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RESEARCH PAPER

Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths

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Abstract

Plant reproduction is highly vulnerable to global climate change components such as carbon dioxide concentration ($[CO_2]$), temperature (T), and ultraviolet-B (UV-B) radiation. The objectives of this study were to determine the effects of season-long exposure to treatments of $[CO_2]$ at 360 (control) and 720 μmol mol^{-1} (+CO_2), temperature at 30/22 °C (control) and 38/30 °C (+T) and UV-B radiation 0 (control) and 10 kJ m^{-2} d^{-1} (+UV-B) on flower and pollen morphology, pollen production, germination, and tube lengths of six soybean genotypes (D 88-5320, D 90-9216, Stalwart III, PI 471938, DG 5630RR, and DP 4933RR) in sunlit, controlled environment chambers. The control treatment had 360 μmol mol^{-1} $[CO_2]$ at 30/22 °C and 0 kJ UV-B. Plants grown either at +UV-B or +T, alone or in combination, produced smaller flowers with shorter standard petal and staminal column lengths. Flowers so produced had less pollen with poor pollen germination and shorter tube lengths. Pollen produced by the flowers of these plants appeared shrivelled without apertures and with disturbed exine ornamentation even at +CO_2 conditions. The damaging effects of +T and +UV-B were not ameliorated by +CO_2 conditions. Based on the total stress response index (TSRI), pooled individual component responses over all the treatments, the genotypes were classified as tolerant (DG 5630RR, D 88-5320: TSRI >−790), intermediate (D 90-9216, PI 471938: TSRI <−790 to >−1026), and sensitive (Stalwart III, DP 4933RR: TSRI <−1026). The differences in sensitivity identified among genotypes imply the options for selecting genotypes with tolerance to environmental stresses projected to occur in the future climates.

Key words: Carbon dioxide, pollen germination, pollen tube length, soybean, temperature, ultraviolet-B radiation.

Introduction

Reproduction plays an important role in the survival and succession of seeded crop plants. The onset of the reproductive phase, its duration, and the quality and quantity of reproductive products are regulated by abiotic factors. Of the various abiotic factors, atmospheric CO_2 concentration ($[CO_2]$), temperature, and ultraviolet-B (UV-B) radiation are projected to change in the near future. Current $[CO_2]$ of 360 μmol mol^{-1} could reach anywhere between 560 and 970 μmol mol^{-1} by the middle or later part of the 21st century (Houghton *et al*., 2001). As a consequence of increased $[CO_2]$, the projected increase in global mean air temperature could range from 1.4 °C to 5.8 °C by 2100 (Houghton *et al*., 2001). Global terrestrial UV-B radiation levels range between 2 kJ and 12 kJ m^{-2} d^{-1} on a given day with near equator and mid-latitudes receiving higher doses (Total ozone mapping spectrometer 2002, http://toms.gsfc.nasa.gov/ery-uv/euv.html). This includes the 6–14% increase of UV-B radiation since the 1970s at the Earth's

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surface due to chlorofluorocarbons released into the atmosphere during the past few decades (UNEP, 2002). Relative to the 1979–1992 conditions, for the 2010–2020 time period, the GISS model results indicate a springtime enhancement of erythemal UV doses of up to 14% in the Northern hemisphere and 40% in the Southern hemisphere (Taalas *et al*., 2000).

Interactions between these climate stress factors may exacerbate the rate and direction of individual climate stress factors and their effects on terrestrial ecosystems. The yield increases experienced at elevated [CO_2] (Reddy and Hodges, 2000, and references therein) were not observed when the plants were grown in combination with high temperature (Reddy *et al*., 1997; Wheeler *et al*., 2000; Prasad *et al*., 2003) or increased UV-B radiation (Teramura *et al*., 1990; Gwynn-Jones *et al*., 1997; Sullivan, 1997; Tosserams *et al*., 2001; Zhao *et al*., 2003). The resulting yield decrease due to high temperature and UV-B radiation could be due to the effect on reproduction at both organ and process levels. Recent *in vitro* studies showed that high temperature and elevated UV-B radiation inhibit pollen germination and pollen tube growth, and genotypes differ in their sensitivity (Huan *et al*., 2000; Kakani *et al*., 2002).

The climate change factors being tested in this study modify reproductive organs and processes. Elevated [CO_2] and high temperature increased flower production in soybean (Nakamoto *et al*., 2001; Zheng *et al*., 2002) while elevated UV-B radiation altered cotton flower morphology, producing smaller flowers with fewer anthers (Kakani *et al*., 2003). Elevated [CO_2] (720 μmol mol^{-1}) increased stand-level ragweed-pollen production by 61% (Wayne *et al*., 2002), but did not affect peanut pollen viability (Prasad *et al*., 2003). By contrast, high temperature reduced pollen viability in peanut (Prasad *et al*., 2003). Similarly, both high temperatures (36 °C and 40 °C) and high doses of UV-B irradiation (900–1500 μW cm^{-2}) reduced pollen viability in tall fescue (Wang *et al*., 2004). However, studies with *Ipomea purpurea* suggested that flavonoids (compounds that block UV-B radiation) alleviate the effect of heat stress on fertilization success (Coberly and Rausher, 2003). Therefore, studies are needed to provide insights into understanding and evaluating the reproductive performance of plants, so that suitable genotypes and management practices can be developed for future climates.

In reality, plants in nature are exposed to multiple environmental conditions concomitantly, and their performance can be assessed only when plants are grown in multiple stress conditions. The objectives of the research were to determine the interactive effects of atmospheric [CO_2], temperature, and UV-B radiation on flower and pollen morphology, pollen production, pollen germination, and pollen tube lengths of soybean and to understand genotypic variation, if any, in the soybean response to multiple environmental stresses.

Materials and methods

Experimental conditions, cultivars and plant husbandry

The study was conducted in eight sunlit Soil-Plant-Atmosphere-Research (SPAR) chambers located at the RR Foil Plant Science Research Facility of Mississippi State University (33°28′ N, 88°47′ W), Mississippi, USA in 2003. Each SPAR chamber consists of an upper Plexiglas unit that is 2.5 m tall by 2 m long by 1.5 m wide and a lower steel soil bin of 1 m deep by 2 m long by 0.5 m wide. The Plexiglas blocks solar UV radiation wavelengths below 385 nm, but transmits 96.6±0.5% of incoming PAR (wavelength 400–700 nm) (Zhao *et al*., 2003). During the experiment, the ambient solar radiation (285–2800 nm) measured with a pyranometer (Model 4–48, The Eppley Laboratory Inc., Newport, Rhode Island, USA) was 21.2±0.5 MJ m^{-2} d^{-1}. Each SPAR unit consists of a heating and a cooling system, and an environment monitoring and control system. The SPAR facility has the capability to control temperature and [CO_2] precisely at predetermined set points for plant growth studies under near ambient levels of photosynthetically active radiation (PAR). Details of the operation and control of SPAR chambers have been described by Reddy *et al*. (1992, 2001).

Six soybean genotypes, Delsoy (D) 88-5320 (maturity group VI, non-glyphosate-tolerant), and D 90-9216 (maturity group VII, non-glyphosate-tolerant), Stalwart III (maturity group III, non-glyphosate-tolerant), Plant Introduction (PI) 471938 (maturity group V, non-glyphosate-tolerant), Deltagrow (DG) 5630RR (maturity group V, glyphosate-tolerant), and Deltapine (DP) 4933RR (maturity group IV, glyphosate-tolerant) were sown on 5 August 2003 in pots filled with fine sand. Thirty 2.5 l pots (five pots for each genotype) were arranged randomly in each SPAR chamber. Plants were watered three times a day with half-strength Hoagland's nutrient solution (Hewitt, 1952) delivered at 08.00, 12.00, and 16.00 h to ensure favourable nutrient and water conditions for plant growth through an automated and computer-controlled drip system. Variable-density black shade cloths around the edges of plants were adjusted regularly to match plant height in order to simulate natural shading in the presence of other plants.

Treatments

The chambers were maintained at temperatures of 30/22 °C (day/night) up to 10 d after sowing (DAS). Thereafter, each chamber was set at one of the eight treatments until 60 DAS. The treatments included combinations of two CO_2 levels of 360 μmol mol^{-1} and 720 μmol mol^{-1} (+CO_2), two levels of temperature 30/22 °C and 38/30 °C (+T) and two daily biologically effective UV-B radiation intensities of 0 and 10 (+UV-B) kJ m^{-2} d^{-1}. Control treatment consists of 30/22 °C, 360 μmol mol^{-1} [CO_2], and 0 kJ UV-B treatments. Air temperature in each SPAR chamber was monitored and adjusted every 10 s throughout the day and night and maintained within ±0.5 °C of the treatment set points measured with shielded, aspirated thermocouples. The daytime temperature was initiated at sunrise and returned to the night-time temperature 1 h after sunset. Constant humidity was maintained by operating solenoid valves that injected chilled water through the cooling coils located outside the air handler of each chamber. These cooling coils condensed excess water vapour from the chamber air in order to regulate the relative humidity at 60% (McKinion and Hodges, 1985).

The chamber [CO_2] was measured with a dedicated infrared gas analyser (Li-Cor, model LI-6252, Lincoln, Nebraska, USA) from the gas sample that was drawn through the lines run underground from SPAR units to the field laboratory building. Moisture was removed from the gas sample by running the sample lines through a refrigerated water tap (4 °C) that was automatically drained and through a column of magnesium perchlorate. Chamber [CO_2] was maintained by supplying pure CO_2 from a compressed gas cylinder through a system

that includes a pressure regulator, solenoid and needle valves, and a calibrated flow meter (Reddy *et al*., 2001).

Square-wave UV-B supplementation systems were used to provide the required UV-B radiation in this study under near ambient PAR. The UV-B radiation was delivered to plants for 8 h d^{-1}, from 08.00 h to 16.00 h by eight fluorescent UV-313 lamps (Q-Panel Company, Cleveland, Ohio, USA) powered by 40 W variable dimming ballasts. The lamps were wrapped with presolarized 0.07 mm cellulose diacetate film to filter UV-C (<280 nm) radiation. The cellulose diacetate film was changed at 3–4 d intervals. The UV-B energy delivered at the top of the plant canopy was checked daily at 09.00 h with a UVX digital radiometer (UVP Inc., San Gabriel, California, USA) and calibrated against an Optronic Laboratory (Orlando, Florida, USA) Model 754 Spectroradiometer, which was used initially to quantify lamp output. The lamp output was adjusted, as needed, to maintain the respective UV-B radiation levels. A distance of 0.5 m from lamps to the top of plants was always maintained throughout the experiment. The actual biologically effective UV-B radiation was measured during the crop growth period at six different locations in each SPAR unit corresponding to the pots arranged in rows. The weighted total biologically effective UV-B radiation levels received at the top of the plants were 9.8±0.16 for +UV-B, 9.6±0.07 for +T+UV-B, 9.6±0.07 for +CO_2+UV-B, and 9.5±0.10 kJ m^{-2} d^{-1} for +CO_2+T+UV-B treatments using the generalized plant response action spectrum normalized at 300 nm (Caldwell, 1971).

Measurement of floral morphology

Soybean, belonging to the family Fabaceae and subfamily Papilionoideae, has a flower with a tubular calyx of five unequal sepal lobes and a five-parted corolla consisting of a posterior standard petal, two lateral wing petals, and two anterior keel petals in contact with each other but not fused (Carlson and Larsten, 1987). Lengths of flower, standard petal, and staminal column were measured on 20 fresh flowers randomly picked from five plants of each genotype, 60 DAS. Flower length was measured from the tip of the standard petal to the base of the calyx. The standard petal was stretched out before measuring the length, and the length was measured from the point of insertion to the distal end. The staminal column was separated from the flower and the length was measured.

Measurement of pollen number

Mature anthers were collected from five different flowers from five plants a day before anthesis to determine the number of pollen grains produced per anther, 60 DAS. Pollen number was counted by placing a single anther in a water drop on a glass slide and squashed with a needle, and the pollen grains dispersed in the drop of water were counted (Bennett, 1999). The number of pollen grains from five anthers per genotype under each treatment was counted.

Measurement of pollen germination and pollen tube lengths

Pollen germination was determined through *in vitro* pollen germination on a medium consisting of 15 g sucrose ($C_{12}H_{22}O_{11}$), 0.03 g calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$], and 0.01 g boric acid (H_3BO_3) dissolved in 100 ml of deionized water (Gwata *et al*., 2003, with modifications). To this liquid medium, 0.6 g agar was added and slowly heated on a hot plate. After the agar was completely dissolved, 10 ml of the germinating medium was poured into five Petri dishes for each genotype in each treatment and allowed to cool for about 15 min so that the agar would solidify. Soybean flowers were randomly collected from five plants in each genotype in the morning between 09.00 h and 10.00 h 55–60 DAS, air-dried for 2 h and pollen from each flower was dusted onto the germination medium to allow a good uniform distribution of grains on the surface of the medium (Salem *et al*., 2004). The plates were then covered and incubated at 30 °C (Precision Instruments, New York, USA) for 3 h. Pollen grains were counted for pollen germination (five fields in each Petri dish), and a pollen grain was considered germinated when its tube length equalled the grain diameter (Luza *et al*., 1987) using a microscope (Nikon SMZ 800 microscope, Nikon Instruments, Kanagawa, Japan) with a magnification of 6.3×. Percentage pollen germination was calculated by counting the total number of pollen grains, an average of 140 pollen grains, and germinated pollen in each Petri dish. Pollen tube length was measured from 20 pollen tubes randomly selected from each Petri dish using a microscope. The lengths were measured with an ocular micrometer fitted to the eyepiece of the microscope. A total of 100 pollen tubes were measured for each treatment.

Cumulative stress response index and total stress response index

The cumulative stress response index (CSRI) is calculated as the sum of the relative individual component responses at each treatment and is similar to the combined response index quoted in other UV-B studies (Dai *et al*., 1994). CSRI was calculated to evaluate the reproductive response of soybean to the treatments under study (+CO_2, +T, +UV-B, +CO_2+UV-B, +CO_2+T, and +CO_2+T+UV-B). The CSRI was calculated as follows:

$$CSRI = \left[\frac{(PG_t - PG_c)}{PG_c} + \frac{(PTL_t - PTL_c)}{PTL_c} + \frac{PN_t - PN_c)}{PN_c} + \frac{(FL_t - FL_c)}{FL_c} \times 100\right]$$

where *CSRI* is the cumulative stress response index, *PG* is the pollen germination percentage, *PTL* is the pollen tube length, *PN* is the pollen number $anther^{-1}$, and *FL* is the flower length under *t* (treatment) and *c* (control). Genotypes were classified into tolerant, intermediate and sensitive, based on the total stress response index (*TSRI*), the sum of *CSRI* over all the treatments.

Pollen morphology

Based on the TSRI, two genotypes (DP 5630RR and DP 4933RR) with low and high TSRI values were selected for pollen morphological studies. For this study, fresh flowers collected between 19.00 h and 21.00 h, 1 d before anthesis and stored in FAA (formaldehyde: glacial acetic acid:ethyl alcohol) solution were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 overnight at 4 °C for SEM. After fixation, specimens were rinsed in buffer, post-fixed in 2% osmium tetroxide in 0.1 M phosphate buffer for 2 h, then rinsed in distilled water, dehydrated in an ethanol series, and critical point dried in a Polaron E 3000 Critical Point Dryer (Quorum Technologies, Newhaven, UK). Specimens were mounted on aluminium stubs, sputter-coated with gold in a Polaron E 5100 sputter coater (Quorum Technologies), and viewed in a LEO Stereoscan 360 SEM (LEO Electron Microscopy, Thornwood, NY, USA) at an accelerating voltage of 15 kV. Images were recorded on Polaroid Type 55 film (Polaroid, Cambridge, Massachusetts, USA).

Data analysis

To test the significance of atmospheric [CO_2], temperature, UV-B radiation, and genotypes, and their interactive effects on flower morphology, pollen number per anther, pollen germination percentage, and pollen tube lengths, data were statistically analysed using analysis of variance (ANOVA) by Genstat 6 for Windows (Genstat 6 Committee, 1997). Pollen germination percentage data were transformed using the arcsin transformation before the analysis. The least significant difference (LSD) tests at P=0.05 were employed to distinguish treatment difference means of the parameters measured in this study. Genotypes were classified based on TSRI of all the treatments as tolerant (>minimum TSRI −1 standard deviation (sd)),

intermediate (>minimum TSRI −2 sd and <minimum TSRI −1 sdv), and sensitive (<minimum TSRI − 2 sd).

Results

Floral morphology

Plants grown under +UV-B conditions showed reduced standard petal lengths either alone or in combination with +T and +CO_2 conditions (Fig. 1A). Similar responses were observed for staminal column lengths. The standard petals were significantly larger in the flowers produced in the plants grown at control and +CO_2 treatments. Significant [CO_2]×T, [CO_2]×UV-B, and [CO_2]×T×UV-B interactions were observed for standard petal length and the T×UV-B interaction was significant for staminal column lengths (Table 1). Significant differences between genotypes were observed in their response to these stressors. Flowers of DP 4933RR had significantly shorter staminal columns under +UV-B, +CO_2+UV-B, +T+UV-B, and +CO_2+T+UV-B conditions compared with the control. The reductions in staminal column with the +CO_2+T+UV-B treatment ranged from 28% (D 90-9216) to 81% (DP 4933RR) (Fig. 1B) compared with the control.

Similarly, plants grown under UV-B in combination with either +CO_2 or +T or both produced smaller flowers with 31–38% less flower length than those produced under control conditions (Fig. 2A). Flowers produced on plants grown under elevated [CO_2] conditions had significantly longer flower lengths in most of the genotypes, while temperature had no effect on flower length in all the genotypes, whereas UV-B radiation had significant effects on the flower length with significant genotypic differences (Table 1). Averaged across all the genotypes, UV-B decreased flower lengths by

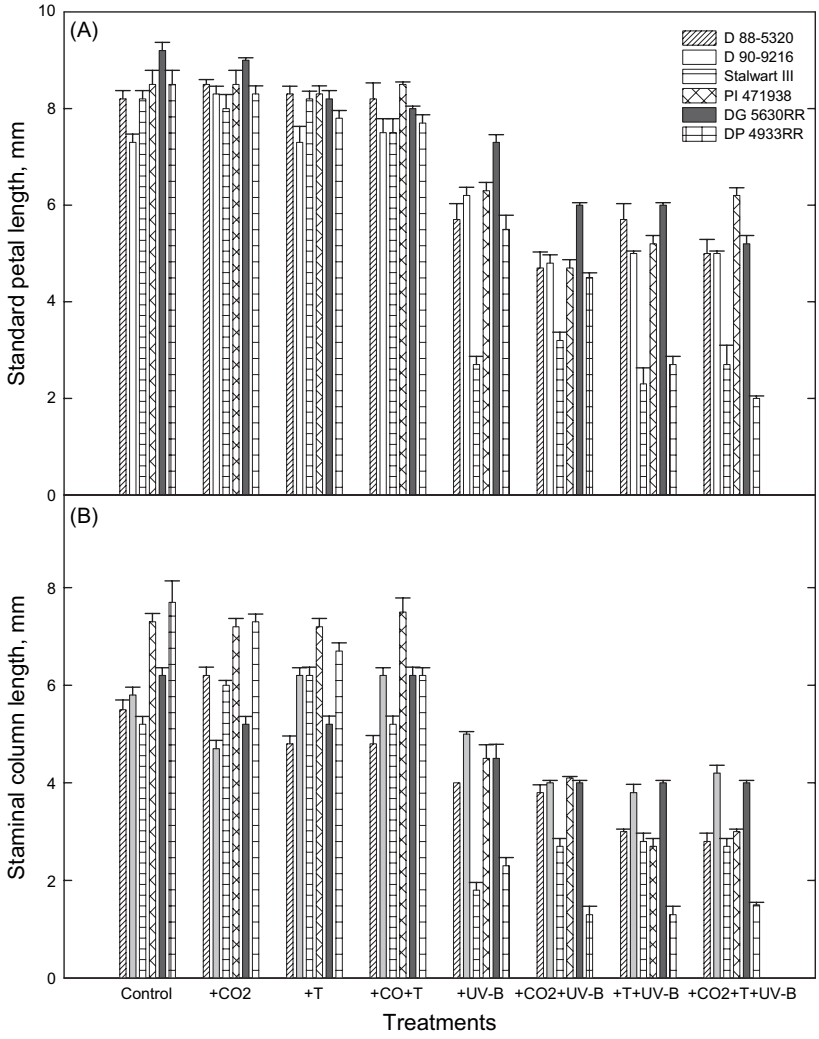

Fig. 1. Influence of carbon dioxide concentration (control, 360 μmol mol^{-1} and +CO_2, 720 μmol mol^{-1}), temperature (control, 30/22 °C and +T, 38/30 °C), UV-B radiation (control, 0 and +UV-B, 10 kJ m^{-2} d^{-1}), and their interactions on lengths of standard petal and staminal column for six soybean genotypes (D 90-9216, Stalwart III, PI 471938, DP 4933RR, D 88–5320, and DG 5630RR). Bars represent standard errors (n=25).

Table 1. *Mean squares of the analysis of variance across the treatments of carbon dioxide concentration ([CO_2]), temperature (T), UV-B radiation (UV-B), and genotype (G) and their interactions on soybean flower morphology, pollen production, pollen germination, and tube lengths*

Source of variation	Flower length[a]	Standard petal length[a]	Staminal column length[a]	Pollen production[a]	Pollen germination[a]	Pollen tube length[a]
Carbon dioxide ([CO_2])	0.01***	2.78*	0.6^{NS}	159.5^{NS}	991.3**	121***
Temperature (T)	10.45^{NS}	11.67***	4.24*	379 229***	80 287***	977.6***
[CO_2]×T	0.02***	0.69*	0.89^{NS}	45^{NS}	69.9^{NS}	72.9**
UV-B radiation (UV-B)	330.6***	416.8***	292.69***	187 812***	46 108***	3 996***
[CO_2]×UV-B	2.56**	2.78**	0.012^{NS}	1 674^{NS}	1 370***	31.5**
T×UV-B	2.45***	0.84^{NS}	1.12*	73 125***	13 576***	808.6***
[CO_2]×T×UV-B	0.023**	2.78**	0.195^{NS}	4 437**	215.9*	0.0^{NS}
Genotype (G)	80.82***	0.84^{NS}	6.32***	213 894***	1 159.7***	425.2***
[CO_2] ×G	2.44***	2.78**	0.387^{NS}	1 409**	365.1***	58.3***
T×G	15.56***	2.69***	1.76***	19 562***	1 009.7***	132.8***
UV-B×G	44.89***	8.16***	13.85***	12 582***	206.14**	365.9***
[CO_2]×T×G	6.96***	0.54**	1.59***	5 879***	344.13***	56.7***
[CO_2]×UV-B×G	2.54**	0.81***	0.167^{NS}	2 690***	205.54**	49.2***
T×UV-B×G	7.85***	1.08***	1.29***	17 916***	793.78***	149.6***
[CO_2]×T×UV-B×G	3.53***	0.35*	0.36^{NS}	4 077***	365.6***	129.7***

[a] Significance levels are indicated by ***, **, * and NS representing P <0.001, P <0.01, P <0.05, and P >0.05, respectively.

28%. Significant [CO_2]×UV-B and T×UV-B interactions were observed for flower length (Table 1).

Pollen production

Significant T×UV-B and [CO_2]×T×UV-B interactions were observed in the number of pollen produced per anther. Plants grown under +T+UV-B and +CO_2+T+UV-B treatments produced significantly less pollen. In DG 5630RR, the 20% more pollen produced for plants grown at +CO_2 was not observed when temperatures and UV-B were increased as well as [CO_2]. Compared with control plants, pollen production in plants grown in the +CO_2+T+UV-B treatment was significantly less, and reduced by 68% (DG 5630RR) and 18% (PI 471938) (Fig. 2B). When averaged over the genotypes, pollen production was reduced by about 30–50% with +T, +CO_2+T, +T+UV-B, and +CO_2+T+UV-B treatments. Genotypes varied significantly in their response to elevated [CO_2] for pollen production. Elevated [CO_2] either increased (7% and 20% in DG 5630RR and PI 471938, respectively), decreased (15% and 17% in D 88-5320 and DP 4933RR, respectively) or had no effect (D 90-9216 and Stalwart III) (Table 1). On the other hand, +T and +UV-B significantly decreased pollen production.

Pollen germination and tube lengths

Significant [CO_2]×UV-B, T×UV-B, and [CO_2]×T×UV-B interactions for pollen germination percentage and [CO_2]×T, [CO_2]×UV-B, and T×UV-B interactions for pollen tube length were observed. Maximum pollen germination percentage was observed at either control conditions (92, 86, and 92% for PI 471938, DG 5630RR, and DP 4933RR, respectively) and at +CO_2 conditions (88, 93, and 82% for D 88-5320, D 90-9216, and Stalwart III, respectively). Both temperature and UV-B reduced pollen germination drastically when imposed alone or in combination with [CO_2]. Temperature had a tremendous influence on pollen germination in D 90-9216, where more than 90% reduction was observed at +T, +CO_2+T, +T+UV-B, and +CO_2+T+UV-B treatments. Pollen produced under +CO_2+T+UV-B conditions, had reductions of 73 (D 88-5320) and 96% (D 90-9216) in pollen germination percentage (Fig. 3A).

Pollen produced with the +CO_2+T+UV-B treatment had significantly shorter tube lengths, ranging from 126 μm (DG 5630 RR) to 235 μm (D 90-9216) (Fig. 3B). Pollen produced for plants grown under +CO_2 treatment, when germinated, had longer pollen tubes than that of the control pollen and the tube lengths ranged from 223 μm (DP 4933RR) to 325 μm (D 90-9216). Genotypes varied significantly in their response to these treatments (Table 1).

Cumulative stress response index

CSRI, which is the sum of individual component relative responses, showed that soybean is sensitive to both temperature and UV-B radiation, and the impact is much more when they occur together. Most of the genotypes had positive CSRI under +CO_2 condition, but when plants were grown under +CO_2 in interaction with negative stressors such as +T and +UV-B radiation, the responses were negative in all the genotypes (Table 2). Out of all the treatments, averaged over all the genotypes, the highest negative CSRI was observed at both +T+UV-B (−184.2) and +CO_2+T+UV-B (−206.3) compared with +T (−126.2) and +UV-B (−146.9) treatments. The reproductive ability of the plants was reduced in these treatments. Plants produced smaller flowers producing less pollen and with poor germination and tube growth. Based on TSRI, the total response over the treatments (Table 2), the genotypes were classified: DG 5630RR and D 88-5320 as tolerant (TSRI > −790); D 90-9216 and PI 471938 as intermediate

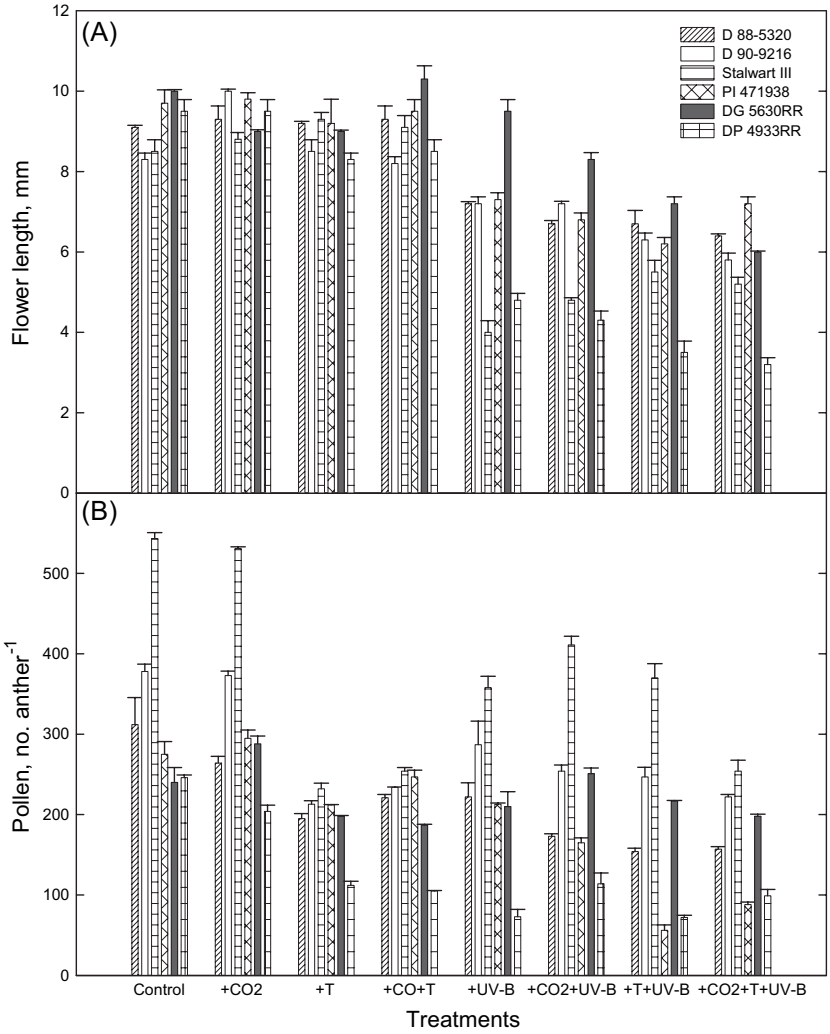

Fig. 2. Influence of carbon dioxide concentration (control, 360 μmol mol^{-1} and +CO_2, 720 μmol mol^{-1}), temperature (control, 30/22 °C and +T, 38/30 °C), UV-B radiation (control, 0 and +UV-B 10 kJ m^{-2} d^{-1}), and their interactions on flower length and pollen production for six soybean genotypes (D 90-9216, Stalwart III, PI 471938, DP 4933RR, D 88-5320, and DG 5630RR). Bars represent standard errors (n=9 for pollen production and n=25 for flower lengths).

(TSRI −790 to −1026); Stalwart III and DP 4933RR as sensitive (TSRI < −1026) (Table 2).

Pollen morphology

Based on the CSRI, two genotypes, one tolerant (DG 5630RR) and one sensitive (DP 4933RR) were studied for pollen morphology. No morphological differences in the pollen grown under control conditions were visible between the sensitive and tolerant genotypes. Pollen abnormalities, however, were observed in both the genotypes with increasing temperatures and UV-B. These morphological differences were similar at both ambient and elevated [CO_2] conditions, and therefore only differences under ambient [CO_2] in combination with other environmental factors are presented. The abnormalities at +T, +UV-B, and +T+UV-B were more evident in DP 4933RR compared with DG 5630RR. A large proportion of the pollen was shrivelled under the +T+UV-B treatment compared with the pollen from plants grown under control conditions (Fig. 4D, H). There were no apertures on the pollen grains from +UV-B and +T plants (Fig. 4B, C, D, F, G, H), while the pollen produced in plants grown under control conditions were triporate, i.e. they had three protruding apertures (Fig. 4A, E). The abnormalities were more evident in the sensitive genotype rather than in the tolerant genotype. Differences in pollen exine structure were also observed (Fig. 5A–H). The columellae heads of the exine were less at +T and +UV-B treatments in both sensitive and tolerant genotypes, and the differences in the columellae heads were more pronounced with the +T+UV-B treatment. Both sensitive and tolerant genotypes had an altered appearance of the exine, while on sensitive genotype the abnormalities were more evident.

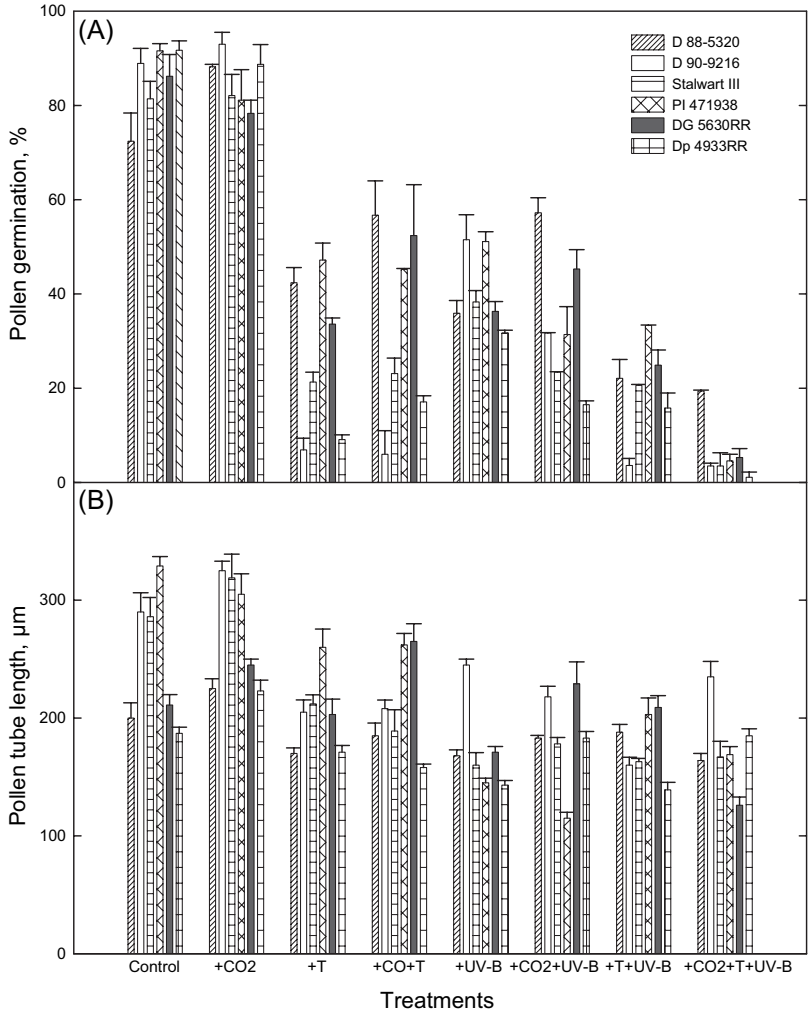

Fig. 3. Influence of carbon dioxide concentration (control, 360 µmol mol^{-1} and +CO_2, 720 µmol mol^{-1}), temperature (control, 30/22 °C and +T, 38/30 °C), UV-B radiation (control, 0 and +UV-B, 10 kJ m^{-2} d^{-1}), and their interactions on pollen germination percentage and pollen tube lengths for six soybean genotypes (D 90-9216, Stalwart III, PI 471938, DP 4933RR, D 88-5320, and DG 5630RR). Bars represent standard errors (n=25 for pollen germination and n=100 for pollen tube lengths).

Discussion

This is the first report to show that there were no beneficial interactions between the three important global change factors ([CO_2], temperature, and UV-B radiation) on the reproductive processes of soybean. Flower morphology, pollen production, pollen germination, pollen tube lengths, and pollen morphology were all negatively affected by +T and +UV-B treatments alone or in combination at both control and +CO_2. Similar to these results, previous investigations on the interactive effects of temperature and [CO_2] on other legumes (Ahmed *et al*., 1992; Prasad *et al*., 2002, 2003) showed that the positive interactions observed between [CO_2] and temperature on vegetative growth cannot be translated to reproductive processes. Significant negative correlation between pollen production and temperature were found in groundnut (Prasad *et al*., 1999). Lower seed yield at high temperatures under both ambient and elevated [CO_2] conditions was shown to be due to decreased pollen viability in groundnut and bean (Prasad *et al*., 2002, 2003).

Smaller flowers with smaller flower-component parts such as standard petal and staminal column lengths were observed in all the genotypes for plants grown in treatment conditions where +T or +UV-B were involved either alone or in combination. Similarly, flowers produced under these treatments also had reduced pollen production even under elevated [CO_2] conditions. Kakani *et al*. (2003) showed similar flower length reductions in cotton under enhanced levels of UV-B radiation. Prasad *et al*. (1999) found strong negative linear relationships between day temperatures over the range of 28–48 °C and fruit number, fruit set, pollen production, and germination in peanut. When +T and +CO_2 acted together, the combined effect on pollen number and pollen germination was additive, being higher than the

Table 2. *Cumulative stress response index (CSRI), sum of relative individual component responses at a given treatment; and total stress response index (TSRI), total CSRI over all the treatments of six soybean genotypes in response to elevated carbon dioxide (720 μmol mol^{-1}) (+CO_2), high temperature (38/30 °C) (+T) and increased UV-B radiation (10 kJ m^{-2}d^{-1}) (+UV-B) and their interactions*

CSRI is the sum of relative responses with treatments in comparison to control (360 μmol mol^{-1} [CO_2], 30/22 °C and 0 kJ m^{-2} d^{-1}) observed for reproductive parameters studied (flower length, pollen production, pollen germination and pollen tube length).

Treatments	Cumulative stress response index (CSRI)					
	D 88-5320	D 90-9216	Stalwart III	PI 471938	DG 5630RR	DP 4933RR
+[CO_2]	+21.1	+35.8	+13.7	−10.5	+16.9	−1.1
+T	−92.8	−162.8	−147.6	−97.5	−90.9	−165.7
+[CO_2]+T	−97.6	−160.8	−151.7	−83.3	−32.7	−164.7
+UV-B	−116.1	−94.9	−184.0	−147.4	−94.3	−208.8
+[CO_2]+UV-B	−100.4	−135.2	−176.9	−200.7	−51.3	−192.5
+T+UV-B	−152.5	−199.5	−184.9	−217.7	−109.6	−242.3
+[CO_2]+T+UV-B	−170.6	−186.4	−229.2	−237.4	−191.6	−221.9
TSRI	**−708.9**	**−903.8**	**−1060.6**	**−994.5**	**−553.5**	**−1197.0**

single stress effects. This concurs with the studies of Prasad *et al*. (2002, 2003), where they showed that elevated [CO_2] did not counteract the negative effects of high temperature on pollen production and viability.

High temperatures during micro and macrosporogenesis reduced pollen viability and pollen number (Prasad *et al*., 1999) at day temperatures >33 °C. Talwar and Yanagihara (1999) have also shown that day/night temperatures of 35/25 °C increased the length of the hypanthium and reduced the rate of pollen tube growth in heat-susceptible genotypes of groundnut when compared with 25/20 °C, both these factors reduce the chances of successful fertilization. In this study, significant reductions in pollen germination in all the genotypes were observed. It seems very likely, however, that pollen germination is affected by environmental stresses during the pre-anthesis period because this is the time during which development of the anther and pollen grains occur. Prasad *et al*. (2002) showed that groundnut flowers were most sensitive to high temperature during microsporogenesis and anthesis. Similar pollen germination inhibitions at high temperatures (Peet *et al*., 1998; Prasad *et al*., 2002, 2003; Cross *et al*., 2003; Young *et al*., 2004) and at higher UV-B levels (Demchik and Day, 1996; Torabinejad *et al*., 1998; Musil *et al*., 1999; Feng *et al*., 2000) were previously reported. Microspore meiosis occurs 9 d before anthesis and differentiation of the stomium, tapetum, middle layers, and endothecium occurs before meiosis (Goldberg *et al*., 1993). If the differentiation processes of those organs are sensitive to high temperature and UV-B radiation, then pollen viability could well be affected since the stomium is important for anther dehiscence (Koltunow *et al*., 1990). Pollen sterility and pollen production at high temperatures may also be associated with early degeneration of the tapetal layer of pollen (Porch and Jahn, 2001). The exact physiological reasons of pollen viability loss are not clearly known and need further investigation.

This study also demonstrated that the morphology of pollen was affected when plants were subjected to high temperatures and enhanced UV-B radiation alone or in combination with either control or +CO_2 conditions, which resulted in flattened and collapsed microspores. The pollen produced under control conditions were equipped with three apertures that act as exits for the germinating pollen and also allows recognition of protein signals, which initiates development of a pollen tube (Heslop-Harrison, 1971). These apertures were missing in pollen produced under both high temperature and high UV-B radiation treatments. In addition, high temperature and enhanced UV-B had a marked effect on the exine ornamentation of both sensitive (DP 4933RR) and tolerant (DG 5630RR) genotypes. The pollen morphological aberrations observed at these climate change stress factors might have resulted in poor pollen germination and shorter tube lengths in the sensitive genotypes. High temperature stress (Cross *et al*., 2003) and water deficit stress (Shen and Webster, 1986) in *Phaseolus vulgaris* showed microsporogenesis to be the most detrimental process during reproductive development, resulting in abnormal exine with deeply pitted and smooth regions. The exine originates from the tapetum (Dickinson and Potter, 1976) and normal pollen development depends on a close interaction with the tapetal tissue that composes the innermost layer of the anther. Although premature degeneration of the tapetal layer (Ahmed *et al*., 1992) could explain the abnormalities observed. All these and other unknown factors might have contributed to the poor pollen germination observed in plants grown under high temperatures, and enhanced UV-B radiation. The stress-induced flower and pollen morphological changes may need further investigation.

These data also showed genotypic variation in the reproductive response to the environmental factors studied. Even though the direction of the stress effect was the same for all the six genotypes, the genotypes varied in the magnitude of their response. Based on TSRI, the six genotypes were classified into tolerant, intermediate, and sensitive. The differences can be due to different adaptive or defensive

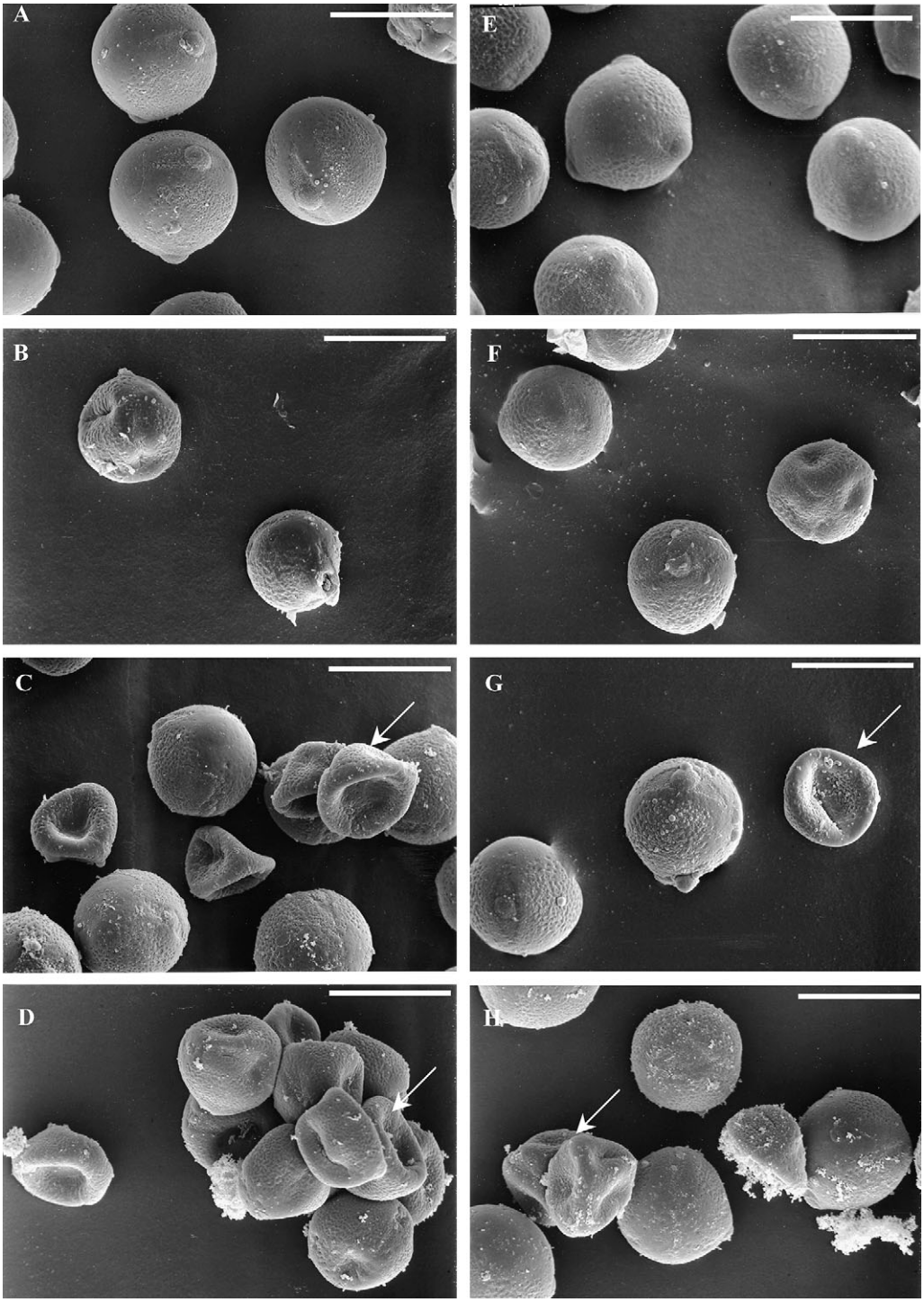

Fig. 4. Scanning electron microscopy images of pollen grains from pollen of two soybean genotypes, DP 4933RR (UV-B sensitive) (A–D) and DG 5630RR (UV-B tolerant) (E–H), grown under (A) control (30/22 °C and 0 kJ UV-B), (B) +T (38/30 °C and 0 kJ UV-B), (C) +UV-B (30/22 °C and 10 kJ UV-B), (D) +T+UV-B (38/30 °C and 10 kJ UV-B) at ambient carbon dioxide concentration (360 μmol mol^{-1}). Arrows indicate collapsed pollen grains without apertures. Scale bar=20 μm.

mechanisms to high temperatures and UV-B radiation, such as the inherent capacity of repair systems such as membrane stability of genotypes.

In summary, there were no beneficial interactions between [CO_2], temperature, and UV-B radiation on reproductive developmental processes such as pollen production, germination, and tube lengths of soybean. Temperatures of 38/30 °C and UV-B radiation levels of about 10 kJ m^{-2} d^{-1} alone or in combination at both the control and +CO_2 conditions produced smaller flowers and fewer pollen

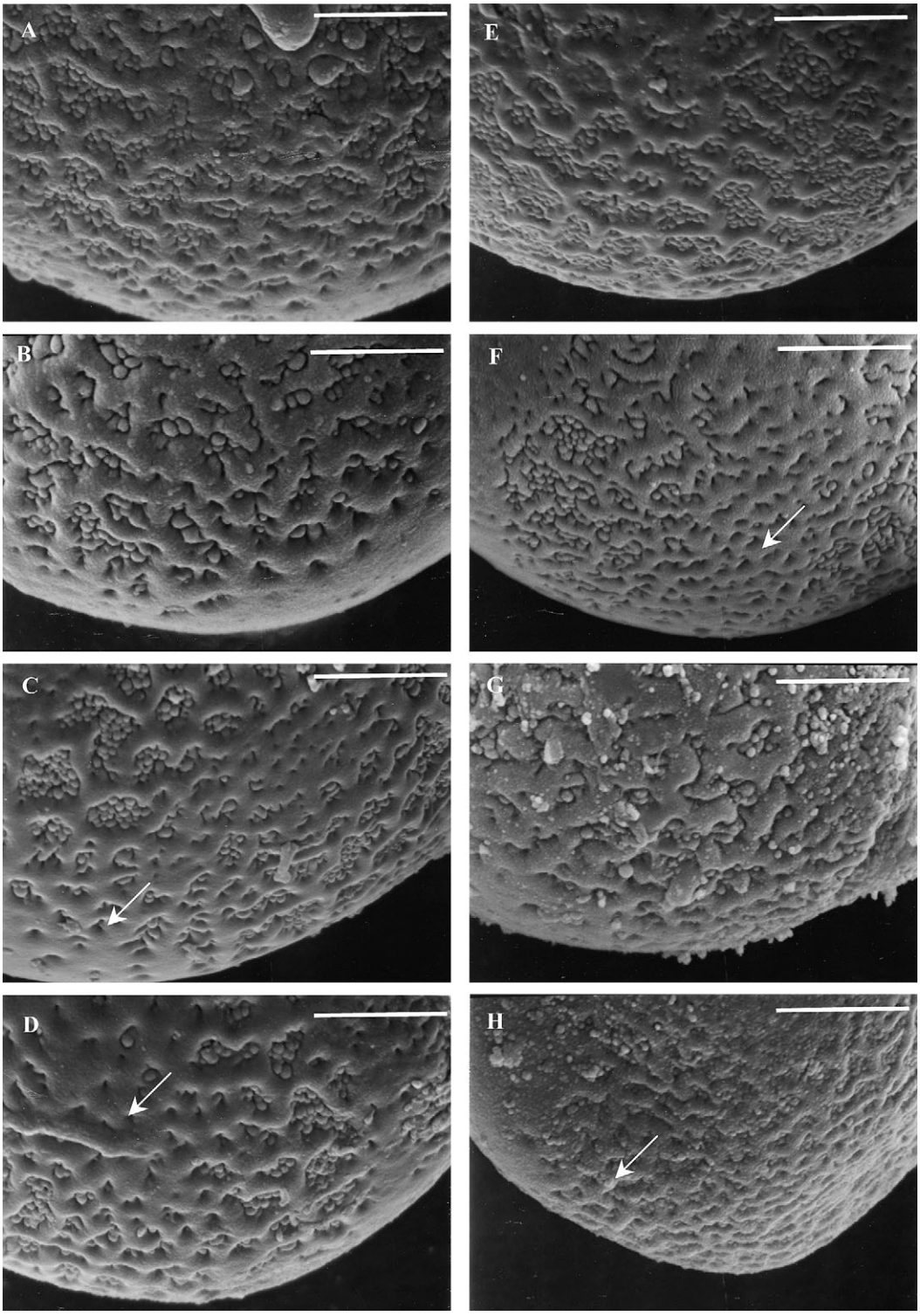

Fig. 5. Scanning electron microscopy images of pollen grain surface from pollen of soybean genotypes, DP 4933RR (UV-B sensitive) (A–D) and DG 5630RR (UV-B tolerant) (E–H), grown under (A, D) control (30/22 °C and 0 kJ UV-B), (B, E) +T (38/30 °C and 0 kJ UV-B), (C, G) +UV-B (30/22 °C and 10 kJ UV-B), (D, H) +T+UV-B (38/30 °C and 10 kJ UV-B) at ambient carbon dioxide concentration (360 μmol mol^{-1}). Arrows indicate disturbed exine ornamentation of pollen. Scale bar=2 μm.

grains per flower together with lower pollen germination. In addition, pollen grains produced at these high temperature and UV-B treatments were shrivelled resulting in further deterioration in pollen germination. The clear effect of high temperature and UV-B radiation on pollen production and pollen grain germination will have major implications on the fertilization process and fruit set in sensitive crops under future climates. In conclusion, pollen germination can be used as a screening tool for selecting cultivars for high temperature and UV-B radiation environments as it is more responsive to those variables. Variability among soybean genotypes in their responses to [CO_2], temperature, and UV-B radiation indicates the possibility of selecting soybean cultivars for higher yields and/or stability in a warmer temperature and higher UV-B, most likely in a future higher CO_2 world. However, the genetic bases for these differences must be further examined.

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