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ORIGINAL ARTICLE

Static magnetic field treatment enhanced photosynthetic performance in soybean under supplemental ultraviolet-B radiation

Sunita Kataria[1] · **Meeta Jain**[1] · **Anshu Rastogi**[2,3] · **Marian Brestic**[4,5]

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Abstract

The study was performed to analyze the impact of seed pretreatment by static magnetic field (SMF) of 200 mT for 1 h on photosynthetic performance of soybean (*Glycine max*) seedlings under ambient ($_{a}$UV-B) and supplemental ultraviolet-B ($_{a+s}$UV-B) stress. Ambient and supplemental UV-B were found to decrease the plant growth, chlorophyll concentration, PSII efficiency, selected JIP-test parameters such as F_v/F_m, φEo, $\Delta V(I–P)$, PI_{ABS}, PI_{total}, and rate of photosynthesis in the leaves of soybean seedlings emerged from untreated (UT) seeds. $_{a}$UV-B and $_{a+s}$UV-B were observed to increase the synthesis of UV-B-absorbing substances (UAS), reactive oxygen species (ROS) like superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), antioxidants like ascorbic acid and α-tocopherol and decrease the nitrate reductase (NR) activity; subsequently, it results in a decreased rate of photosynthesis, biomass accumulation, and yield. However, our results provided evidence that SMF pretreatment increased the tolerance of soybean seedlings to UV-B radiation by increased NO content and NR activity; higher efficiency of PSII, higher values of φEo, $\Delta V(I–P)$, PI_{ABS}, and PI_{total}, decreased intercellular CO_2 concentration, lower amount of UAS, ROS, and antioxidants that consequently improve the yield of soybean plants under $_{a}$UV-B as well as $_{a+s}$UV-B stress. Thus, our results suggested that SMF pretreatment mitigates the adverse effects of UV-B stress by the enhancement in photosynthetic performance along with higher NO content which may be able to protect the plants from the deleterious effects of oxidative stress caused by UV-B irradiation.

Keywords Growth · Photosynthesis · PSII efficiency · Chl fluorescence · Nitric oxide · UV-B

Abbreviations

ASA	Ascorbic acid
Chl	Chlorophyll
F_v/F_m	The maximum quantum yield (efficiency) of PS II photochemistry
FW	Fresh weight
NO	Nitric oxide
NR	Nitrate reductase
φEo	Quantum yield of electron transport
PI_{ABS}	Performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors
PI_{total}	Performance index (potential) for energy conservation from the photons absorbed by PSII to the reduction of photosystem I electron end-acceptors
ROS	Reactive oxygen species
$_{a}$UV-B	Ambient UV-B
$_{a+s}$UV-B	Supplemental or enhanced ultraviolet-B
UAS	UV-B absorbing substances
ΔV ($I–P$)	Relative amplitude of the $I–P$ phase of Chl*a* fluorescence

✉ Sunita Kataria
sunita_kataria@yahoo.com

1 School of Biochemistry, Devi Ahilya University, Khandwa Road, Indore, M.P. 452001, India

2 Laboratory of Bioclimatology, Department of Ecology and Environmental Protection, Poznan University of Life Sciences, Piątkowska 94, 60-649 Poznan, Poland

3 Faculty of Geo-Information Science and Earth Observation (ITC), University of Twente, 7500 AE Enschede, The Netherlands

4 Department of Plant Physiology, Slovak University of Agriculture, A. Hlinku 2, 94976 Nitra, Slovak Republic

5 Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, 16500 Prague, Czech Republic

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Introduction

Plants growing in natural environments are exposed to different environmental signals that regulate responses at the plant level. Of those environmental signals, sunlight is of utmost importance as a source of energy for plants. In sunlight, wavelengths in the UV region are divided into UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm) radiations (Björn 2015). However, wavelengths below 290 nm are absorbed by the ozone layer and atmospheric oxygen, therefore are not detectable at the Earth's surface (Rowland 2006). As a consequence of human activities worldwide, the chemical composition of the atmosphere is changing. A reduction in the stratospheric ozone layer is dangerous for most of the Earth's population as it leads to a higher level of UV-B radiation on Earth's surface (Bais et al. 2019; Bornman et al. 2019). Due to the high sustainability of chloro-fluorocarbons (an industrial gas), the ozone layer is under the threat, and even if all the nations execute the Montreal Protocol, it is not possible to reduce the UV-B level reaching on the Earth surface to its pre-industrialization era by 2050 (Mohammed and Tarpley 2010). Though in the solar spectrum UV-B radiations consist of only a small part, it's high energy can degrade vital molecules like lipids, proteins, nucleic acids, and phytohormones (Jansen et al. 1998; Singh et al. 2012; Kataria et al. 2014a, b; Vanhaelewyn et al. 2016). The enhanced UV-B radiation drastically hampered the physiological, morphological, and biochemical development of numerous plant species (Kakani et al. 2003a, b; Caldwell et al. 2007; Kataria et al. 2014a, b) and eventually decrease the crop yield (Liu et al. 2013; Kataria et al. 2014a, b). The morphological alterations by UV-B, such as decrease in plant height, leaf area and leaf length, thicker leaves, reduced internode length, curling of cotyledons/leaves, bronzing/glazing of leaves, chlorosis and necrotic spots in leaves, delayed seedling emergence and flowering have been reported in numerous crop plants (Caldwell et al. 1995, 2007; Robson et al. 2015; Suchar and Robberecht 2015).

One of the most sensitive physiological processes in plants affected by UV-B exposure is photosynthesis which is closely related to biomass accumulation and crop yield. A reduction in plant growth parameters due to UV-B exposure was observed in several plant species (Kakani et al. 2003a, b; Kataria et al. 2013) which eventually reduced the crop productivity (Searles et al. 2001; Zuk-Golaszewska et al. 2003). UV-B radiations caused a reduction in photosynthetic activity due to the damage to carotenoids and chlorophyll, destruction of PSII proteins, decreased activity of Rubisco and sedoheptulose 1,7-biphosphatase (Allen et al. 1998; Kataria et al. 2013), and damage to PSII efficiency (Nogues and Baker 1995; Allen et al. 1998; Yu et al. 2013). In the last few decades, chlorophyll fluorescence observation has established itself as a reliable technique for the detection of photosynthetic processes (Kalaji et al. 2014, 2018). The 'JIP' test is a chlorophyll-*a* fluorescence-based method used to evaluate the status of photosynthetic apparatus under different abiotic stresses (Rastogi et al. 2019a, b, 2020; Akhter et al. 2021).

Due to their survival nature, plants evolved with time to reduce UV-induced damages. Some of the protection mechanisms include higher production and accumulation of phenolic compounds which shield different organelles, whereas DNA photolyase protects damages to DNA (Jenkins 2009). Exposure to UV leads to the generation of reactive oxygen species (ROS) like superoxide anion radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO^-) (Rastogi and Pospisil 2013). Plants have developed several enzymatic and non-enzymatic mechanisms for the purpose to protect themselves from different ROS molecules (Jain et al. 2004; Hasanuzzaman et al. 2012; Rastogi et al. 2014). The non-enzymatic molecules include ascorbic acid (AsA), glutathione, α-tocopherol, and carotenoids (Munné-Bosch and Alegre 2002; Jain et al. 2004), whereas enzymatic scavenger include Halliwell/Asada pathway enzymes, superoxide dismutase, and catalase (Jain et al. 2004). A number of studies have suggested the participation of nitric oxide (NO) in response to UV-B radiation (An et al. 2005; Zhang et al. 2011).

Considering the increasing influence of UV-B radiation, it is required to look into different techniques for the purpose to improve crop yield efficiently. Among different available techniques, seed priming is one of the most commonly used processes for enhancing different stress tolerance (Sytar et al. 2019; Prajapati et al. 2020; Shah et al. 2020). The SMF priming is one of the techniques which is less explored for its interaction with UV-B radiation. Some of the earlier studies have indicated that SMF pretreatment alleviates the response of different abiotic stress factors on number of plant speciecs (Anand et al. 2012; Thomas et al. 2013; Kataria et al. 2017, 2019, 2020; Baghel et al. 2018).

The impact of ambient and enhanced UV-B radiation on soybean yield has been previously studied by several researchers (Liu et al. 2013; Baroniya et al. 2011, 2014). Some of the studies have indicated that the pretreatment of seeds by SMF enhances productivity under ambient UV-B stress conditions (Kataria et al. 2017, 2020). However, the mitigation of the adverse effects of enhanced or supplemental UV-B radiation on growth, the efficiency of PSII, JIP-test parameters, photosynthesis, and yield of soybean through magnetopriming have not been investigated yet. Therefore, the present study aims to evaluate the impact of SMF pretreatment on photosynthetic performance and yield of soybean under enhanced UV-B stress.

Materials and methods

Soybean (*Glycine max* (L.) variety JS-335) seeds were obtained from the ICAR-Indian Institute of Soybean Research in Indore, India. The experiments were conducted in October to January'2018 under ambient conditions at the University campus (latitude 22°43′N) in Indore, India.

Magnetic field generation and magnetic treatment

The magnetic field was generated by a fabricated electromagnetic field generator ("AETec" Academy of Embedded Technology, Delhi, India) as described in Kataria et al. (2020). The SMF of 200 mT was generated and provided to soybean seeds for an hour in a sample holder (transparent plastic) of capacity 42 cm^3 (2.7 $L \times 2.6$ $B \times 7.3$ H) at 25 ± 5 °C. The untreated (UT) seeds were kept far away from the influence of the electromagnetic field generator. The local geomagnetic field was observed to be < 10 mT.

Supplementary UV-B treatments

The SMF-treated (MT) and untreated (UT) seeds were sown in nursery bags of 34 × 34 cm, filled with a 5 kg mixture of homogeneous black soil, sand, and farm-yard manure (2:2:1 by volume). Before sowing, the recommended fungicides Bevistin (Ankur Agro company, Etawah, India) and Diathane M-45 (Sagan Agro Industries, Meerut, India) (2 g kg^{-1} seeds) were applied to all the seeds. These seeds were also properly mixed with *Rhizobium* culture (National Fertilizer limited, New Delhi, India) at 3 g kg^{-1} seeds. Six seeds of similar size were packed in plastic nursery bags and after germination three plants were maintained in each bag. The black soil used was affluent in lime, iron, alumina, and magnesia. The average temperature ranged from 27 to 30 °C, relative humidity ranged from 55 to 75% during the experimental period.

From the time of germination, soybean seedlings emerged from UT and MT seeds were exposed to ambient $_{a}$UV-B (0.136 $mWcm^{-2}$ s^{-1}) and enhanced UV-B ($_{a+s}$UV-B, 0.335 $mWcm^{-2}$ s^{-1}) radiation *via* UV-B lamps (a UV-B fluorescent tube,TL40W/12, Philips, Eindhoven, The Netherlands) which exhibited its emission > 280 nm to a maximum at 312 nm and fitted on steel frames at a distance of 45 cm directly above the plant canopy of nursery bags (34 × 34 cm) that was maintained constant throughout the experimental period.

The experimental site was organized in randomised way and divided into three blocks containing three biological replications, $n = 3$. This split-plot design allowed us to test the effects of ambient UV-B and for enhanced UV-B (ambient + supplemental, $_{a+s}$UV-B; 280–315 nm) radiation on soybean plants.The intensity of UV-B radiation was measured by a radiometer, solar light Co. Inc. (PMA 2100), Glenside, PA, USA.

Measurement of growth, chlorophyll, PSII efficiency, and gas exchange parameters

The sampling was done from 45-day-old soybean plants for the growth and biochemical parameters.The plant height and leaf area were measured. A portable laser leaf area meter, CI-202 (CID Inc., Camas, WA USA), was used for the measurement of the area of third trifoliate leaves. The specific leaf weight was measured according to Hunt (1982). The chlorophyll content was measured via the dimethyl sulfoxide (DMSO) method (Hiscox and Israelstam 1979). The total chlorophyll concentration was calculated by equation of Wellburn's (Wellburn and Lichtenthaler 1984) and expressed as mg g^{-1} leaf fresh weight. For chlorophyll *a* (Chl*a*) fluorescence analysis, the handy PEA fluorimeter (Plant Efficiency Analyzer, Hansatech Instruments, Norfolk, UK) was used with the standard protocol of 30 min dark adaptation (Strasser et al. 2000; Kalaji et al. 2016, 2018). The transients were induced by red light (peak at 650 nm) of 600 Wm^2 (3200 µE m^{-2} s^{-1}) supply through a range of six light-emitting diodes, fixed on the leaf surface in the clips on a spot of 4 mm diameter to provide homogenous illumination over the exposed area of the sample. Data were recorded for 1 s with 12-bit resolution; the data acquisition was done at every 10 µs for the first 2 ms and every 1 ms thereafter (Strasser et al. 2000). The JIP-test parameters such as F_v/F_m, the maximum quantum yield (efficiency) of PSII photochemistry, φEo, the quantum yield of electron transport, ΔV(*I*–*P* phase), the amplitude of the relative contribution of the *I*-to-*P* rise to the OJIP transient, PI_{ABS}, performance index at absorption basis, and PI_{total} total performance index were calculated according to the equations reviewed by Bussotti et al. (2020) and Banks (2017). The Biolyzer HP3 software (Bioenergetics Laboratory) was used for the calculation of the photosynthetic parameters.

The LI-6200 photosynthetic system (LICOR Inc., USA), was used to measure the rate of photosynthesis (*Pn* µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mmol H_2O m^{-2} s^{-1}), and internal CO_2 concentration (µmol CO_2 mol^{-1}) in third trifoliate leaves of 45-day-old soybean plants. The measurement was conducted around noon according to the protocol as described previously by Fatima et al. (2020).

Biochemical analyses

All the biochemical analyses were made in third trifoliate leaves of 45-day-old soybean plants from untreated and

SMF-treated seeds grown under $_{a}$UV-B as well as $_{a+s}$UV-B stress.

UV-B absorbing substances (UAS)

UAS accumulation in leaves of soybean was determined spectrophotometrically (Shimadzu-UV 1601) from acidified methanol extract by the method of Mazza et al. (1999) from the leaf disk of 0.50 cm diameter and expressed as units mg^{-1} fresh weight of leaves.

Hydrogen peroxide (H_2O_2)

H_2O_2 was estimated by the formation of titanium-hydroperoxide complex (Mukherjee and Choudhuri 1983) from500 mg of soybean leaves and expressed as µmol H_2O_2 g^{-1} fresh weight of leaves.

Superoxide anion radical ($O_2^{\cdot-}$)

The standard nitroblue tetrazolium chloride (NBT) reduction method was used to quantify $O_2^{\cdot-}$ in soybean leaves (100 mg) following the method of Chaitanya and Naithani (1994) and expressed as µmol g^{-1} fresh weight of leaves.

Estimation of ascorbic acid (ASA)

Total ASA was estimated following the protocol explained in Arakawa et al. (1981). A total of 200 mg leaf tissue was used to determine the reduction of dehydroascorbic acid (DHA) to ASA by dithiothreitol and expressed as nmol g^{-1} leaf fresh weight.

Estimation of α-tocopherol

α-Tocopherol was estimated from 500 mg of leaf tissue following the slightly modified procedure from Pearson et al. (1970) and described before in Kataria et al. (2019) and represented as mg g^{-1} leaf fresh weight.

Nitric oxide (NO) determination

The procedure from Zhou et al. (2005) was used to estimate NO content from 500 mg of soybean leaves. The NO content was expressed in nmol g^{-1} fresh weight of leaves.

Nitrate reductase (NR) activity

The procedure of Jaworski (1971) was followed to estimate the enzymatic activity of NR from 250 mg of soybean leaf tissue and expressed in µmol NO_2 g^{-1} leaf fresh weight h^{-1}.

Yield

After the crop harvest at 120 DAE, the number of pods and seeds, pod weight, and seed yield per plant were measured.

Statistical analysis

Statistical analysis was performed on Microsoft Excel and Prism 4 (GrafPad Software, La Jolla, California) software where mean and standard errors were calculated, and the analysis of variance (ANOVA) followed by post hoc Newman–Keuls Multiple Comparison Test were performed. $^{###}p < 0.001$; $^{##}p < 0.01$; $^{#}p < 0.05$ indicate the significant difference among the seedlings emerged from untreated (UT) seeds grown-up in ambient UV-B with ambient + supplemental UV-B conditions; $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$ indicate significant difference among the seedlings emerged from SMF pretreated (MT) seeds with the seedlings of untreated (UT) seeds grown-up in ambient and ambient + supplemental UV-B conditions.

Results

The effects of UV-B radiation on morphological parameters of SMF pretreated seeds in 45-day-old soybean plants are presented in Fig. 1. Enhanced UV-B ($_{a+s}$) significantly reduced the plant height (15%), leaf area (27%), total biomass (22%), and specific leaf weight (27%) in plants obtained from untreated soybean seeds as compared to the plants from untreated seeds grown in ambient UV-B conditions; while all of these parameters were significantly increased by SMF pretreatment (Fig. 1b–d). The plant height maximally enhanced by 34% and 67% (Fig. 1a); leaf area enhanced by 60% and 115% (Fig. 1b), and specific leaf weight was also increased by 60% and 96% (Fig. 1d), respectively, under $_{a}$UV-B as well as $_{a+s}$UV-B exposure by the SMF treatment as compared to their respective untreated ones.

Total chlorophyll content of leaves was decreased by 25% in enhanced UV-B irradiation plants in comparison to untreated seeds grown under only ambient UV-B, while it was significantly increased (21%) by SMF treatment even after the enhanced UV-B irradiation (Fig. 2a).

UV-B absorbing compounds were found to be increased in the leaves of soybean plant under the $_{a}$UV-B and $_{a+s}$UV-B (Fig. 2b). UAS contents in the leaves of plants from untreated seeds showed a significant increase of 28% under $_{a+s}$UV-B, whereas a significant reduction of 32% and 30% were recorded respectively under $_{a}$UV-B and $_{a+s}$UV-B as compared to their untreated ones (Fig. 2b).

ChlF data indicate that maximum fluorescence (F_m) decreased in $_{a+s}$UV-B treated plants when compared with

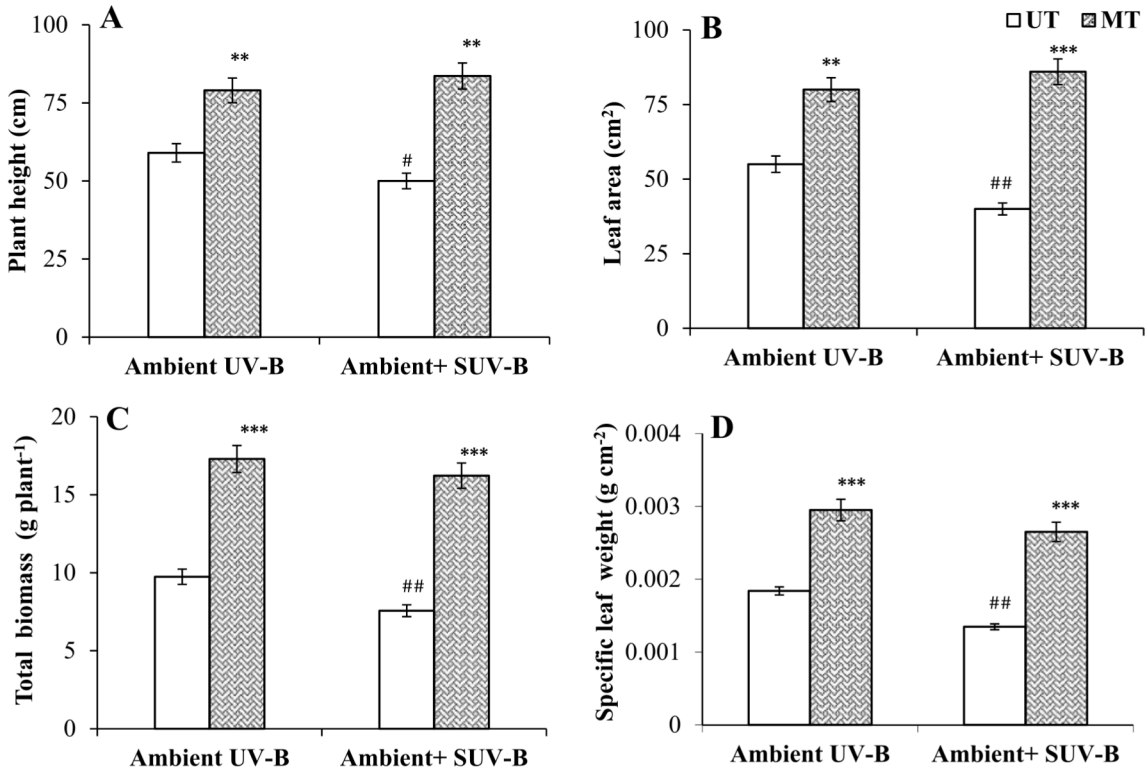

Fig. 1 Effect of SMF-priming (200 mT for 1 h) on plant height (**a**), leaf area (**b**), total biomass (**c**), and specific leaf weight (**d**) of soybean plants under ambient and supplemental UV-B conditions. MT = seedlings from SMF pretreated and UT = seedlings from untreated seeds

ChlF from plants of untreated seeds grown under ambient UV-B. The transient fluorescence curve (OJIP) in plants exposed to ambient or $_{a+s}$UV-B after SMF pretreatment is shown in Fig. 3. The OJIP curve showed an increase in the *I–P* phase due to SMF treatment as compared to the leaves of plants that emerged from UT seeds under ambient as well as $_{a+s}$UV-B conditions (Fig. 3).

The photosynthetic parameters such as F_v/F_m, φEo = ETo/ABS, ΔV (*I–P*), $PI_{ABS,}$ and PI_{total} were decreased in plants from untreated seeds grown under $_a$UV-B and $_{a+s}$UV-B exposure (Fig. 4a–e). SMF treatment caused a slight increase in F_v/F_m as compared to their UT ones under the presence of UV-B stress (Fig. 4a). However, SMF pretreatment significantly increased the value of φEo by 50% and 113% and ΔV (*I–P*) by 12.9% and 13%, respectively, under $_a$UV-B and $_{a+s}$UV-B in comparison to their UT ones (Fig. 4c).Values of PI_{abs} and PI_{total} parameter derived from ChlF records (Fig. 4d, e) confirmed much higher responsiveness compared to F_v/F_m. A tremendous increase was found in PI_{ABS} after SMF treatment under $_a$UV-B (145%) and $_{a+s}$UV-B (254%) (Fig. 4d). SMF pretreatment also caused a significant increase of 43% and 68% in PI_{total}, respectively under $_a$UV-B and $_{a+s}$UV-B as compared to their UT ones (Fig. 4e).

Significant inhibition of the net rate of photosynthesis (23%) and stomatal conductance (42%) was observed under the enhanced UV-B, whereas internal CO_2 concentration was observed to be increased by 15% in the leaves of soybean plants emerged from untreated seeds as compared to the plants grown in ambient UV-B condition (Fig. 5a–c). On the other hand, *Pn* was increased by 36% and 46%, respectively, under $_a$UV-B and $_{a+s}$UV-B in the plants from SMF-treated seeds in comparison to the plants obtained from untreated seeds (Fig. 5a). SMF pretreatment caused 38% and 64% enhancement in stomatal conductance, respectively, under $_a$UV-B and $_{a+s}$UV-B in comparison to their untreated seedlings (Fig. 5b). However, SMF pretreatment caused a reduction of 56% and 52% in internal CO_2 concentration, respectively, for $_a$UV-B and $_{a+s}$UV-B conditions (Fig. 5c).

Superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) content was observed to be higher in the leaves of plants that emerged from UT seeds under $_a$UV-B and $_{a+s}$UV-B conditions when compared with plants from SMF pretreated seeds (Fig. 6a, b). Most of the antioxidant activities were found to be increased in presence of $_{a+s}$UV-B depicting a better defensive response of the soybean plants under $_{a+s}$UV-B exposure. Among non-enzymatic antioxidants, ascorbic acid showed a significant increase of 24% and α-tocopherol showed an increase of 52% by $_{a+s}$UV-B as compared to their untreated plants grown under $_a$UV-B

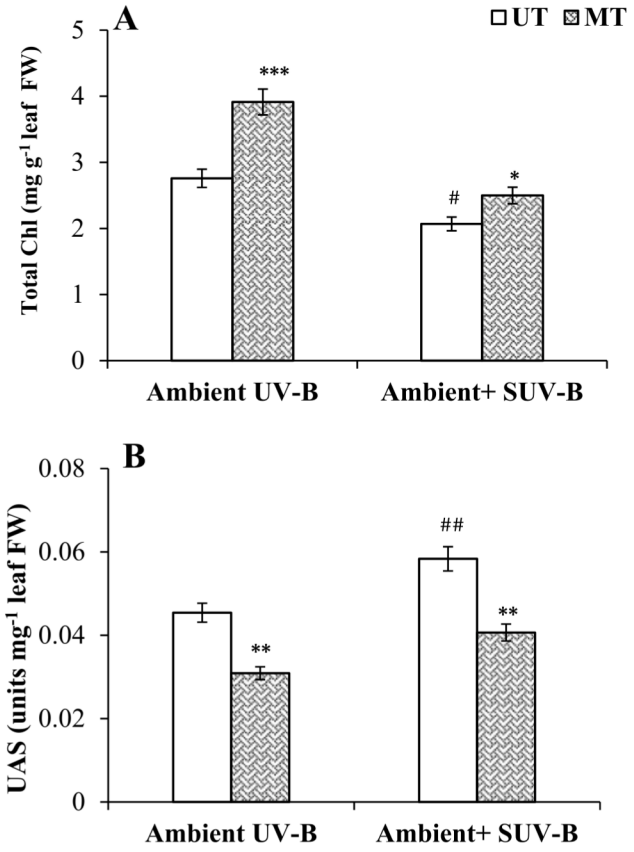

Fig. 2 Effect of SMF-priming (200 mT for 1 h) on Total Chlorophyll (**a**), and UAS (**b**) in third trifoliate leaves of soybean plants under ambient and supplemental UV-B conditions. MT = seedlings from SMF pretreated and UT = seedlings from untreated seeds

condition (Fig. 6c, d), while SMF pretreatment caused a significant decrease in ASA and α-tocopherol content under $_{a}$UV-B and $_{a+s}$UV-B (Fig. 6c, d).

Nitric oxide (NO) content was remarkably increased by 63% under $_{a+s}$UV-B in plants from untreated seeds as compared to their plants grown under ambient UV-B stress (Fig. 7a). SMF treatment increased the NO content by 58% and 23%, respectively, in $_{a}$UV-B and $_{a+s}$UV-B conditions as compared to their UT ones (Fig. 7a). NR activity was decreased by $_{a+s}$UV-B, while SMF-treatment significantly enhanced the NR activity in $_{a}$UV-B and $_{a+s}$UV-B conditions as compared to their respective UT ones (Fig. 7b).

A significant difference in yield parameters such as the number of pods/seeds and the weight of pods/seeds per plant was observed at crop maturity (Fig. 8a–d). We observed a negative effect of $_{a+s}$UV-B stress on the yield of soybean, but the plants that emerged from SMF-treated seeds showed a significant increase of 35% in the number of pods, 39% in pod weight, 45% in the number of seeds, and 54% in seed weight per plant in comparison to their respective UT ones under $_{a+s}$UV-B stress conditions (Fig. 8a–d).

Discussion

In this study plant growth, the efficiency of PSII, performance indices, photosynthesis, and yield was negatively impacted by enhanced UV-B, whereas SMF pretreatment was observed to mitigate the UV-B-induced oxidative stress in soybean plants. A significant decline in several morphological and physiological characteristics (plant height, dry mass, leaf area, chlorophyll content, the efficiency of PSII, and photosynthesis) was observed in soybean after its exposure to $_{a}$UV-B together with $_{s}$UV-B. A reason behind the morphological and physiological changes in the plant can be related to an enhanced level of ROS ($O_2^{\cdot -}$ and H_2O_2) observed in this study and it can be related to previous studies (Reddy et al. 2004; Shine and Guruprasad 2012; Rastogi and Pospisil 2013; Kataria et al. 2020). The decline in dry mass and leaf area due to enhanced UV-B could be due to a reduction in the cytokinin content, the extent of cell division, and elongation as described in previous studies (Hopkins et al. 2002; Kataria et al. 2014a, b; Singh et al. 2014; Kataria and Guruprasad 2018). Reduction in plant height by enhanced UV-B could have been due to photo-oxidative damage of the phytohormone indole acetic acid followed by lower cell wall extensibility, as established in sunflower seedlings (Ros and Tevini 1995). On the other hand, our results revealed that SMF pretreatment significantly enhanced the plant height, leaf area, specific leaf weight, and total biomass accumulation even under $_{a+s}$UV-B. Some previous studies demonstrated that SMF pretreatment could ameliorate the inhibition of growth, photosynthesis, and yield caused by the ambient UV-B stress in soybean and maize (Shine and Guruprasad 2012; Kataria et al. 2017, 2020; Baghel et al. 2015). A significant increase in SLW after SMF pretreatment influenced the plant's higher biomass and an increase in leaf thickness. This is the first study showing that SMF (200 mT for 1 h) pretreatment considerably increased efficiency of PSII, the quantum yield of electron transport, performance indices (PI_{abs} and PI_{total}), rate of photosynthesis, NR activity, and NO content as compared to the plants that emerged from UT seeds grown under enhanced UV-B stress (a + sUV-B) conditions.

A decrease in total chlorophyll concentration under enhanced UV-B stress observed is in agreement with studies under elevated UV-B radiation (Zhao et al. 2003; Reddy et al. 2004). UV-B might have caused the

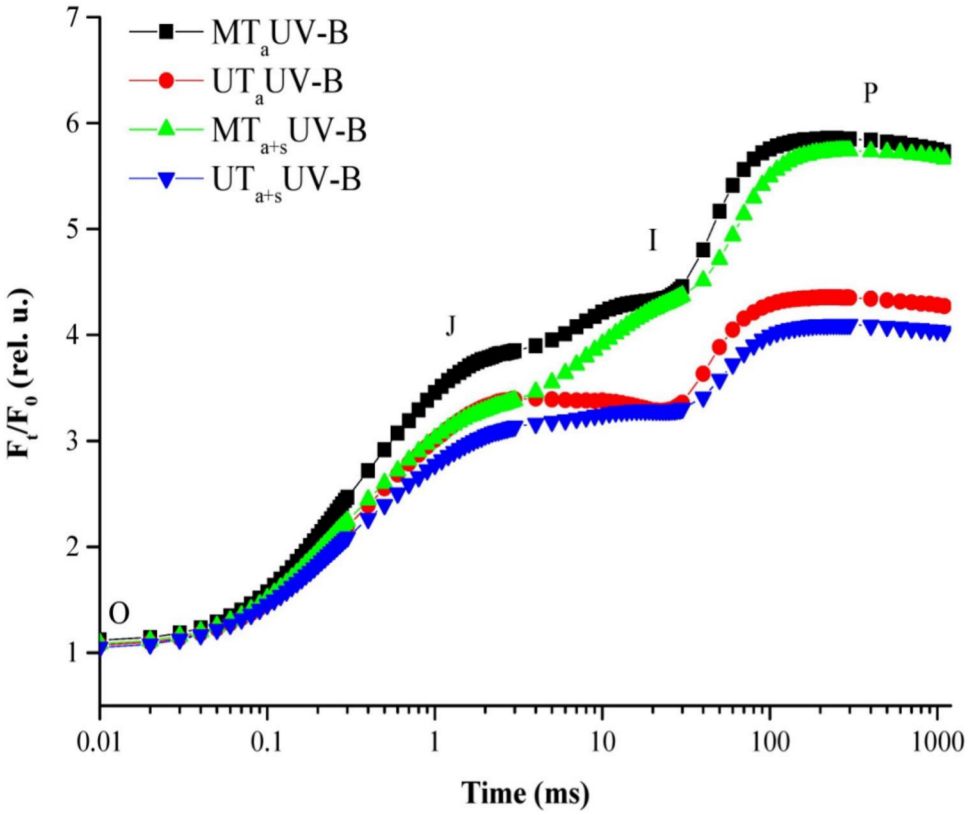

Fig. 3 Effect of SMF-priming (200 mT for 1 h) on chlorophyll fluorescence emission transients in third trifoliate leaves of the soybean seedlings grown under ambient and supplemental UV-B conditions. MT = seedlings from SMF pretreated and UT = seedlings from untreated seeds. $MT_aUV\text{-}B$ = plants emerged from SMF-treated seeds grown under ambient UV-B; $UT_aUV\text{-}B$ = plants emerged from UT seeds grown under ambient UV-B; $MT_{a+s}UV\text{-}B$ = plants emerged from SMF-treated seeds grown under ambient plus supplemental UV-B; and $UT_{a+s}UV\text{-}B$ = plants emerged from UT seeds grown under ambient plus supplemental UV-B stress.The curve was normalized for F_o (fluorescence at time 0) and F_t/F_o (where F_t is fluorescence at time t) against the time

destruction of chloroplast structure, interferes in chlorophyll synthesis through destruction of enzymes, and may have enhanced chlorophyll degradation which may have resulted in a decrease in total chlorophyll concentration observed in this study and supported by previous studies (Sakaki et al. 1983; Kataria et al. 2013, 2014a, b). The exposure of the plant to $_{a+s}$UV-B significantly increased the UV-B absorbing compounds that emerged from untreated seeds in the current study as reported previously (Kakani et al. 2004; Reddy et al. 2004). The increase in flavonoids had been recognized as a common and quick response to UV-B stress (Tiitto et al. 2015). In a study, the total phenolic and flavonoid content was observed to increase in lettuce plants exposed to direct sunlight (UV exposed) in contrast to greenhouse conditions (Low UV) by the fluorescence excitation ratio method (Zivcak et al. 2017). The flavonoids have effective free radical-scavenging capabilities and could contribute directly to enhanced photoprotection against UV-B radiation (Mosadegh et al. 2018). Thus, flavonoids could protect photosynthetic pigments and may help in sustaining the photosynthetic activity (Day and Neale 2002). In this study, the flavonoids concentrations were observed to be decreased while total Chl concentration was increased in combined treatment of SMF with UV-B radiation.

It has been previously reported that the supplemental UV-B irradiance harms the physiological processes of plants including the reduction of photosynthetic efficiency, decrease of leaf stomatal conductance, and transpiration rate (Krupa and Kickert 1989; Chen and Zhang 2007; Kataria et al. 2014a, b). The photosynthetic efficiency of soybean seedlings was determined in terms of chlorophyll fluorescence (JIP-test parameters) (Figs. 3, 4) and gas exchange parameters (Fig. 5). It showed that $_{a+s}$UV-B reduced the *I–P* phase of the OJIP curve in the leaves of plants that emerge from UT seeds, while plants that emerged from SMF-treated seeds showed a significant increase in the *I–P* phase even under $_{a+s}$UV-B stress. The *I–P* phase is related to the electron transfer through PS I (Schansker et al. 2006). The *I–P* phase may decline under several kinds of stresses such as nitrogen deficiency (Nikiforou and Manetas 2011), salinity (Oukarroum et al. 2015), heavy metal (Bernardini et al. 2015), drought (Pollastrini et al. 2014), and ozone pollution (Bussotti et al. 2011). The increase in chlorophyll fluorescence specifically in the *I–P* phase of the OJIP curve is characteristically recognized to the reduction of electron transporters (ferredoxin, intermediary acceptors, and NADP) of thePSI acceptor side (Kalaji et al. 2016). A positive correlation between $\Delta V(I\text{-}P)$ and net photosynthesis (Pn) have been observed in plants (Cascio et al. 2010; Santos et al. 2019). $\Delta V(I\text{-}P)$ is measured as a proxy of the concentration of PSI reaction centers (Schansker et al. 2005), which is implied in the production of $NADPH^+$ for CO_2 fixation.The analysis of

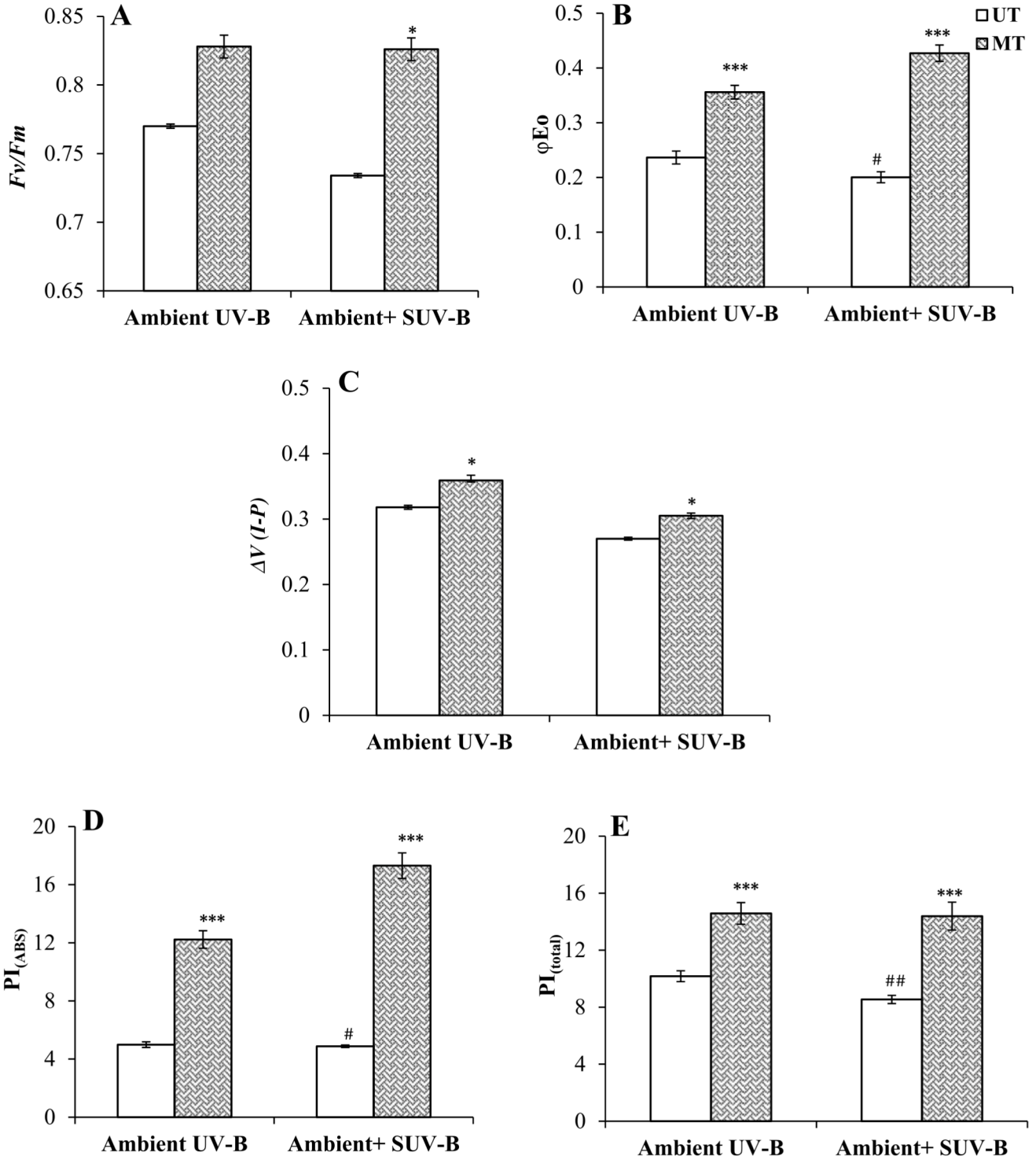

Fig. 4 Effect of SMF-priming (200 mT for 1 h) on maximum photochemical efficiency of PS II, F_v/F_m (**a**), quantum yield of electron transport, φEo (**b**), relative amplitude of the *I–P* phase of Chl*a* fluorescence, ΔV (*I–P*) (**c**), performance index on absorbtion basis, PI_{ABS}, (**d**), and performance index total, PI_{total} (**e**) in third trifoliate leaves of soybean plants under ambient and supplemental UV-B conditions. MT = seedlings from SMF pretreated and UT = seedlings from untreated seeds

fast ChlF transient was applied in numerous studies in crop plants, to study the environmental effects such as salinity, drought, high/low temperature (Stirbet et al. 2018), and light stress (Kalaji et al. 2018). Significant suppression in Chl fluorescence induction curve was observed in salt-sensitive genotypes, whereas for salt-resistant sorghum