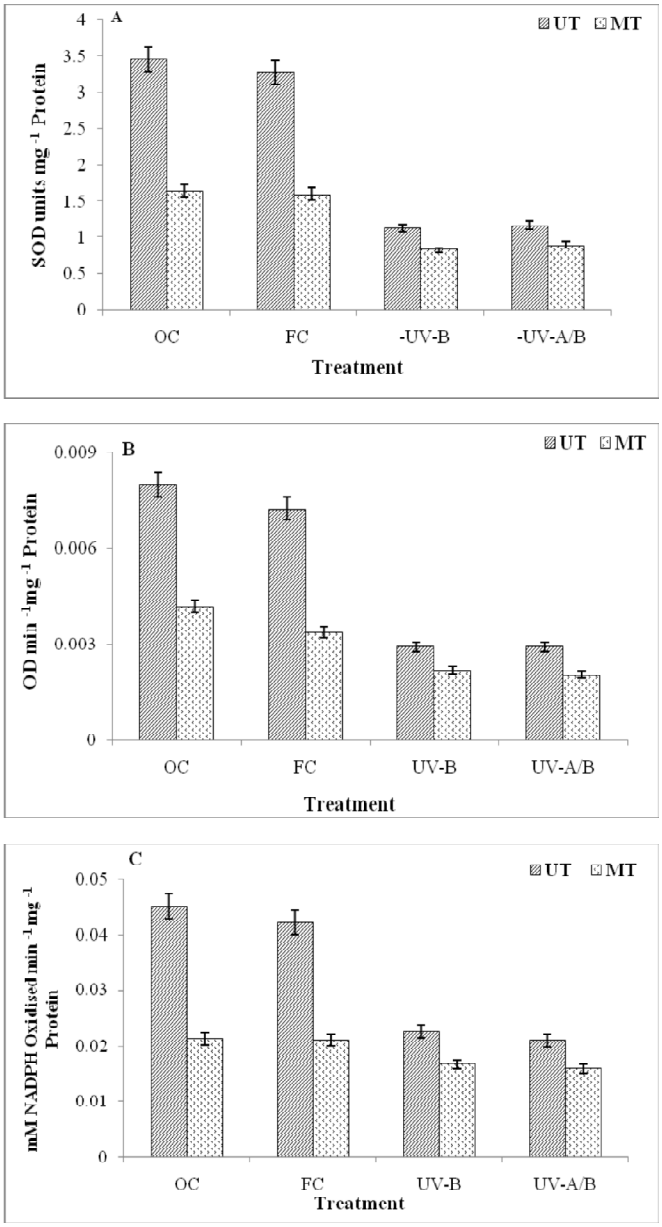

Figure 3: Effect of ambient UV-A/B and SMF pre-treatment (200 mT for 1 h) on (a) SOD, (b) GPX and (c) GR activity in the leaves of maize var. JM-216 at 35 days after emergence of the seedlings (DAE). Vertical bar indicates ±SE of mean

maize seeds alleviates the oxidative stress caused by the ambient UV stress.

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