DOI: 10.1007/s11099-014-0029-6 PHOTOSYNTHETICA 52 (2): 262-271, 2014

Maize growth and developmental responses to temperature and ultraviolet-B radiation interaction

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Abstract

Plant response to the combination of two or more abiotic stresses is different than its response to the same stresses singly. The response of maize (*Zea mays* L.) photosynthesis, growth, and development processes were examined under sunlit plant growth chambers at three levels of each day/night temperatures (24/16°C, 30/22°C, and 36/28°C) and UV-B radiation levels (0, 5, and 10 kJ m^{-2} d^{-1}) and their interaction from 4 d after emergence to 43 d. An increase in plant height, leaf area, node number, and dry mass was observed as temperature increased. However, UV-B radiation negatively affected these processes by reducing the rates of stem elongation, leaf area expansion, and biomass accumulation. UV-B radiation affected leaf photosynthesis mostly at early stage of growth and tended to be temperature-dependent. For instance, UV-B radiation caused 3–15% decrease of photosynthetic rate (P_N) on the uppermost, fully expanded leaves at 24/16°C and 36/28°C, but stimulated P_N about 5–18% at 30/22°C temperature. Moreover, the observed UV-B protection mechanisms, such as accumulation of phenolics and waxes, exhibited a significant interaction among the treatments where these compounds were relatively less responsive (phenolics) or more responsive (waxes) to UV-B radiation at higher temperature treatments or *vice versa*. Plants exposed to UV-B radiation produced more leaf waxes except at 24/16°C treatment. The detrimental effect of UV-B radiation was greater on plant growth compared to the photosynthetic processes. Results suggest that maize growth and development, especially stem elongation, is highly sensitive to current and projected UV-B radiation levels, and temperature plays an important role in the magnitude and direction of the UV-B mediated responses.

Additional key words: photosynthesis; phenolic compounds, stem elongation, waxes.

Introduction

Changes projected in concentrations of atmospheric CO_2 and other greenhouse gases are expected to increase global air temperature by 2.5–4.5°C until the end of this century (IPCC 2007). In addition, ground-level ultraviolet-B radiation (UV-B; 280-315 nm) has increased considerably due to emission of ozone-depleting compounds such as chlorofluorocarbons (CFCs), methane, and nitrous oxide. Global distribution of mean erythemal daily doses of UV-B radiation ranges from 2 to 9 kJ m^{-2} between the latitude 40°N and 40°S during summer (McKenzie *et al.* 2007). While crop productivity may benefit from rising CO_2, the increased potential for abiotic stresses, such as heat waves and UV-B radiation, pose a challenge for farmers. Studies suggest that due to climate change, Southern Africa could lose approximately 30% maize production by 2030 and the losses of many regional staples, such as rice and maize, could be up to 10% by this period in the South Asia (Lobell *et al.* 2008).

Received 14 April 2013, *accepted* 12 September 2013
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Abbreviations: BAR – biomass accumulation rate; Car – carotenoids; Chl – chlorophyll; DAE – days after emergence; F_v'/F_m' – quantum efficiency by oxidized (open) PSII reaction center in light or actual PSII efficiency; LA – leaf area; LAER – leaf area expansion rate; MSER – main stem elongation rate; MSNN – main stem node number; PH – plant height; P_N – net photosynthetic rate; SPAR – soil-plant-atmosphere research.
Acknowledgements: This research was funded in part by the USDA-UV-B Monitoring Program at Colorado State University, CO. We also thank Mr. David Brand for technical support. This article is a contribution from the Department of Plant and Soil Sciences, Mississippi State University, Mississippi Agricultural and Forestry Experiment Station, paper no. J–12101.

Corn and all other crops cultivated between 40°N and 40°S latitudes are already experiencing UV-B dosage of 2–10 kJ m^{-2} d^{-1} depending on location and season (Gao *et al.* 2004, McKenzie *et al.* 2007, Reddy *et al.* 2013). Both high and low temperatures and increased UV-B radiation affect adversely plant growth and development and the interaction among these stressors often exacerbates the damaging effects on plant (Teramura 1983, Reddy *et al.* 2004, Qaderi *et al.* 2010, Singh *et al.* 2010).

The effect of temperature and UV-B radiation on crop plants varies depending on the intensity of stress and crop growth stage. The growth and development processes, such as stem elongation, leaf initiation and expansion, leaf area development, photosynthesis, flowering and fruiting of many crops including maize, are highly dependent on growth temperature (Tollenaar 1989a, b; Kim *et al.* 2007, Singh *et al.* 2008a, Li *et al.* 2010). Yield and seed quality of many crops including maize are sensitive to frequently observed temperature extremes, and both to ambient and elevated UV-B radiation levels (Teramura *et al.* 1990, Gao *et al.* 2004, Kim *et al.* 2007, Ballare *et al.* 2011, Yin and Wang 2012). Some of the deleterious effects of UV-B radiation on plants include DNA damage, dilation and disintegration of cellular membranes, photooxidation of leaf pigments and phytohormones, and inhibition of photosynthesis (Ros and Tevini 1995, Mark and Tevini 1997, Correia *et al.* 1999, Li *et al.* 2010, Reddy *et al.* 2013). Moreover, UV-B mediated downregulations of genes associated with photosynthetic processes and phytohormones metabolism have also been reported (Casati and Walbot 2003, Hectors *et al.* 2007). The UV-B protective mechanism in plants involves photoreactivation to restore DNA damage, accumulation of UV-B absorbing compounds (*e.g.*, phenolic compounds) and waxes in leaf epidermis to partially block UV-B radiation (Caldwell *et al.* 1983, Rozema *et al.* 1997, Casati and Walbot 2003).

In nature, abiotic stresses do operate independently, but often interact to produce combined impact on agroecosystems. Understanding the interactive effects of stress factors are particularly important when their combined effect can not be predicted based on evidence from single-stressor studies (Mittler 2006, Singh *et al.* 2010, 2013). The effects of temperature have been studied extensively on many crops, including maize (Tollenaar 1989a,b; Kim *et al.* 2007), but studies addressing the influence of UV-B radiation on maize are limited (Mark and Tevini 1997, Correia *et al.* 1998, 1999). Moreover, experiments on the interactive effect of temperature and UV-B radiation on maize growth and development are scarce (Mark and Tevini 1997). Mark and Tevini (1997) reported that elevated temperature compensated for some of the harmful effects of UV-B radiation in maize. However, the temperature and UV-B radiation interactions have shown to adversely affect growth and development of many crops (Reddy *et al.* 2004, Koti *et al.* 2007, Singh *et al.* 2010).

Maize is one of the most cultivated C_4 crop in the world (FAO 2011). The assessments at regional scales and field studies project significant reduction in maize yield due to every unit increase in temperature (Tao and Zhang 2011) and UV-B radiation (Gao *et al.* 2004, Yin and Wang 2012, Reddy *et al.* 2013) under current and projected environmental conditions. Experiments designed to understand the interaction among the abiotic factors on maize can help to elucidate how the interaction between temperature and UV-B radiation alters maize growth and development. In this study, we examined the interactive effects of temperature and UV-B radiation on maize growth and development, and photosynthetic processes. Accumulation of leaf pigments, ultraviolet absorbing compounds, and waxes were also studied under UV-B and temperature interactions.

Materials and methods

Soil-plant-atmosphere research experimental facility: This experiment was conducted in nine sunlit, soil-plant-atmosphere research (SPAR) units located at the R.R. Foil Plant Science Research Center (33° 28′ N, 88° 47′ W), Mississippi State, Mississippi, USA in 2008. Each SPAR growth chamber has the capability to precisely control CO_2, temperature, UV-B radiation, and desired nutrient and irrigation regimes at determined set points under near ambient levels of PAR. Each SPAR chamber consists of a steel soil bin (1 m deep, 2 m long, 0.5 m wide) to accommodate the root system, a Plexiglas chamber (2.5 m tall, 2 m long, 1.5 m wide) to accommodate aerial plant parts and a heating and cooling system, and an environmental monitoring and control system. The Plexiglas chambers are completely opaque to solar UV-B radiation, but transmit 12% UV-A and >95% incoming PAR (wavelengths among 400–700 nm; Zhao *et al.* 2003). Uniformity tests of SPAR units have indicated no statistical difference in SPAR chambers for sorghum (Reddy, personal communication) and wheat growth parameters (Fleisher *et al.* 2009). Many details of the operations and controls of SPAR chambers have been described by Reddy *et al.* (2001) and mentioned elsewhere (Singh *et al.* 2010).

Plant culture: Maize (*Zea mays* L.) cv. DKC 65-44 seeds were sown on 16 July 2008 in the soil bins, filled with fine sand, of nine SPAR units. Emergence was observed 5 d after sowing. Four days after emergence (DAE), plants were thinned so that each SPAR unit had 11 rows of 5 plants per row, with each row 18.2 cm apart. Six and two rows of plants (5 plants per row) were harvested at 15 and 23 DAE, respectively, to avoid competition and to determine aboveground biomass and total leaf area at the early growth stages. Thus, 3 rows of 15 plants per m^2 were retained till 43 DAE with 66.7 cm row spacing and 10 cm between plants within the row. Plants were irrigated three times a day with a full-strength Hoagland's nutrient solution delivered at 8:00, 12:00, and 17:00 h with an

automated, computer-controlled drip system to provide favorable nutrient and water conditions for plant growth. Variable density shade cloths, designed to simulate canopy spectral and intensity properties, placed around the edges of the plant canopy, were adjusted regularly to match canopy height and to eliminate the need for border plants.

Treatments: The SPAR units were maintained at 30/22°C (day/night) until 4 DAE. Thereafter, day/night air temperatures in the units were maintained at 24/16, 30/22, or 36/28°C, until the end of experiment at each UV-B treatment as described below. The daytime temperature was controlled in a square-wave fashion and initiated at sunrise and returned to the night-time temperature 1 h after sunset. The UV-B radiation treatments of zero (no UV-B) and a total daily dose of biologically effective UV-B radiation of 5 and 10 kJ m^{-2} d^{-1} were imposed at each temperature level from 4 DAE. The daily dosage of UV-B radiation in USA ranges from 0.02 to 8.75 kJ m^{-2} d^{-1} depending upon season and cloud cover (USDA, UV-B-Monitoring and Research Program, Colorado State University, CO, USA; (http://uvb.nrel.colostate.edu/UVB). Therefore, the imposed UV-B doses were expected to reflect near ambient and projected UV-B in the near future climate. The square-wave supplementation systems were used to provide desired UV-B radiation doses which were delivered from 0.5 m above the plant canopy for 8 h, each day, from 8:00 to 16:00 h by 8 fluorescent *UV-B-313* lamps (*Q-Panel Company*, Cleveland, OH, USA) mounted horizontally on a metal frame inside each SPAR chamber. To filter-out and avoid the germicidal effects of UV-C radiation (<280 nm) (Mercier *et al.* 2001), the lamps were wrapped with presolarized (kept under UV-B light for 48 h to stabilize transmission) 0.07 mm cellulose diacetate (CA) film (*JCS Industries Inc.*, La Mirada, CA, USA). The CA film was changed every 3–4 d to account for the degradation of CA properties. The UV-B radiation delivered at the top of the plant canopy was monitored at 10 different locations daily, and it was adjusted as needed. The mean data for temperatures and the weighted total biologically effective UV-B radiation at the top of the plant canopy during the experiment, and daytime CO_2 for a typical day are presented in Table 1. Other details were the same as described by Singh *et al.* (2008b).

Growth and developmental measurements: Plant heights (PH), the number of main stem nodes (MSNN), and leaf length at each node were determined on 9 plants (3 center plants in each row) at 4-d interval from emergence. The leaf-length measurements were subsequently converted to leaf areas (LA) by developing a relationship between the lengths of different leaves and leaf areas measured using a *LI-3100* leaf-area meter (*Li-COR, Inc.*, Lincoln, NE, USA) from the plants at 3 different harvests (15, 23, and 43 DAE) for each temperature treatments separately. The LA was calculated using the quadratic equations, $y = 0.12485\ x + 0.06734\ x^2$ ($r^2 = 0.96$, $n = 162$; 24/16°C), $y = 0.4474\ x + 0.05268\ x^2$ ($r^2 = 0.92$, $n = 227$; 30/22°C) and, $y = 0.096549\ x + 0.05166\ x^2$ ($r^2 = 0.93$, $n = 168$; 36/28°C) where y is area and x is the length. Plants were separated into leaves and stems at each harvest, and MSNN, LA, and dry mass (DM) of leaves and stems were determined.

Photosynthesis and chlorophyll (Chl) fluorescence: These measurements were made on the upper, most fully expanded leaves (25 DAE and 40 DAE) and 6th leaves (25 DAE) from the stem apex between 10:00 and 13:00 h on 3 plants. The leaf net photosynthetic rate (P_N) and PSII efficiency [quantum efficiency by oxidized (open) PSII reaction center in light, F_v'/F_m'] of light-adapted leaves were measured simultaneously using a *LI-COR 6400* photosynthetic system (*LI-COR Inc.*, Lincoln, NE, USA) with an integrated Chl fluorescence chamber head (*LI-COR 6400-40* Leaf Chamber Fluorometer). Photosynthesis is driven by the actinic light sources of the instrument that uses blue (centered 475 nm) and red light emitting diodes (LEDs) (centered 630 nm). The measurements were taken, when a steady state (around 4–6 min) was obtained, at PAR intensity of 1,500 µmol m^{-2} s^{-1} and cuvette block temperature was maintained at the respective treatment daytime temperature. For the Chl fluorescence measurement, the steady-state fluorescence (F_s) was measured first followed by maximal fluorescence (F_m') by providing a 0.8 s of saturating light flash of > 8,000 µmol m^{-2} s^{-1} using 27 red LEDs. Immediately, in the following step, the minimal fluorescence of a light-adapted leaf (F_0') was obtained by providing a dark flash by turning off the actinic light briefly while using far-red LEDs (centered wavelength 740 nm). The dark interval of 6 s (actinic light off) began with the far-red light 1 s before the actinic light goes off (pre time) and finished by 1 s after (post time, before the actinic light turns on). The details of the fluorescence measurements and other operation are available in *LI-6400 Instruction Manual,* (*version 5, LI-Cor Inc.*, Lincoln, NE, USA). The far-red radiation drives PSI to help drain PSII electrons. The F_v'/F_m' was estimated using the equation $F_v'/F_m' = (F_m' - F_0')/F_m'$ following Genty *et al.* (1989).

Pigments, phenolics and cell membrane thermostability: The total leaf Chl, carotenoids (Car), and phenolics (UV-B absorbing compounds) were extracted and determined from the uppermost, fully-expanded leaves at 18 DAE on five 0.38 cm^2 leaf disks from three replications. The disks were placed in a vial containing either 5 ml of dimethyl sulfoxide for pigments extraction or 10 ml of a mixture of methanol, distilled water, and hydrochloric acid in 79:20:1 ratio for phenolics extraction. After incubation in the dark for 24 h, the concentration of the extract was determined at 664, 648, and 470 nm for estimation of total Chl and Car concentration, and at 300 nm for estimation of phenolic compounds by using a *Bio-Rad UV/VIS* spectrophotometer (*Bio-Rad Laboratories*, Hercules, CA, USA).

Table 1. The set treatments, day/night temperatures (T), and daily dosage of UV-B radiations, and measured CO_2 from a typical day, temperature (day, night, and mean), daily mean UV-B radiation dosage, and daytime vapor pressure deficit (VPD) during the experimental period for each treatment. Each value represents the mean ± SD for one typical day for CO_2, and 25 July to 31 August 2008 for temperature, UV-B, and VPD.

Set treatments		Measured variables					
$T_{day/night}$ [°C]	UV-B [kJ m^{-2} d^{-1}]	UV-B [kJ m^{-2} d^{-1}]	T_{day} [°C]	T_{night} [°C]	T_{mean} [°C]	CO_2 [µmol mol^{-1}]	VPD [kPa]
24/16	0	0	23.78 ± 0.32	16.42 ± 0.63	20.55 ± 0.43	403 ± 4	1.11 ± 0.13
24/16	5	5.13 ± 0.51	23.92 ± 0.32	16.51 ± 0.63	20.67 ± 0.43	407 ± 5	1.29 ± 0.15
24/16	10	10.06 ± 0.36	23.98 ± 0.27	16.59 ± 0.39	20.72 ± 0.32	408 ± 6	1.36 ± 0.20
30/22	0	0	29.06 ± 0.43	21.70 ± 0.10	25.84 ± 0.28	406 ± 7	2.17 ± 0.14
30/22	5	5.03 ± 0.58	29.41 ± 0.26	21.99 ± 0.09	26.17 ± 0.21	409 ± 9	2.25 ± 0.13
30/22	10	10.09 ± 0.32	29.36 ± 0.25	21.84 ± 0.12	26.07 ± 0.24	406 ± 5	2.48 ± 0.18
36/28	0	0	34.24 ± 0.29	26.92 ± 0.66	31.03 ± 0.32	408 ± 5	3.72 ± 0.16
36/28	5	5.13 ± 0.34	34.50 ± 0.34	26.98 ± 0.62	31.20 ± 0.40	412 ± 5	3.95 ± 0.07
36/28	10	10.04 ± 0.50	34.39 ± 0.39	26.89 ± 0.64	31.11 ± 0.41	409 ± 5	3.41 ± 0.13

The equations of Lichtenthaler (1987) were used to estimate the Chl and Car concentrations, whereas the phenolic concentration was estimated by using the method of Kakani *et al.* (2004) and expressed as an equivalent of *p*-coumaric acid. The leaf cell membrane thermostability (CMT) was assessed on uppermost, fully-expanded leaves at 18 DAE according to Martineau *et al.* (1979) with minor modifications as described in detail by Singh *et al.* (2010).

Quantification of leaf epicuticular waxes: The extraction and quantitative analysis of leaf epicuticular waxes were carried out as per the method of Ebercon *et al.* (1977) with minor modifications. Fifteen leaf discs constituting an area of 26.51 cm^2 from uppermost, fully-expanded leaves were used for each of the three replications. Leaf waxes were removed and extracted as described by Singh and Reddy (2011). The wax content was expressed on a leaf area basis [µg cm^{-2}] by using a standard curve developed from the wax obtained from *Vigna sp.* (Singh and Reddy 2011).

Data analysis: The growth data grouped into categories for PH, LA, MSNN, aboveground DM, and their relationship with time (DAE) were used to estimate main stem expansion rate (MSER), leaf area expansion rate (LAER), node addition rate (NAR), and biomass accumulation rate (BAR), respectively. The rates (MSER, LAER, and NAR) were calculated for each DAE except first measurement using equation as rate on a specific DAE = $[(V_c - V_p)/(D_c - D_p)]$, where V_c – current measured value and V_p – the previous measured value, D_c – current DAE, and D_p – previous DAE. Since the rapid growth for these traits was mainly associated with linear part of the curves residing between last four measurements, the average rate of last four measurement was taken as the representative of average MSER, LAER and NAR and these rates were used in the analysis of variance as described later. A single linear regression equation was fit using *PROC REG* procedure of *SAS* (*SAS Institute Inc*., 2008, Cary, NC, USA) between the total aboveground DM and DAE (15, 23, and 43 DAE) for each treatment and the slope was taken as an estimate of BAR. The commonality of slopes was tested and contrasts were used to compare the slopes across treatments using *PROC GLM* procedure of *SAS*. The slopes that were not significantly different from one another between treatments, were noted in the Table 2.

The growth parameters at the end of experiment (PH, LA, and MSNN) were analyzed along with biomass and other observations. Two-way analysis of variance was performed to test the significance of temperature and UV-B radiation and their interactions on the studied parameters using *PROC GLM* procedure of *SAS*. Least significant differences (LSD, $\alpha = 0.05$) for comparing treatment means were calculated only for the variable with significant *F*-test for the main effects (UV-B radiation and temperature) and/or their interaction.

Results

Leaf photosynthesis and Chl fluorescence: Leaf photosynthesis exhibited a significant temperature × UV-B radiation interaction only at 25 DAE (Fig. 1*A*,*B*). At 25 DAE, 10 kJ UV-B mostly decreased P_N in both the uppermost, fully expanded leaves and older leaves with maximum percentage decrease (22%) in 30/22°C treatment (Fig. 1*B*). The UV-B radiation did not show a significant effect on P_N in 40-d-old plants, whereas, plants grown at higher temperatures exhibited 10% (30/22°C) and 6% (36/28°C) higher P_N as compared to the plants grown at 24/16°C treatments, when averaged across UV-B treatments (Fig. 1*C*). The effect of UV-B on F_v'/F_m' was only significant in older leaves at 36/28°C treatment (Fig. 1*E*).

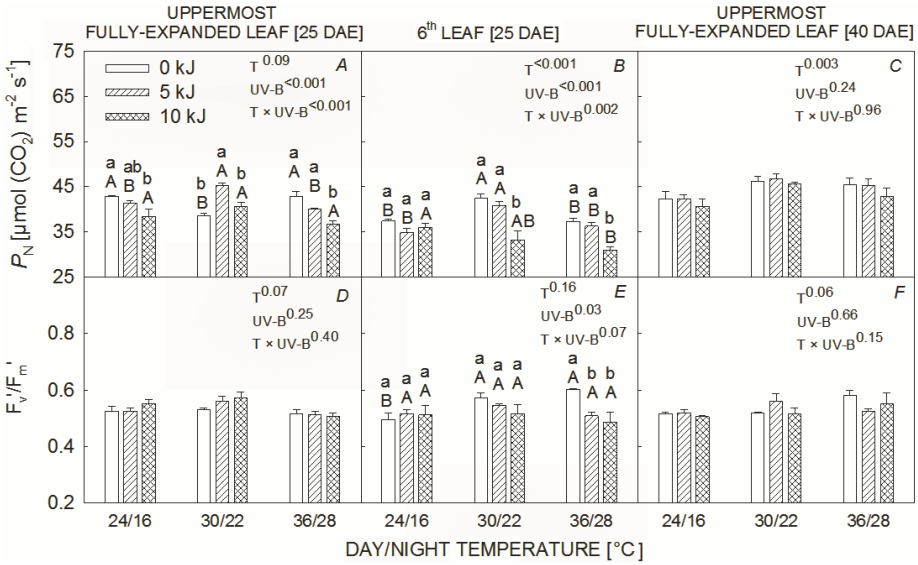

Fig. 1. The effect of temperature (T, day/night) and ultraviolet-B (UV-B) radiation on maize leaf photosynthesis (P_N) and chlorophyll fluorescence (F_v'/F_m') 25 and 40 d after emergence (DAE). The error bars show the SE from three replicates. The *P*-values of the two-way analysis of variance are shown. The letter grouping for means separation using LSD ($\alpha = 0.05$) were only shown when both the main effects (T and UV-B) and/or the interaction were significant ($P \leq 0.05$). The means are not significantly different when followed by *the same small letter* (for UV-B at a given temperature) and *capital letters* (for temperature at a given UV-B level) at $\alpha = 0.05$.

Leaf pigments and phenolics concentrations: The Chl concentration and Car content did not vary significantly among UV-B treatments (Fig. 2*A,B*). However, a significant temperature *vs*. UV-B radiation interaction was observed for phenolics concentration (Fig. 2*C*). Increase in temperature from 24/16°C caused a significant reduction in phenolic concentrations at a given UV-B radiation. In contrast, plants grown with UV-B radiation resulted in higher concentration of phenolic compounds as compared to the plants grown without UV-B radiation. The maximum percentage increase (51%) was observed at 10 kJ UV-B (24/16°C), whereas the minimum (10%) increase in phenolic concentration was recorded at 5 kJ UV-B (30/22°C) as compared with 0 kJ UV-B treatments at a given temperature.

Leaf membrane relative injury and leaf wax content: A significant temperature effect was observed for leaf membrane relative injury (RI) (Fig. 2*D*). Averaged over UV-B treatments, RI varied among temperature treatments as 33% (24/16°C), 21% (30/22°C), and 23% (36/28°C). The leaf epicuticular wax content exhibited a significant temperature *vs*. UV-B radiation interaction (Fig. 2*E*). Except for plants grown at 24/16°C treatment, increased UV-B radiation caused significant increase in wax content at other two temperatures. The highest percentage increase (143%) in wax concentration was found at 30/22°C (5 kJ UV-B) as compared with 0 kJ m^{-2} d^{-1} UV-B treatment. In plants grown without UV-B radiation, wax content decreased as growth temperature increased; 24/16°C (17.8 µg cm^{-2}), 30/22°C (8.2 µg cm^{-2}) and 36/28°C (6.5 µg cm^{-2}).

Plant growth and development: An exponential growth pattern was observed for PH, LA, and MSNN development during the course of experiment (Fig. 3). At the final harvest, PH and LA showed significant temperature *vs*. UV-B interaction (Table 2). However, MSNN was only affected by the temperature. Plants grown at 10 kJ UV-B combined with 36/28°C temperature recorded maximum decrease in final PH (36%) and MSNN (one node less) as compared to no UV-B treatment. Compared to the 0 kJ m^{-2} d^{-1} UV-B, the maximum decrease (22%) in LA was found at 10 kJ m^{-2} d^{-1} UV-B under 24/16°C temperature. Averaged across UV-B treatments, plants grown at 24/16°C temperature were the shortest with the smallest leaf area and fewer node numbers (Table 2, Fig. 3). The interaction terms were also significant for MSER and LAER (Table 2). Irrespective of the growth temperature, UV-B caused significant reduction in averaged MSER and LAER except at 30/22°C for LAER. The averaged NAR did not differ significantly among the UV-B-treated plants, however, they increased with temperature.

Total aboveground DM showed significant effects of temperature and UV-B treatments at all harvest dates (Table 3). A significant temperature *vs*. UV-B interaction was observed at the first 2 harvests but not at the 43 DAE. Plant DM were negatively affected by UV-B regardless of the temperature and harvest date. Compared to the 0 kJ m^{-2} d^{-1} UV-B-treated plants, 10 kJ m^{-2} d^{-1} treatments exhibited lower BAR regardless of the growth temperatures.

There was no significant temperature *vs*. UV-B interaction for leaf/stem DM ratio at all harvests (Fig. 4*A*,*C*). Irrespective of treatments, this ratio decreased

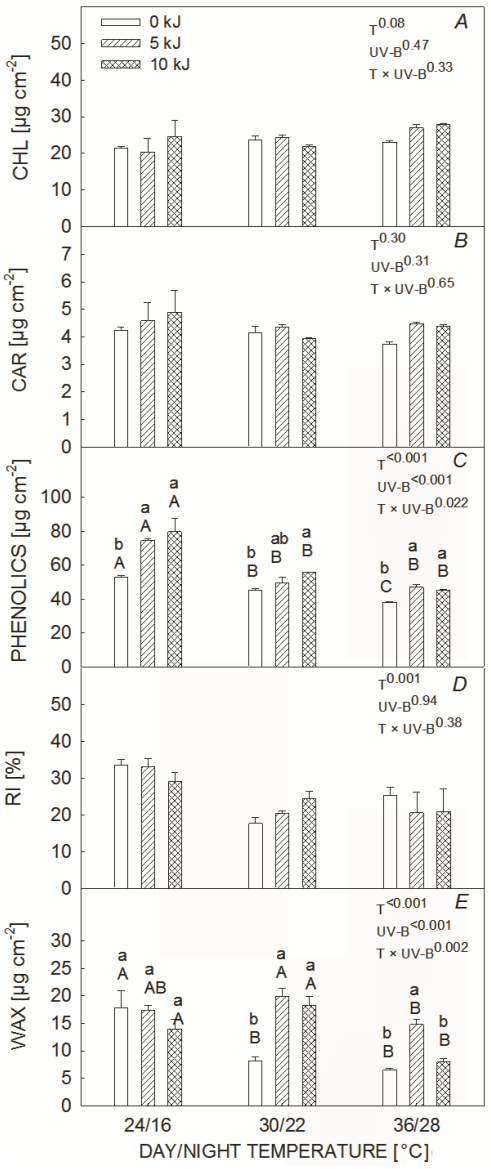

Fig. 2. The effect of temperature (T, day/night) and ultraviolet-B (UV-B) radiation on maize leaf chlorophyll concentration, carotenoids, phenolic concentration, percentage of leaf membrane relative injury (RI), and wax content 18 d after emergence. Other details are as in Fig. 1.

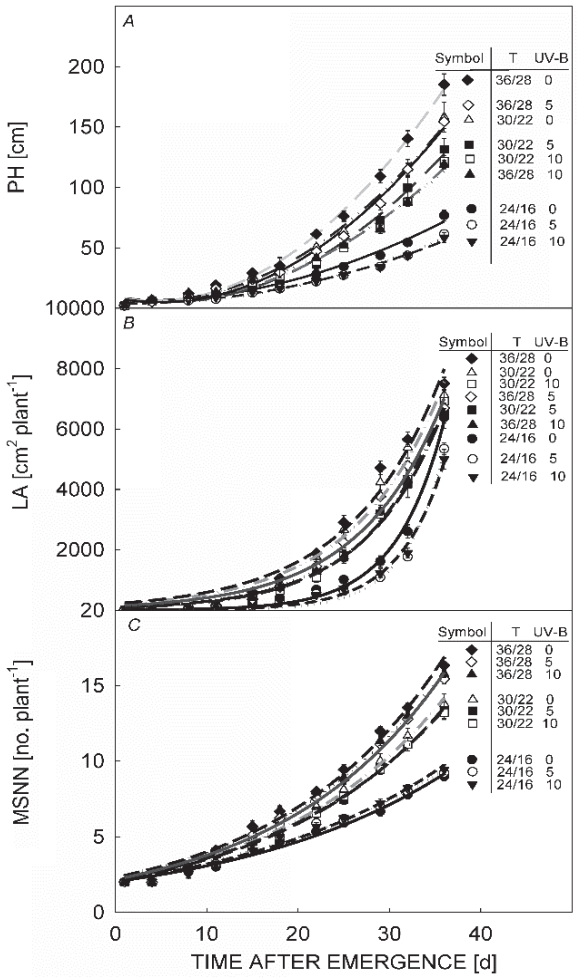

Fig. 3. The effect of temperature (T, day/night) and ultraviolet-B (UV-B) radiation on maize plant height (PH), leaf area (LA), and main stem node number (MSNN) during experiment. The error bars show the standard error from nine replicates. *Lines* are the fit of the exponential growth equations.

as the growth period advanced. At the final harvest, the UV-B did not affect leaf/stem ratio, whereas higher temperatures decreased the ratio. At the end of experiment, the LA distributions at the main stem nodes were still increasing at 24/16°C whereas, it started to decline at 9 and 10 node at 30/22°C and 36/28°C, respectively (Fig. 4*D*,*F*). Regardless of temperature treatments, plants grown without UV-B radiation tended to have larger LA mostly at all main stem nodes as compared to the plants grown with UV-B radiation treatment.

Discussion

Leaf photosynthetic responses: Leaf photosynthesis was sensitive to UV-B radiation, especially at the early stage of plant growth. However, this effect of enhanced UV-B was temperature-dependent. For instance, UV-B radiation caused 3–15% decrease in P_N on the uppermost, fully expanded leaves at 24/16°C and 36/28°C, but stimulated about 5–18% P_N at 30/22°C temperature. The older leaves (6^{th} leaf from the top) appeared to be more sensitive than uppermost leaves exhibiting a consistent reduction in P_N regardless of the temperature treatments. This may be attributed to the longer exposure of the older leaves to UV-B radiation as compared to the younger leaves contributing to early leaf senescence (Kakani *et al.* 2004, Reddy *et al.* 2004). The stimulation of ethylene evolution

Table 2. The growth parameters observed at the end of experiment for plant height (PH), leaf area (LA), main stem node number (MSNN), and their rates as mainstem elongation rate (MSER), leaf-area expansion rate (LAER), and node addition rate (NAR) in maize grown at 24/16, 30/22, and 36/28°C day/night temperatures each at 0, 5, and 10 kJ m^{-2} d^{-1} UV-B radiation treatments. Values are average of 9 measurements. The mean and least significant differences (LSD, α=0.05) along with the analysis of variance among temperature and UV-B radiation are also shown. *, ***significant at P<0.05, P<0.001, respectively; NS indicate not significant (P>0.05). † – within columns, means followed by the same small letters and within rows means followed by the same capital letter are not significantly different at P = 0.05.

	PH [cm plant^{-1}]			MSER [cm plant^{-1} d^{-1}]			LA [cm^2 plant^{-1}]			LAER [cm^2 plant^{-1} d^{-1}]			MSNN [no. plant^{-1}]			NAR [no. plant^{-1} d^{-1}]		
UV-B	24/16	30/22	36/28	24/16	30/22	36/28	24/16	30/22	36/28	24/16	30/22	36/28	24/16	30/22	36/28	24/16	30/22	36/28
0	76.8^{aC}†	158.0^{aB}	185.2^{aA}	3.42^{aC}	7.59^{aB}	8.7^{aA}	6378^{aB}	7122^{aA}	7503^{aA}	384^{aA}	378^{aA}	392^{aA}	9	13.8	16.3	0.26	0.48	0.58
5	61.4^{bC}	131.5^{bB}	154.5^{bA}	2.77^{bC}	6.79^{abB}	7.56^{bA}	5342^{bB}	6560^{aA}	6687^{bA}	316^{bB}	364^{aA}	365^{abA}	9.2	13.3	15.4	0.23	0.47	0.55
10	58.5^{bB}	121.7^{bA}	119.0^{cA}	2.54^{bB}	5.89^{bA}	5.53^{cA}	5000^{bB}	6915^{aA}	6472^{bA}	299^{bC}	404^{aA}	356^{bB}	9.4	13.2	15.8	0.27	0.47	0.54
Mean	65.6^{C}	137.1^{B}	152.9^{A}	2.91^{C}	6.76^{B}	7.27^{A}	5574^{B}	6866^{A}	6887^{A}	333^{B}	382^{A}	371^{A}	9.2^{C}	13.4^{B}	15.9^{A}	0.26^{C}	0.47^{B}	0.56^{A}
LSD	6.64	15.48	10.87	0.42	1.00	0.62	507.91	589.49	328.98	35.04	42.11	28.02	0.477	0.955	0.621	0.26	0.48	0.58
T	***			***			***			***			***			***		
UV-B	***			***			***			***			NS			NS		
T × UV-B	***			***			*			***			NS			NS		

from leaves under high UV-B radiation may also contribute to the leaf senescence processes causing lower photosynthetic activity (Qaderi *et al.* 2010). Prior studies have also reported stimulating (Mark and Tevini 1997) or reducing (Correia *et al.* 1999) effects of UV-B radiation on maize photosynthesis. The UV-B mediated changes in P_N were not associated with the changes observed in F_v'/F_m' under the same treatment conditions. These results agree with the other studies and support the view questioning the key role of PSII functioning in response to UV-B radiation (Nogués and Baker 1995, Singh *et al.* 2008b). While the physiobiochemical mechanisms are not clearly understood, previous studies and reviews indicated that UV-B radiation can induce a decline in the light-saturated photosynthetic capacity without major inhibition of the PSII efficiency. Among the contributing factors, this UV-B-mediated photosynthetic inhibition may partly be attributed to the decline in the ribulose-1,5-bisphosphate regeneration and the loss in some Calvin cycle enzymes (reviewed by Allen *et al.* 1998). Averaged across UV-B radiation levels, the increased P_N at higher temperature especially at 30/22°C was in accordance with the reported optimum temperature (31–34°C) for photosynthesis in maize (Tollenaar 1989b, Kim *et al.* 2007).

Leaf pigments, phenolics, and waxes: The concentrations of Chl and Car in maize leaves were not significantly influenced by either UV-B radiation or temperature treatments. Previous studies also reported no significant change, an increase or decrease in Chl concentration under UV-B radiation or temperature treatments in maize and other species, which may be attributed to the variation in total leaf area and leaf thickness (Mark and Tevini 1997, Kakani *et al.* 2004, Hectors *et al.* 2007). Mark and Tevini (1997) reported the increase in leaf area-based Chl concentration under UV-B-exposed maize plants. However, the total Chl concentration per plant basis was reduced. The small or significant changes in the Chl concentration were also reported depending upon the leaf position and UV-B or temperature treatments (Mark and Tevini 1997, Kakani *et al.* 2004).

In general, similarly to the observations made in this study, UV-B radiation stimulates biosynthesis of phenolic compounds and waxes in leaves as a protective response of plants (Caldwell *et al.* 1983, Mark *et al.* 1996, Rozema *et al.* 1997, Casati and Walbot 2003, Gao *et al.* 2004, Hectors *et al.* 2007, Koti *et al.* 2007). Casati and Walbot (2003) found that maize line lacking in UV-B-absorbing compounds was more dramatically affected by UV-B radiation, confirming shielding role of these compounds. The epicuticular wax protects plant by physically avoiding the entry of UV-B radiation into the leaf internal tissues. On the contrary, the increased temperature tended to suppress both phenolics and waxes particularly in the leaves not exposed to UV-B radiations. Similar observations were also reported in other crops (Koti *et al.* 2007, Singh *et al.* 2010). However, this response was

Table 3. Total aboveground biomass for three harvest dates corresponding to 15, 23, and 43 d after emergence (DAE) and biomass accumulation rate (BAR) in maize grown at 24/16, 30/22, and 36/28°C day/night temperatures each at 0, 5, and 10 kJ m^{-2} d^{-1} UV-B radiation. Values are averages of 30 (15 DAE), 10 (23 DAE), and 15 (43 DAE) plants. The mean and least significant differences (LSD, α=0.05) along with the analysis of variance among temperature and UV-B radiation are also provided. **, ***significant at $P<0.01$, $P<0.001$, respectively; NS – not significant ($P>0.05$). † Within columns, means followed by *the same small letters* and within rows means followed by *the same capital letters* are not significantly different at $P = 0.05$. Analysis of variance was not performed for BAR.

	Total aboveground biomass [g $plant^{-1}$]									BAR [g $plant^{-1}$ d^{-1}]		
	15 DAE			23 DAE			43 DAE					
UV-B	24/16	30/22	36/28	24/16	30/22	36/28	24/16	30/22	36/28	24/16	30/22	36/28
0	2.09^{aC}†	4.63^{aB}	5.12^{aA}	7.69^{aC}	19.1^{aB}	21.5^{aA}	79.2^{aB}	111.9^{aA}	111.4^{aA}	2.74^{aB}	3.82^{aA}	3.79^{aA}
5	1.61^{bC}	2.63^{cB}	3.23^{cA}	6.26^{bB}	11.8^{bA}	13.5^{bA}	74.2^{aB}	104.3^{aA}	109.2^{aA}	2.58^{aB}	3.62^{abA}	3.78^{aA}
10	1.65^{bC}	3.26^{bB}	3.96^{bA}	7.01^{abB}	12.9^{bA}	14.1^{bA}	72.8^{aC}	98.6^{aB}	86.2^{bA}	2.53^{aB}	3.39^{bA}	2.93^{bB}
Mean	1.78^{C}	3.51^{B}	4.10^{A}	6.99^{C}	14.6^{B}	16.3^{A}	75.4^{B}	104.9^{A}	102.3^{A}	2.62^{B}	3.61^{A}	3.5^{A}
LSD	0.21	0.45	0.50	1.02	2.4	2.2	14.4	19.7	12.2	–	–	–
T	***			***			***				–	
UV-B	***			***			**				–	
T×UV-B	***			***			NS				–	

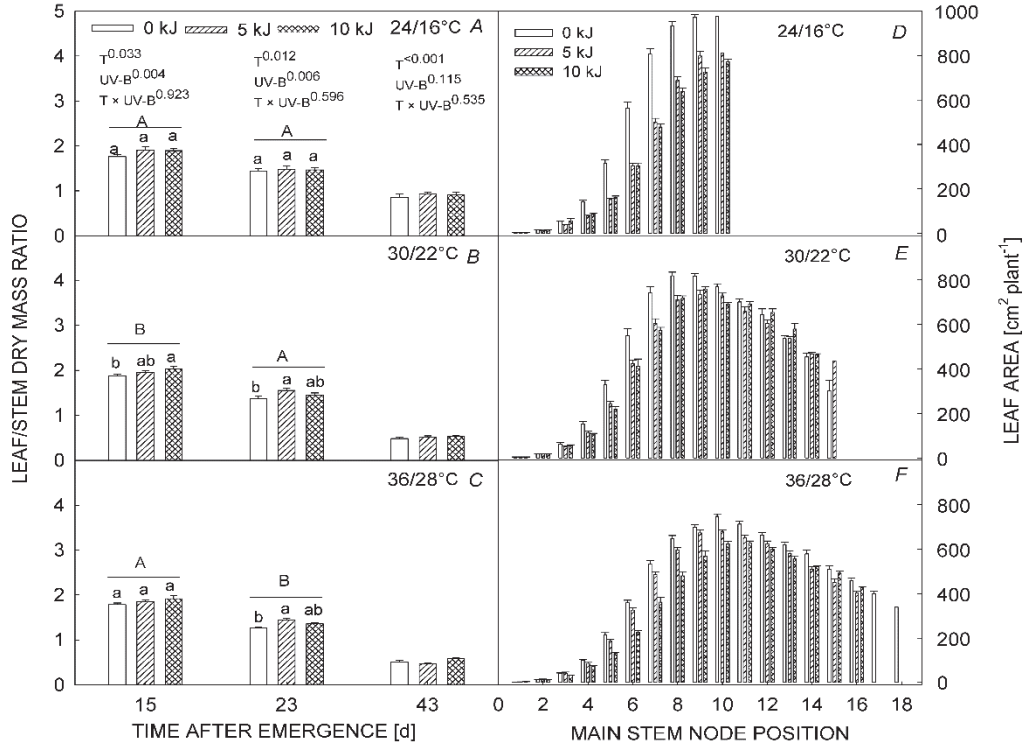

Fig. 4. Changes in leaf to stem dry mass ratio over time (*A–C*) and in individual leaf area by main stem node positions (*D–F*) as affected by temperature (T, day/night) and ultraviolet-B (UV-B) radiation in maize. The error bars (*A–C*) show the SE from 30 (15 d after emergence, DAE), 10 (23 DAE) and 15 (43 DAE) plants measured at each harvest. The error bars (*D–F*) show the SE from 9 plants. The *ANOVA* is performed for each day separately. Other details are as in Fig. 1.

dramatically altered in the presence of UV-B radiation. For instance, UV-B stimulated biosynthesis of phenolics at all temperatures, in a manner of lower percentage increase as temperature treatments, increased from 24/16 to 36/28°C, indicating reduction in the UV-B protection by phenolics as temperature increases. In contrast, biosynthesis of waxes was not stimulated by UV-B treatment at low temperature (24/16°C), but it was greatly enhanced at higher temperatures. The decrease in leaf membrane relative injury at higher temperatures might be attributed to the plant acclimation to higher growth temperatures as observed in other studies (Singh *et al.* 2010).

Growth and development: Plant growth parameters were highly sensitive to both, UV-B radiation and temperature. Irrespective of the temperature treatments, UV-B radiation

decreased many growth parameters with greater effects at the highest temperature treatments. The reduced total DM was primarily caused by a lower plant stature due to slow rate of stem elongation and leaf area expansion, and lower LA without a significant effect on leaf/stem ratio. Moreover, UV-B radiation affected LA in similar way at all main stem nodes regardless of the temperature. UV-B-mediated decreases in these parameters were also reported in other studies (Mark and Tevini 1997, Reddy *et al.* 2003, 2004; Singh *et al.* 2008b). Correia *et al*. (1998) also reported a decrease in plant DM under UV-B radiation due to reduction in LA, leaf duration, and photosynthesis.

Among the measured growth rates, the MSER exhibited the greatest reduction in response to the UV-B radiation at all temperatures. Maize PH was reported to be highly sensitive to UV-B radiation at early stages of development (Mark and Tevini 1997, Yin and Wang 2012). The mechanisms of reduction in stem elongation and leaf expansion under UV-B radiation are suggested due to alteration in phytohormone metabolism, primarily auxins (Ros and Tevini 1995, Li *et al.* 2010), decrease in cell wall loosening (Qu *et al.* 2006), and downregulation of genes participating in the biosynthesis of auxins (Hectors *et al.* 2007). Auxins play important roles in plant developmental processes, such as apical dominance and stem elongation. Hectors *et al*. (2007) found that several genes involved in auxin biosynthesis pathway, such as nitrilases *NIT3* and *NIT1*, were downregulated in UV-B-acclimated plants. Also, the accumulation of phenolic compounds as observed in this study may induce catabolism of auxins and their transport (Jansen 2002).

In summary, the interaction between temperature and UV-B radiation was significant for many growth and developmental processes. The effect of temperature was significant for almost all the parameters studied. Among the various growth processes, stem elongation rate and leaf area expansion rate were the processes most sensitive to UV-B radiation. A growth temperature either above or below 30/22°C reduced the averaged leaf photosynthesis. The UV-B radiation also inhibited photosynthetic processes especially at early stage of plant growth and in older leaves. Smaller plants and reduced leaf area mainly resulted in lower biomass in UV-B treated plants. The results from this study indicated that maize vegetative growth and developmental processes (MSER, LAER, and total DM) were more sensitive to UV-B radiation levels compared to the photosynthetic processes. Moreover, the observed UV-B protection mechanisms, such as accumulation of phenolics and waxes, exhibited a significant interaction among the treatments, where these compounds were relatively less responsive (phenolics) or more responsive (waxes) to UV-B radiation at higher temperature treatments or *vice versa*. The 10 kJ m^{-2} d^{-1} UV-B radiation treatments in association with higher temperature appeared to have greater negative effect on maize growth, development, and photosynthetic processes. These results corroborate that maize growth and development are sensitive to both current and projected UV-B radiation and growth temperature has a profound impact on maize sensitivity to the natural UV-B radiation.

References

Allen, D.J., Nogues, S., Baker, N.R.: Ozone depletion and increased UV-B radiation: Is there a real threat to photosynthesis? – J. Exp. Bot. **49**: 1775-1788, 1998.

Ballare, C.L., Caldwell, M.M., Flint, S.D., Robinson, A., Bornman, J.F.: Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. – Photoch. Photobio. Sci. **10**: 226-241, 2011.

Caldwell, M.M., Robberecht, R.D., Flint, S.: Internal filters: prospects for UV-acclimation in higher plants. – Physiol. Plantarum **58**: 445-450, 1983.

Casati, P., Walbot, V.: Gene expression profiling in response to ultraviolet radiation in maize genotypes with varying flavonoid content. – Plant Physiol. **132**: 1739-1754, 2003.

Correia, C.M., Areal, E.L.V., Torres-Pereira, M.S., Torres-Pereira, J.M.G.: Intraspecific variation in sensitivity to ultraviolet-B radiation in maize grown under field conditions. I. Growth and morphological aspects. – Field Crop. Res. **59**: 81-89, 1998.

Correia, C.M., Areal, E.L.V., Torres-Pereira, M.S., Torres-Pereira, J.M.G.: Intraspecific variation in sensitivity to ultraviolet-B radiation in maize grown under field conditions: II. Physiological and biochemical aspects. – Field Crop. Res. **62**: 97-105, 1999.

Ebercon, A., Blum, A., Jordan, W.R.: A rapid colorimetric method for epicuticular wax content of sorghum leaves. – Crop Sci. **17**: 179-180, 1977.

FAO, 2011: Food and Agriculture Organization of United Nations (online). http://faostat3.fao.org/home/index.html #DOWNLOAD. FAO, Rome, Italy.

Fleisher, D.H., Timlin, D.J., Yang, Y., Reddy, V.R., Reddy, K.R.: Uniformity of soil-plant-atmosphere-research chambers. – T. ASABE **52**: 1721-1731, 2009.

Gao, W., Zheng, Y.F., Slusser, J.R., *et al*.: Effects of supplementary ultraviolet-B irradiance on maize yield and qualities: A field experiment. – Photochem. Photobiol. **80**: 127-131, 2004.

Genty, B., Briantais, J.M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Hectors, K., Prinsen, E., De Coen, W., Jansen, M.A.K., Guisez, Y.: *Arabidopsis thaliana* plants acclimated to low dose rates of ultraviolet B radiation show specific changes in morphology and gene expression in the absence of stress symptoms. – New Phytol. **175**: 255-270, 2007.

IPCC. Climate Change 2007: The Physical Science Basis. - In.: Solomon, S., Qin, D., Manning, M. *et al*. (ed.): Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Pp- 999. Cambridge University Press, Cambridge 2007.

Jansen, M.A.K.: Ultraviolet-B radiation effects on plants:

induction of morphogenic responses. – Physiol. Plantarum **116**: 423-429, 2002.

Kakani, V.G., Reddy, K.R., Zhao, D., Gao, W.: Senescence and hyperspectral reflectance of cotton leaves exposed to ultraviolet-B radiation and carbon dioxide. – Physiol. Plantarum **121**: 250-257, 2004.

Kim, S.H., Gitz, D.C., Sicherb, R.C., *et al*.: Temperature dependence of growth, development, and photosynthesis in maize under elevated CO_2. – Environ. Exp. Bot. **61**: 224-236, 2007.

Koti, S., Reddy, K.R., Kakani, V.G., Zhao, D., Gao, W.: Effects of carbon dioxide, temperature and ultraviolet-B radiation and their interactions on soybean (*Glycine max* L.) growth and development. – Environ. Exp. Bot. **60**: 1-10, 2007.

Li, Y., He, L., Zu, Y.: Intraspecific variation in sensitivity to ultraviolet-B radiation in endogenous hormones and photosynthetic characteristics of 10 wheat cultivars grown under field conditions. – S. Afr. J. Bot. **76**: 493-498, 2010.

Lichtenthaler, H.K.: Chlorophylls and carotenoids: Pigments of photosynthesis. – Method. Enzymol. **148**: 350-382, 1987.

Lobell, D.B., Burke, M.B., Tebaldi, C., *et al*.: Prioritizing climate change adaptation needs for food security in 2030. – Science **319**: 607-610, 2008.

Mark, U., Saile-Mark, M., Tevini, M.: Effects of solar UVB radiation on growth, flowering and yield of central and southern european maize cultivars (*Zea mays* L.). – Photochem. Photobiol. **64**: 457-463, 1996.

Mark, U., Tevini, M.: Effects of solar ultraviolet-B radiation, temperature and CO_2 on growth and physiology of sunflower and maize seedlings. – Plant Ecol. **128**: 224-234, 1997.

Martineau, J.R., Williams, J.H., Specht, J.E.: Tolerance in soybean. II. Evaluation of segregating populations for membrane thermostability. – Crop Sci. **19**: 79-81, 1979.

McKenzie, R.L., Aucamp, P.J., Bais, A.F., Björn, L.O., Ilyas, M.: Changes in biologically active ultraviolet radiation reaching the Earth's surface. – Photoch. Photobio. Sci. **6**: 218-231, 2007.

Mercier, J., Baka, M., Reddy, B., Corcuff, R., Arul, J.: Shortwave ultraviolet irradiation for control of decay caused by *Botrytis cinerea* in bell pepper: Induced resistance and germicidal effects. – J. Am. Soc. Hortic. Sci. **126**: 128-133, 2001.

Mittler, R.: Abiotic stress, the field environment and stress combination. – Trends Plant Sci. **11**: 15-19, 2006.

Nogués, S., Baker, N.R.: Evaluation of the role of damage to photosystem II in the inhibition of CO_2 assimilation in pea leaves on exposure to UV-B radiation. – Plant Cell Environ. **18**: 781-787, 1995.

Qaderi, M. M., Basraon, N. K., Chinnappa, C.C., Reid, D. M.: Combined effects of temperature, ultraviolet-B radiation, and watering regime on growth and physiological processes in canola (*Brassica napus*) seedlings. – Int. J. Plant Sci. **171**: 466-481, 2010.

Qu, Y., Feng, H.Y., Wang, Y.B., *et al*.: Nitric oxide functions as a signal in ultraviolet-B induced inhibition of pea stems elongation. – Plant Sci. **170**: 994-1000, 2006.

Reddy, K.R., Hodges, H.F., Read, J.J., *et al*.: Soil-Plant-Atmosphere-Research (SPAR) facility: A tool for plant research and modeling. – Biotronics **30**: 27-50, 2001.

Reddy, K.R., Kakani, V.G., Zhao, D., Mohammed, A.R., Gao, W.: Cotton responses to ultraviolet-B radiation: experimentation and algorithm development. – Agr. Forest. Meteorol. **120**: 249-265, 2003.

Reddy, K.R., Kakani, V.G., Zhao, D., Koti, S., Gao, W.: Interactive effects of ultraviolet-B radiation and temperature on cotton physiology, growth, development and hyperspectral reflectance. – Photochem. Photobiol. **79**: 416-427, 2004.

Reddy, K.R., Singh, S.K., Koti, S., *et al*.: Quantifying corn growth and physiological responses to ultraviolet-B radiation for modeling. – Agron. J. **105**: 1367-1377, 2013.

Ros, J., Tevini, M.: UV-radiation and indole-3-acetic acid: Interactions during growth of seedlings and hypocotyl segments of sunflower. – J. Plant Physiol. **146**: 295-302, 1995.

Rozema, J., van de Staaij, J., Björn, L.O., Caldwell, M.: UV-B as an environmental factor in plant life: stress and regulation. – Trends Ecol. Evol. **12**: 22-28, 1997.

Singh, S.K., Kakani, V.G., Brand, D., Baldwin, B., Reddy, K.R.: Assessment of cold and heat tolerance of winter-grown canola (*Brassica napus* L.) cultivars by pollen-based parameters. – J. Agron. Crop Sci. **194**: 225-236, 2008a.

Singh, S.K., Surabhi, G.-K., Gao, W., Reddy, K.R.: Assessing genotypic variability of cowpea (*Vigna unguiculata* [L.] Walp.) to current and projected ultraviolet-B radiation. – J. Photoch. Photobio. B **93**: 71-81, 2008b.

Singh, S.K., Kakani, V.G., Surabhi, G.K., Reddy, K.R.: Cowpea (*Vigna unguiculata* [L.] Walp.) genotypes response to multiple abiotic stresses. – J. Photoch. Photobio. B **100**: 135-146, 2010.

Singh, S.K., Reddy, K.R.: Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (*Vigna unguiculata* [L.] Walp.) under drought. – J. Photoch. Photobio. B **105**: 40-50, 2011.

Singh, S.K., Badgujar, G., Reddy, V.R., Fleisher, D.H., Bunce, J.A.: Carbon dioxide diffusion across stomata and mesophyll and photobiochemical processes as affected by growth CO_2 and phosphorus nutrition in cotton. – J. Plant Physiol. **170**: 801-813, 2013.

Tao, F., Zhang, Z.: Impacts of climate change as a function of global mean temperature: Maize productivity and water use in China. – Climatic Change **105**: 409-432, 2011.

Teramura, A.H.: Effects of ultraviolet-B radiation on the growth and yield of crop plants. – Physiol. Plantarum **58**: 415-427, 1983.

Teramura, A.H., Sullivan, J.H., Lydon, J.: Effects of UV-B radiation on soybean yield and seed quality: a 6-year field study. – Physiol. Plantarum **80**: 5-11, 1990.

Tollenaar, M.: Response of dry matter accumulation in maize to temperature: I. Dry matter partitioning. – Crop Sci. **29**: 1239-1246, 1989a.

Tollenaar, M.: Response of dry matter accumulation in maize to temperature: II. Leaf photosynthesis. – Crop Sci. **29**: 1275-1279, 1989b.

Yin, L.N., Wang, S.W.: Modulated increased UV-B radiation affects crop growth and grain yield and quality of maize in the field. – Photosynthetica **50**: 595-601, 2012.

Zhao, D., Reddy, K.R., Kakani, V.G., Read, J.J., Sullivan, J.H.: Growth and physiological responses of cotton (*Gossypium hirsutum* L.) to elevated carbon dioxide and ultraviolet-B radiation under controlled environmental conditions. – Plant Cell Environ. **26**: 771-782, 2003.