Assessing genotypic variability of cowpea (*Vigna unguiculata* [L.] Walp.) to current and projected ultraviolet-B radiation

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ABSTRACT

The current and projected terrestrial ultraviolet-B (UV-B) radiation affects growth and reproductive potential of many crops. Cowpea (*Vigna unguiculata* [L.] Walp.), mostly grown in tropical and sub-tropical regions may already be experiencing critical doses of UV-B radiation due to a thinner ozone column in those regions. Better understanding of genotypic variability to UV-B radiation is a prerequisite in developing genotypes tolerant to current and projected changes in UV-B radiation. An experiment was conducted in sunlit, controlled environment chambers to evaluate the sensitivity of cowpea genotypes to a range of UV-B radiation levels. Six cowpea genotypes [Prima, California Blackeye (CB)-5, CB-27, CB-46, Mississippi Pinkeye (MPE) and UCR-193], representing origin of different geographical locations, were grown at 30/22 °C day/night temperature from seeding to maturity. Four biologically effective ultraviolet-B radiation treatments of 0 (control), 5, 10, and 15 kJ m^{-2} d^{-1} were imposed from eight days after emergence to maturity. Significant genotypic variability was observed for UV-B responsiveness of eighteen plant attributes measured. The magnitude of the sensitivity to UV-B radiation also varied among cowpea genotypes. Plants from all genotypes grown in elevated UV-B radiation were significantly shorter in stem and flower lengths and exhibited lower seed yields compared to the plants grown under control conditions. Most of the vegetative parameters, in general, showed a positive response to UV-B, whereas the reproductive parameters exhibited a negative response showing the importance of reproductive characters in determining tolerance of cultivars to UV-B radiation. However, all cultivars, except MPE, behaved negatively to UV-B when a combined response index was derived across parameters and UV-B levels. Based on the combined total stress response index (C-TSRI) calculated as sum of individual vegetative, physiological and reproductive component responses over the UV-B treatments, the genotypes were classified as tolerant (MPE), intermediate (CB-5, CB-46 and UCR-193) and sensitive (CB-27 and Prima) to UV-B radiation. The differences in sensitivity among the cowpea genotypes emphasize the need for selecting or developing genotypes with tolerance to current and projected UV-B radiation.

1. Introduction

Even though ultraviolet-B (UV-B, 280–320 nm) represents a small fraction of total electromagnetic spectrum, exposure to UV-B at the current and projected levels is known to elicit a variety of responses by all living organisms including crop plants [1,2]. The amount of UV-B radiation received on the Earth's surface is closely correlated with the thickness of the stratospheric ozone (O$_3$) column. Relative to the 1970s, the midlatitudes O$_3$ column losses for the 2002–2005 periods are approximately 3% in the Northern and 6% in the Southern hemispheres [3]. Current global distribution of mean erythemal daily doses of UV-B radiation between the latitude 40 °N and 40 °S during summer ranges from 2 to 9 kJ m^{-2} [4] which are comparatively higher than the earlier measurement of 2–6 kJ m^{-2} d^{-1} in 1994 [5]. The three-dimensional Chemistry-Climate models estimates indicate that ground-level UV-B radiation is currently near its maximum levels and is expected to revert to the pre-1980s level at the midlatitudes by 2040–2070, if all member countries implement the Montreal Protocol [3]. Non-compliance by member countries to implement the protocol would delay the recovery or even prevent the recovery of the ozone layer. Therefore, depletion of stratospheric O$_3$ and consequent increase in the terrestrial UV-B radiation has and will continue to raise interest in understanding the deleterious effects of UV-B radiation on plants.

Cowpea plays an important role in the cropping systems of tropical and sub-tropical, arid and semi-arid regions that cover a wide range of latitudes (45 °N–35 °S) on the globe [6,7]. Typically, the daily doses of UV-B radiation in the cowpea growing regions in

USA ranges from 0.02 to 8.75 kJ m^{-2} d^{-1} [8], however, on the global scale, maximum UV-B radiation could reach up to 8–10 kJ m^{-2} d^{-1} [9].

Previous reviews and published studies clearly demonstrate the extent of damage caused by both ambient [10–13] and elevated UV-B radiation [2,11,14–16] on morphological, physiological, biochemical, and molecular level processes of crop plants which varied widely among species and among cultivars of the same species. In a recent review, Kakani et al. [2] reported that enhanced UV-B radiation affects most crop growth processes directly through several first order effects including reductions in photosynthesis and vegetative growth, leading to lower yield. Moreover, UV-B in combination with other abiotic stressors can drastically modify the magnitude and direction of plant responses [14]. Premkumar and Kulandaivelu [17] reported that enhanced UV-B, simulating 20% O$_3$ depletion, markedly alleviated the adverse effect of magnesium deficiency in cowpea, whereas, the impacts of elevated UV-B aggravated the negative effects of temperature on growth and development of soybean [18].

In general, plants may tolerate small increases in UV-B by protective mechanisms such as reducing the transmittance of UV-B through the epidermis by producing UV-B absorbing compounds, scattering and reflecting light, quenching free radicals and photorepair of sensitive systems such as nucleic acids [12,15,17]. Most defense mechanisms appeared to be light dependent such as photo-repair system for DNA and the biosynthesis of UV-B absorbing compounds [15,19,20]. Despite the known importance of photosynthetically active radiation (PAR), studies utilizing an unrealistic and unbalanced UV-B and PAR ratio for plant growth are not uncommon resulting in unrealistic plant responses [21]. However, many species appeared to be more sensitive to the UV-B radiation than others even under ambient PAR and such crop species may already be experiencing UV-B stress [10].

Crop economic yield is an important trait for selection of cultivar for a niche environment. Increased concern about the UV-B radiation effects on crops has prompted developing screening tools and methods for tolerance in crop populations [2,22]. The large differences among cultivar responses to UV-B radiation offer a valuable tool for selection process in response to UV-B radiation [2]. Many crops have been screened using various UV-B response indices which were derived from short-term plant growth responses to UV-B [18,22,23]. The reproductive growth and seed yield are important components of plant growth responses to UV-B radiation [18], but have received little attention. Therefore, a season-long UV-B exposure on crop plants is needed to understand the mechanisms and causes for crop yield losses.

Noticeable uncertainties exist concerning influence of UV-B radiation on tropical legumes including cowpea plants exposed to both above and below ambient levels of UV-B radiation [9,10,13,24–29]. For instance, cowpea plants did not exhibit a significant change in plant height, leaf area and dry matter when grown under elevated UV-B simulating 15–25% O$_3$ depletion [26,27]. Contrary to this, studies simulating a similar O$_3$ depletion caused pronounced decrease in biomass production and photosynthesis [10,28,29]. These inconsistencies could be partially explained by genotypic differences, different growth environments, intensity and duration of UV-B supplementation [2,21]. The supplied UV-B radiation in these studies represents very small additions of absolute energy capable of inducing a variety of responses in biological systems.

Cowpea, a traditional source of livelihood to many rural African populations, has been reported as highly sensitive to UV-B radiation [14,28]. Musil et al. [28] found that cowpea was exceptionally sensitive to UV-B (15% O$_3$ depletion) among the evaluated 17 species native to or largely grown in South Africa. Earlier studies evaluating the UV-B responsiveness of cowpea represented a smaller set of

plant attributes usually measured from part of a plant organ and/or growth stage involving either vegetative, physiological and/or molecular responses expressed for a part of a growing season [17,26–29]. To our knowledge, there are no reports on screening the responses of cowpea genotypes to UV-B radiation based on both vegetative and reproductive growth processes. We hypothesized that UV-B tolerant characteristics are present in cowpea with genotypic variability and when exposed to UV-B, the vegetative traits respond dissimilarly in comparison to the reproductive characteristics. The objectives of this study were to determine the vegetative, physiological and reproductive responses of cowpea genotypes to a range of UV-B radiation and to identify the genotypic variability using several plant attributes and statistical methods.

2. Materials and methods

2.1. Experimental facility

The experiment was conducted in four sunlit, controlled environment chambers known as soil–plant–atmosphere-research (SPAR) units located at the R.R. Foil Plant Science Research Center, Mississippi State (33° 28′ N 88° 47′ W), Mississippi, USA. SPAR units have the capacity to precisely control temperature, CO$_2$ concentration, UV-B radiation, and the recommended nutrient and irrigation regimes at determined set points for plant growth studies under near ambient levels of PAR. Each SPAR chamber consists of a steel soil bin (1 m deep by 2 m long by 0.5 m wide) to accommodate the root system, a Plexiglas chamber (2.5 m tall by 2 m long by 1.5 m wide) to accommodate aerial plant parts and a heating and cooling system connected to air ducts that pass the conditioned air through the plant canopy with sufficient velocity (4.7 km h^{-1}) to cause leaf flutter, mimicking field conditions. Variable density black shade cloths around the edge of the plant canopy were adjusted regularly to match the height and to eliminate the need for border plants. The Plexiglas chambers are completely opaque to solar UV-B radiation and transmit 12% UV-A, and more than 95% incoming PAR [30]. During the experiment, the incoming solar radiation (285–2800 nm) outside of the SPAR units measured with a pyranometer (Model 4–8; The Eppley Laboratory Inc., Newport, RI, USA) ranged from 1.5 to 24 MJ m^{-2} d^{-1} with an average of 18 ± 4 MJ m^{-2} d^{-1}. The measured solar radiation on most of the days except few cloudy days were above 15 MJ m^{-2} d^{-1}, 3 days <10 MJ m^{-2} d^{-1} or 6 days <15 MJ m^{-2} d^{-1}. The data acquisition and control systems are networked to provide automatic acquisition and storage of the data from the SPAR units, monitoring the SPAR environments every 10 s throughout the day and night. The operational details and controls of the SPAR chambers have been described by Reddy et al. [31].

2.2. Plant culture

Six genotypes of cowpea representing diverse sites of origin; California blackeye (CB)-5 and CB-46 (University of California, Davis, USA), CB-27 (University of California, Riverside, USA), Mississippi Pinkeye; MPE (Mississippi State University, Mississippi, USA), Prima (Nigeria), and UCR-193 (India) were used in present study [32–34]. The genotypes were seeded in 15 cm diameter and 15 cm deep plastic pots filled with fine sand on 26 July, 2005. After emergence, seven days after sowing, thirty pots having healthy plants, five pots for each genotype and three plants in each pot, were transferred and arranged randomly into each SPAR chamber. The temperature and CO$_2$ were maintained at 30/22 °C (day/night) and 360 μL L^{-1}, respectively, in all chambers. Plants were watered three times a day with full-strength Hoagland's nutrient solution delivered 8:00, 12:00, and 17:00 h to ensure opti-

mum nutrient and water conditions for plant growth through an automated and computer-controlled drip irrigation system.

2.3. UV-B radiation protocol

A UV-B radiation (280–320 nm) treatment of 0 (control; no UV-B) and three total daily doses of biologically effective UV-B radiation intensities of 5, 10, and 15 kJ m^{-2} d^{-1} were imposed from eight days after emergence (DAE) to maturity. The square-wave supplementation systems were used to provide desired UV-B radiation which was delivered with a constant rate from 0.5 m above the plant canopy for 8 h, each day, from 8:00 to 16:00 h by eight fluorescent UV-313 lamps (Q-Panel Company, Cleveland, OH, USA) driven by 40 W dimming ballasts, horizontally mounted on a metal frame inside the SPAR chambers. To filter UV-C radiation (<280 nm), the lamps were wrapped with pre-solarized 0.07 mm cellulose diacetate (CA) film (JCS Industries Inc., La Mirada, CA, USA). The CA films were changed every 3–4 days to account for the degradation of CA properties. The amount of energy delivered at the top of the plant canopy was checked daily at 10:00 h with a UVX digital radiometer (UVP inc., San Gabriel, CA, USA) and calibrated against an Optronic Laboratory (Orlando, FL, USA; Model 754 Spectroradiometer), which was used initially to quantify the lamp output. The biologically effective doses of UV-B were measured during the plant growth period at 10 different locations in each SPAR chamber corresponding to the pots arranged in the row. During the experiment, the weighted total biologically effective UV-B radiation at the top of the plants were 0, 4.8 ± 0.15, 9.8 ± 0.1, and 14.6 ± 0.2 for the planned 0, 5, 10, and 15 kJ m^{-2} d^{-1} set points, respectively, using generalized plant response spectrum [35] as formulated by Green et al. [36] which was normalized at 300 nm. The simulated O_3 depletion of the four UV-B doses was 0%, 6%, 12%, and 24%, respectively, at this location.

2.4. Vegetative growth parameters

One plant per pot, five pots per genotype, was cut at the soil surface 10 and 18 d after UV-B treatments (DAT) to determine plant height (PH), leaf area (LA) and dry matter (DM) of the leaves and stems separately. LA was determined using an automated leaf area meter (Li-3100 leaf area meter, Li-COR Inc., Lincoln, NE, USA) at both the harvests. Specific leaf area (SLA) was calculated as leaf area per gram of leaf dry mass (cm^2 g^{-1}). The plant components were oven dried for 72 h at 70 °C to obtain DM. The final remaining one plant per pot was harvested at the maturity, 53 DAE, of the crop.

2.5. Photosynthesis and chlorophyll fluorescence parameters

Eighteen days after treatment, leaf net photosynthesis (P$_{net}$), and chlorophyll fluorescence (Fv′/Fm′) were measured between 9:00 and 14:00 h on the 3rd or 4th sunlit leaves from the terminal using an infrared gas analyzer built into a leaf cuvette in an open gas exchange system (Li-COR 6400; Li-Cor Inc., Lincoln, NE, USA) with an integrated fluorescence chamber head (Li-COR 6400-40 Leaf Chamber Fluorometer). The cuvette chamber conditions were adjusted to provide photosynthetic photon flux density of 1500 μmol m^{-2} s^{-1} and cuvette block temperature was maintained at 30 °C to match treatment day-time temperature using a computer controlled Peliter module mounted in the cuvette. Relative humidity inside the cuvette was maintained at approximately 50% and airflow entering the cuvette was maintained at 360 μL L^{-1} CO_2 concentration. The efficiency of energy harvested by oxidized (open) PSII reaction center in light (Fv′/Fm′) was calculated as chlorophyll fluorescence (Fm′ − Fo′)/Fm′, where, Fo′ and Fm′ are the minimal and maximal fluorescence of light saturated leaves. The

actual flux of photons driving photosystem II (PSII), i.e. electron transport rate (ETR), was computed according to the equation [(Fm′ − Fs)/Fm′] × flα$_{leaf}$, where, Fs = steady state fluorescence, f = the fraction of absorbed quanta that is used by PSII, typically, 0.5 for C$_3$ plants (in this study), I = incident photon (μmol m^{-2} s^{-1}) flux density, and α$_{leaf}$ = leaf absorptance (it was constant about 0.85 in this study).

2.6. Chlorophyll and UV-B absorbing compounds (phenolics)

The total leaf chlorophyll (Chl) and UV-B-absorbing compounds were extracted and determined (18 DAT) on five 0.38 cm^{-2} leaf disks for each replication by placing them in a vial containing either 5 ml of dimethyl sulfoxide for chlorophyll extraction or 10 ml of a mixture of methanol, distilled water and hydrochloric acid in 79:20:1 ratio for phenolics extraction and were incubated in dark for 24 h. Thereafter, the concentration of the 1 ml extract was determined at 648 and 662 nm for estimation of total chlorophyll and 320 nm for estimation of phenolic compounds by using Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). The equations of Lichtenthaler [37] was used to estimate the Chl concentration, whereas the phenolic concentration was estimated according to the Kakani et al. [38] and expressed as equivalent of *p*-coumaric acid.

2.7. Cell membrane thermostability (CMT)

The leaf CMT in cowpea genotypes was assessed on 18 DAT according to the procedure described by Martineau et al. [39] with minor modification. In brief, a sample for assay consist of a paired set namely; control (C) set and treatment (T) set, of five leaf disks each 1.3 cm^{-2}, cut from five fully expanded 3rd or 4th randomly selected leaves. Samples were replicated three times each. Prior to assay, the paired set of leaf disks were placed in two separate test tubes and washed thoroughly with four changes of deionized water, 10 ml each time, to remove electrolytes adhering to the cut surface of the leaf disks. After the final wash, both sets of test tubes were filled with 10 ml of deionized water and sealed with aluminum foil to avoid the evaporation of water. The T-set of the test tubes were incubated for 20 min at 50 °C in a temperature controlled-water bath, while the C-set of test tubes were left at room temperature (approximately 25 °C). Then, both sets of test tubes were incubated at 10 °C for 24 h. Initial conductance readings of both sets (CEC1 and TEC1) using an electrical conductivity meter (Corning Checkmate II: Corning Inc., New York, USA) were made after bringing test tubes to room temperature. After which, tubes were again sealed with aluminum foil and autoclaved at 120 °C and 0.15 MPa for 20 min to completely kill the leaf tissue. Autoclaved tubes were cooled to room temperature, contents mixed thoroughly and a final conductance (CEC2 and TEC2) was recorded. The CMT was calculated as shown below

$$CMT \% = \frac{1 - (TEC1/TEC2)}{1 - (CEC1/CEC2)} \times 100,$$

where TEC and CEC are the measure of conductance in treated and controlled test tubes, respectively, at initial = 1 and final = 2 conductance measurements.

2.8. Reproductive parameters

2.8.1. Flower morphology and pollen viability

From the time-series measurement of anther dehiscence (data not shown), we found that cowpea anthers dehisce between 5:00 and 8:00 h. Therefore, this time period was used to collect flowers for both morphological and pollen parameters. Flower length (Fl length), percentage pollen viability (PV) and flower dry weight

(Fl Dwt) were determined on 10 flowers randomly picked from five plants per genotype in each treatment. Flower length was measured from the tip of the standard petal to the base of the calyx. A 3% concentration of 2,3,5-triphenyltetrazolium chloride (TTC) in 20% concentration of sucrose solution was found to be the best for cowpea pollen staining (data not shown). The pollen grains were dusted gently by tapping the flower with an artist brush on the microscope glass slides containing a drop of staining solution as described by Aslam et al. [40]. The preparations were stored at room temperature in the dark, and after 16 h, the number of total as well as TTC-stained pollen grains were counted at two microscopic fields of $2.4 \, mm^2$ having >100 pollen grains from each field by using Nikon SMZ 800 microscope (Nikon Instruments, Kanagawa, Japan). Then, the same flowers were dried in an oven at 75 °C for 72 h and weighed to determine dry weights.

2.8.2. Pod production and yield components

Cowpea plants were cut at the soil surface when most of the pods were mature and dry (53 DAE). The yield components such as total number of pods $plant^{-1}$ (pod no.), total seed weight $plant^{-1}$ (Seed Wt), individual seed weight (g $seed^{-1}$, average of 100 seeds) and number of seeds pod^{-1} (Seeds pod^{-1}) were determined on all five plants from each genotype. The dry weights of pods and seeds were also measured after complete drying at room temperature. Pod shelling percentage (Shelling) was calculated as seed mass over pod weight multiplied by 100.

2.9. Data analysis and classification of genotypes

2.9.1. Analysis of variance (ANOVA)

To test the significance of UV-B and genotype effects on vegetative and reproductive growth components of cowpea, a two way ANOVA was performed using the general linear model "PROC GLIMMIX" procedure of SAS [41]. GLIMMIX produce Type III F-statistics and *P*-values, which are based on likelihood estimations. The GLIMMIX procedure was used to analyze fixed and random effects and in estimating the error distribution within the data. The analysis included genotype, treatment and genotype × treatment as fixed effects and replication nested in treatments as a random effect. The least square means (LSMEANS) comparisons were used to determine significance differences between genotypes means for the levels of UV-B treatments for each parameter measured using PDIFF LINES option (*P* = 0.05).

2.9.2. Multivariate analysis

The multivariate statistical procedure, principal component analysis (PCA), was performed to determine the similarities and differences of the measured parameters in their pattern of response to UV-B radiation among cowpea genotypes [42]. Through the linear orthogonal transformation, PCA creates a new coordinate system for the data sets generating principal component (PC) scores or latent vectors capable of explaining the systematic behavior of the observed variables in a reduced dimension [42]. PCA analysis was performed using PROC PRINCOMP procedure of SAS [41] on the correlation matrix (18 rows × 18 columns) of ultraviolet radiation response index (UVRI) data as described in Section 2.9.3. The UVRI were obtained from the three levels of elevated UV-B radiation treatments (5, 10 and 15 kJ m^{-2} d^{-1}) versus control (0 UV-B) for 6 cowpea genotypes (3 × 6 = 18 rows) of 18 measured response variables (18 columns). The cowpea genotype responses to the three elevated levels of UV-B radiation were then examined by using the biplot of PC1 vs. PC2 and analyzing the positive and negative responses associated with a particular axis. The UVRI of 18 response variables were subsequently regressed with PC1 and PC2 to facilitate the distinction of key plant attributes characterizing the UV-B responses in each dimension.

2.9.3. Cumulative UV-B response index (CUVRI) and total stress response index (TSRI)

CUVRI was calculated as the sum of ultraviolet-B response index (UVRI) of individual plant attribute responses to the three levels of UV-B (5, 10 and 15 kJ m^{-2} d^{-1}) compared to the control (0 UV-B) and is based on the response index concept reported in another UV-B study [22] which was calculated as:

$$UVRI = \frac{RV_t - RV_c}{RV_c} \times 100,$$

where UVRI = ultraviolet-B response index (that could be measured at 5, 10 or 15 kJ m^{-2} d^{-1}), RV = individual response variable (that could be anyone of 18 measured plant response variables) under t = treatment and c = control conditions. The average of two measurements (10 and 18 DAT) for PH, LA, DW, and SLA were used to capture the genotypic variability, if any. TSRI, sum of the CUVRIs over all the response variables, was evaluated for vegetative (V-TSRI) and reproductive (R-TSRI) responses separately and in combination (C-TSRI) based on the following equations:

$$V - TSRI = \left(\frac{PH_t - PH_c}{PH_c} + \frac{DM_t - DM_c}{DM_c} + \frac{LA_t - LA_c}{LA_c} + \frac{SLA_t - SLA_c}{SLA_c}\right.$$
$$+ \frac{P_{net_t} - P_{net_c}}{P_{net_c}} + \frac{ETR_t - ETR_c}{ETR_c} + \frac{Fv'/Fm'_t - Fv'/Fm'_c}{Fv'/Fm'_c}$$
$$\left. + \frac{Chl_t - Chl_c}{Chl_c} + \frac{Phe_t - Phe_c}{Phe_c} + \frac{CMT_t - CMT_c}{CMT_c}\right) \times 100 \quad \text{and}$$

$$R - TSRI = \left(\frac{Fl\,length_t - Fl\,length_c}{Fl\,length_c} + \frac{F\,DWt_t - F\,DWt_c}{F\,DWt_c}\right.$$
$$+ \frac{PV_t - PV_c}{PV_c} + \frac{Pod\,no_t - Pod\,no_c}{Pod\,no_c} + \frac{Seed\,Wt_t - Seed\,Wt_c}{Seed\,Wt_c}$$
$$+ \frac{g\,seed_t^{-1} - g\,seed_c^{-1}}{g\,seed_c^{-1}} + \frac{Seed\,Pod_t^{-1} - Seed\,Pod_c^{-1}}{Seed\,Pod_c^{-1}}$$
$$\left. + \frac{Shelling_t - Shelling_c}{Shelling_c}\right) \times 100$$

where PH = plant height, DM = dry matter of plant shoot, LA = leaf area, SLA = specific leaf area, P_{net} = net photosynthesis, ETR = electron transport rate, Fv'/Fm' = chlorophyll fluorescence, Chl = total leaf chlorophyll, Phe = phenolics concentration, CMT = cell membrane thermostability, Fl length = flower length, Fl Dwt = flower dry weight, PV = pollen viability, Pod no. = pods $plant^{-1}$, Seed Wt = seed weight $plant^{-1}$, g $seed^{-1}$ = individual seed weight, Seeds Pod^{-1} = seed number pod^{-1} and Shelling = pod shelling percentage under t = treatment and c = control conditions. Based on C-TSRI, sum of V-TSRI and R-TSRI, cowpea genotypes were classified as tolerant (⩾minimum C-TSRI + 2 standard deviation; SD), intermediate (⩾minimum C-TSRI + 1 SD and ⩽minimum C-TSRI + 2 SD) and sensitive (⩽minimum C-TSRI + 1 SD) to UV-B radiation.

3. Results

3.1. Vegetative growth and dry matter production

The signature of the UV-B radiation was first appeared on the top leaves starting from five days after treatment. The symptoms included minor yellowing in the veinal and inter-veinal regions that later developed into small chlorotic patches and an upward cupping of the leaves. A significant interaction was found between UV-B radiation and genotypes (UV-B × G) for PH and DM production accompanied with a significant reduction in PH and DM at elevated UV-B levels when averaged across genotypes (Table 1 and Fig. 1A and B). PH reduction was not significant in CB-5, CB-46 and MPE at 10 kJ m^{-2} d^{-1} or higher UV-B levels. Genotypes also varied for DM production from no significant change (Prima) to

Table 1
Combined total stress response index (C-TSRI), sum of CUVRIs (cumulative ultraviolet response indices) over all vegetative, physiological and reproductive plant attributes studied; plant height (PH), dry matter plant^{-1} (DM), leaf area (LA), specific leaf area (SLA), net photosynthesis (P_{net}), electron transport rate (ETR), chlorophyll fluorescence (Fv'/Fm'), total chlorophyll (Chl), phenolics (Phe), cell membrane thermostability (CMT), pod number plant^{-1} (Pod no.), seed weight plant^{-1} (Seed Wt), individual seed weight (g seed^{-1}), seeds number pod^{-1} (Seed pod^{-1}), flower length (Fl length), flower dry weight (Fl Dwt), pollen viability (PV) and shelling percentage (shelling)

Genotype	CUVRI for vegetative and physiological attributes										V-TSRI	CUVRI for reproductive attributes								R-TSRI	C-TSRI
	PH	DM	LA	SLA	P_{net}	ETR	Fv'/Fm'	Chl	Phe	CMT		Fl length	Fl Dwt	PV	Pod no.	Seed Wt	g seed^{-1}	Seeds pod^{-1}	Shelling		
Prima	−86	−5	+11	+51	−76	−106	+13	+16	+152	−56	−85	−38	−43	−9	−84	−91	−34	−1	−13	−312	−397
CB-5	−37	−24	+39	+70	−10	−68	+10	−27	+59	−6	+6	−30	−55	+5	−27	−53	+10	+5	−3	−147	−141
CB-27	−84	−127	−40	+162	−85	−83	+25	−46	+6	−24	−295	−2	−188	−8	−86	−112	−22	+11	+2	−405	−700
CB-46	−42	−96	−36	+77	+63	−64	+26	−42	+21	+52	−39	−25	−45	−5	−40	−56	−16	+13	+13	−160	−199
MPE	−35	+59	+78	+9	+98	+13	+15	−5	+117	−19	+329	−15	−18	−4	+49	−34	−18	−34	−5	−78	+251
UCR-193	−121	−105	−81	+95	+130	+52	+6	+19	+84	+48	+127	−16	−29	−1	−118	−143	−23	−48	−13	−390	−263
ANOVA																					
G	***	***	***	***	**	***	*	**	***	***	–	***	***	***	***	***	***	***	***	–	–
UV-B	***	***	NS	***	**	***	*	*	**	**	–	***	**	***	*	*	**	NS	NS	–	–
UV-B × G	**	***	***	NS	***	***	NS	*	*	***	–	***	NS	**	NS	NS	**	NS	NS	–	–

The significance levels ***, **, *, and NS represent $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$ and $P > 0.05$, respectively.
The CUVRI is the sum of UVRI (ultraviolet response index) which is the relative responses of treatments (3 UV–B levels; 5, 10 and 15 kJ m^{-2} d^{-1}) in comparison to control (0 kJ m^{-2} m^{-1}) observed for individual vegetative, physiological and reproductive plant attributes. Analysis of variance (ANOVA) across the treatment of UV-B radiation and genotypes on cowpea growth.

significant increase (CB-5 and MPE) in response to UV-B radiation. Compared with the control, averaged over UV-B levels, UCR-193 and CB-27 produced 49% and 25% shorter plants, respectively (Fig. 1A). However, this reduction was less in CB-5 (13%) and MPE (2%). A similar trend was also recorded for DM (Fig. 1B). At any given UV-B level, cowpea genotypes showed a significant UV-B × G interaction with non significant increase in LA due to an increase in the leaf expansion per unit of leaf dry weight, SLA (Fig. 1C and D). SLA showed no UV-B × G interaction and increased significantly in all genotypes at elevated UV-B levels compared to control. However, there were no significant differences among the three elevated UV-B levels. The CUVRI, representing the overall UV-B responsiveness of individual traits (Table 1) clearly exhibited that all the genotypes responded negatively for PH and positively for SLA.

3.2. Photosynthesis and chlorophyll fluorescence

P_{net} and ETR of the photosystems exhibited considerable variability among cowpea genotypes and treatments showing highly significant UV-B × G interaction (Table 1). Genotypes Prima and CB-27 recorded a significant reduction in P_{net} across all UV-B treatments while MPE and UCR-193 showed significantly increased P_{net} under elevated UV-B treatments (Fig. 2A). A similar pattern was also observed for ETR, however, the magnitude of reduction in ETR was greater than that of the reduction in P_{net} (Fig. 2B). The Fv'/Fm' values did not show a significant UV-B × G interaction and were significantly higher for the plants exposed to 5 and 10 kJ m^{-2} UV-B compared to the control plants (Fig. 2C). The values at 15 kJ m^{-2}, however, were not significantly different from that of the control plants.

3.3. Leaf chlorophyll and UV-B absorbing compounds (phenolics)

Total leaf chlorophyll and phenolic concentrations exhibited a significant UV-B × G interaction (Table 1). Averaged over UV-B levels, the Chl concentration showed significantly lower value in most of genotypes at 15 kJ m^{-2} d^{-1} UV-B treatments (Fig. 2D). However, this reduction was not significant in Prima and UCR-193. Phenolic concentrations, on the other hand, increased significantly at elevated (5 and 10 kJ m^{-2} d^{-1}) UV-B radiation with a highly significant genotypic variation that ranged from 12% (UCR-193) to 45% (Prima) when averaged over UV-B treatments (Table 1 and Fig. 2E). There was also a significant UV-B × G interaction for phenolic concentration. The CUVRI for phenolic concentration also varied among cowpea genotypes and increased under UV-B treatment (Table 1).

3.4. Cell membrane thermostability

Significant UV-B × G interaction was observed in CMT (Table 1). The elevated UV-B radiation, in general, caused significant decreases in CMT in most of the genotypes except CB-46 and UCR-193 (data not shown). Maximum decrease in CMT was recorded in Prima (18%) followed by CB-27 (8%) when averaged across UV-B treatments.

3.5. Flower morphology and pollen viability

The appearance of the first flower ranged from 25 to 33 DAE across genotypes and treatments. The 15 kJ m^{-2} UV-B treatment delayed flower initiation by three days in CB-5 and CB-46, while it was three days earlier in CB-27 as compared to the control. Variable degree of flower shedding was observed mostly in the plants grown under UV-B treatments. In general, all genotypes under UV-B treatments produced significantly smaller flowers that caused
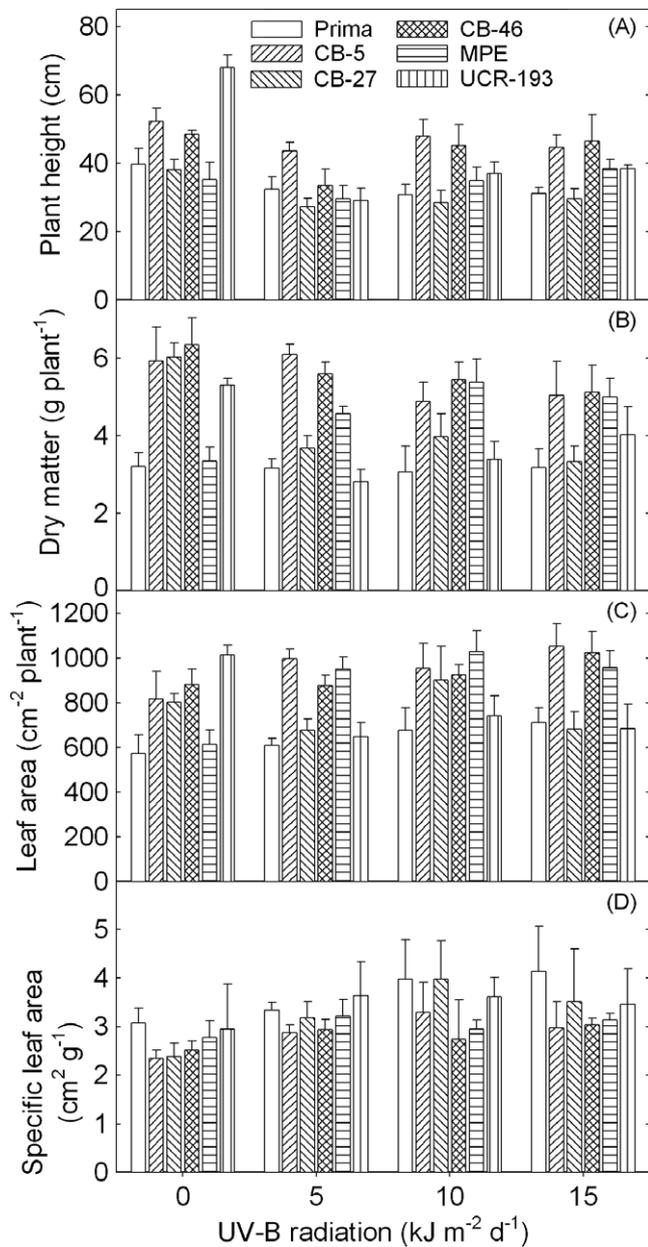
Fig. 1. Influence of UV-B radiation on (A) plant height, (B) plant dry matter, (C) leaf area and (D) specific leaf area of six cowpea genotypes. Error bars show standard deviation from five replicates.

Fig. 2. Influence of UV-B radiation on the leaf (A) photosynthesis (P_{net}), (B) electron transport rate (ETR), (C) chlorophyll fluorescence (Fv′/Fm′), (D) chlorophyll and (E) phenolics concentrations of six cowpea genotypes. Error bars show standard deviation from three replicates.

significant reduction in Fl Dwt across UV-B radiation treatments (Table 1 and Fig. 3A). Flower length ranged from 22.5 (Prima) to 27.4 mm (CB-5) showing a significant UV-B × G interaction (Table 1). PV also showed a significant UV-B × G interaction causing a variable degree of responses among the genotypes (Fig. 3B). PV reduction was more pronounced in CB-27 at 15 kJ m^{-2} d^{-1} as compared to the control, while minimal or no significant reduction was observed in CB-5, MPE and UCR-193. Flowers produced under controlled condition exhibited maximum percentage of pollen viability with a significant variability among genotypes. Mostly, these flower attributes exhibited negative CUVRI in all genotypes (Table 1).

3.6. Pod production and yield components

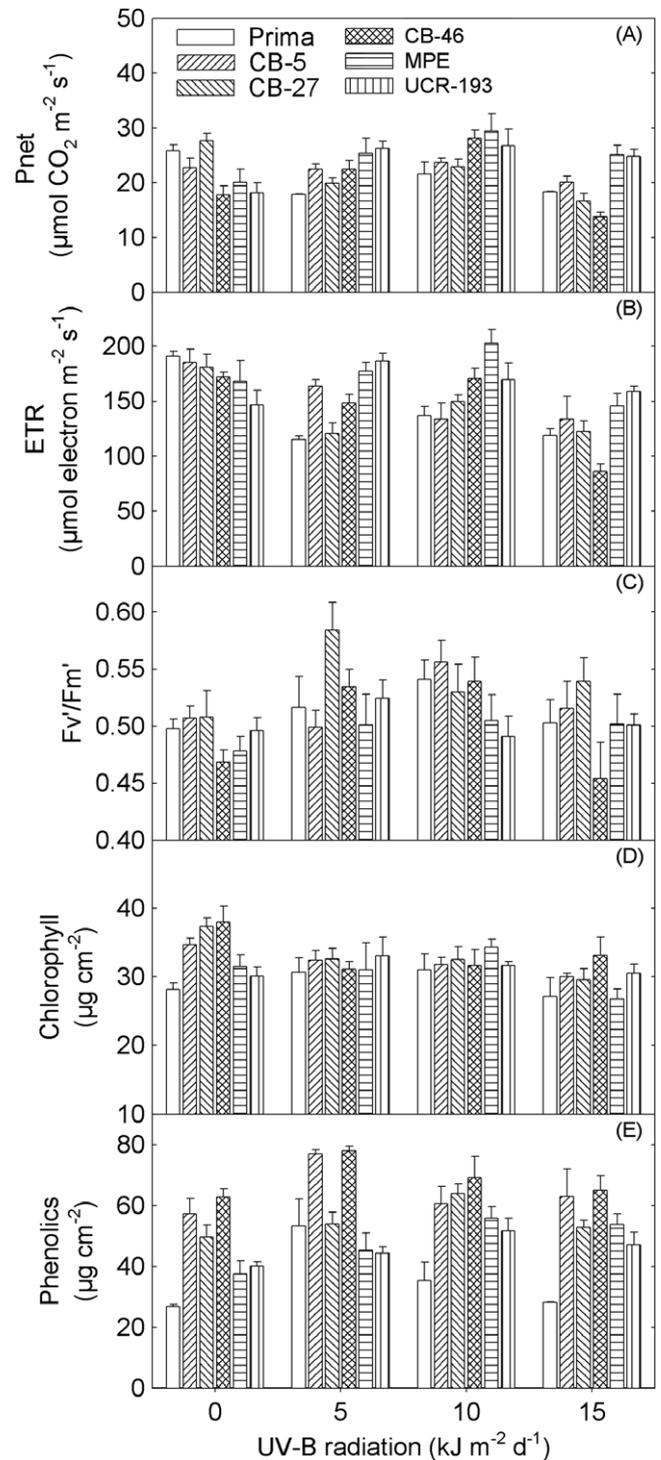Sixty days after sowing, almost all the pods were physiologically mature with a fewer number of small green pods. The number of pods and Seed Wt showed no interactions between UV-B × G. For CB-27, CB-46 and UCR-193, these traits exhibited a linear decrease as UV-B increased from 5 to 15 kJ m^{-2} d^{-1} (Table 1 and Fig. 3C–D). The reductions in average Seed Wt over UV-B treatments varied from 11% (MPE) to 48% (UCR-193) among the genotypes. Individual seed weight (g seed^{-1}) showed a significant UV-B × G interaction and genotypes Prima, CB-27 and UCR-193 exhibited significant reduction when averaged across UV-B levels (Table
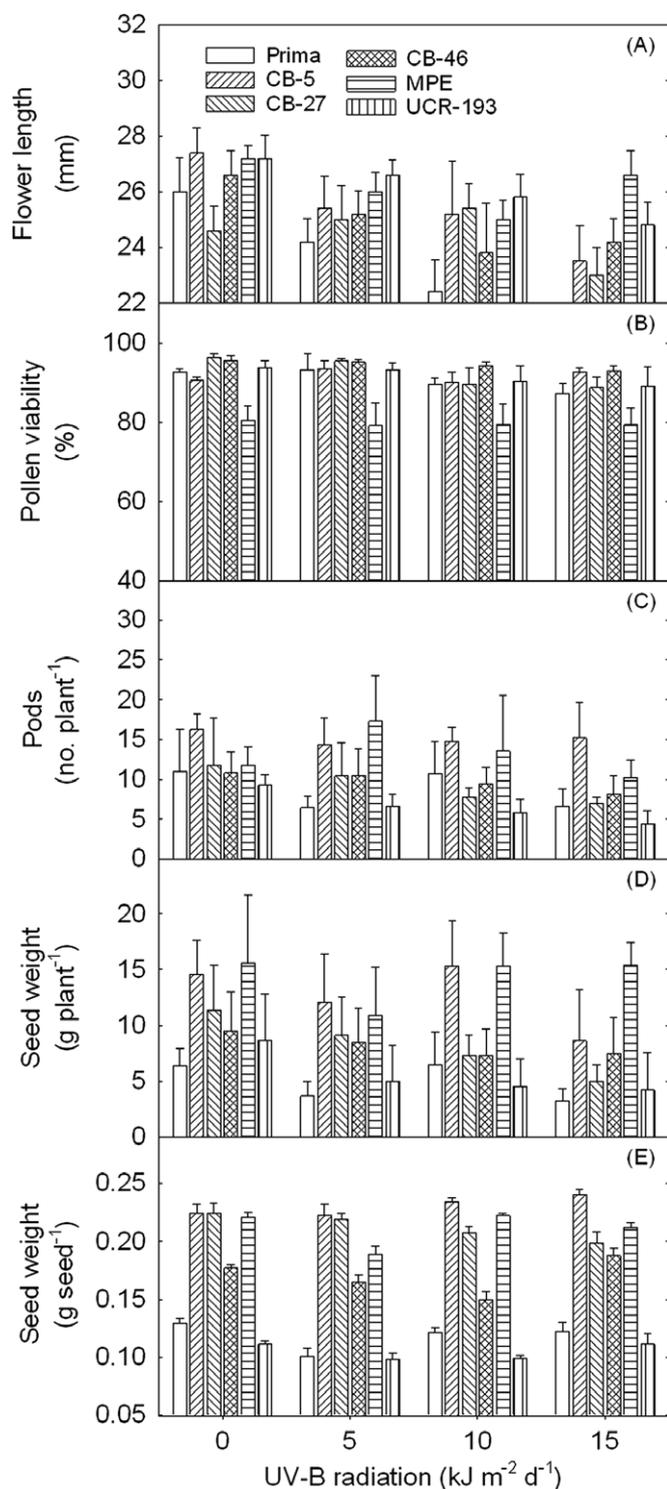
Fig. 3. Influence of UV-B radiation on (A) flower length, (B) pollen viability, (C) pod number, (D) seed weight and (E) individual seed weight (g seed^{-1}) of six cowpea genotypes. Error bars show standard deviation from five replicates.

1 and Fig. 3E). The CUVRI for pod production and seed weight were highly negative for all genotypes except MPE (Table 1).

3.7. Principal component analysis (PCA)

3.7.1. Plant attributes response to UV-B radiation

PCA effectively summarized the total variability (74%) of 18 measured plant attributes into first three principal components (PCs), which individually accounted for by 41% PC1, 26% PC2 and 7% PC3 variability. Because of their high contribution in explaining the variability present in genotypes due to the UV-B effect, PC1 and PC2 were considered the most important dimensions of UV-B responsiveness. Therefore, PC1 and PC2 were regressed against plant attributes to find the contributing traits to the main response patterns of UV-B radiation. Fourteen plant attributes were significantly ($P < 0.05$) correlated with either PC1 or PC2 (Table 2). The plant DM production, Fl Dwt, phenolic concentration and Pod no. were strongly and significantly ($P > 0.001$) associated with changes in PC1 scores indicating the contribution of these plant attributes in determining the responsiveness of cowpea genotypes to UV-B radiation. Similarly, PH, LA, Seed Wt and Seeds pod^{-1} were strongly associated with PC2. The lower score of PC1 and PC2 (the negative proportion of the axis of PC1 and PC2) were characterized by greater decrease in DM production, Fl Dwt, Pod no. and Seed Wt. This was accompanied by short-stature plants, reduced LA, P_{net}, Chl concentration and individual seed weight (Table 1 and Fig. 4). Plant attributes such as Fv′/Fm′, CMT, Fl length and PV did not show any significant correlation with either of the first two PC scores.

3.7.2. Genotype response to UV-B radiation

The PCA also accounted for genotypic variability at all three levels of elevated UV-B treatments separately. The biplot of PC1 vs. PC2 clearly displayed the response patterns between the genotypes and doses of UV-B radiation (Fig. 4). Genotypes exhibiting stronger negative UV-B induced responses were located towards the negative end of the PC1 and PC2, whereas, the positive end of PC1 and PC2 represents the tolerant genotypes. Except CB-27, the three elevated (5, 10 and 15 kJ m^{-2} d^{-1}) levels of UV-B radiation did not show any distinct patterns of reduction among the genotypes studied. However, the collective effect of elevated UV-B radiation appeared to be uniform on each genotype, as deduced from the separation of some genotypes form others (Fig. 4). For instance, pronounced UV-B responsiveness could be observed for MPE (highest scores for PC1; also relatively higher UV-B tolerance), CB-27

Table 2
Pearson's correlation coefficient (r) for the 18 plant attributes representing a measure of UV-B responsiveness to the first two principle components (PC 1 and PC2) and combined total stress response index (C-TSRI)

Plant attribute	Principal component	
	PC1	PC2
Plant height	0.24	0.85[***]
Dry matter plant^{-1}	0.83[***]	0.34
Leaf area plant^{-1}	0.50[*]	0.59[**]
SLA	−0.90[***]	−0.16
P_{net}	0.46[*]	−0.39
ETR	0.42[*]	−0.31
Fv′/Fm′	−0.17	0.19
Chlorophyll	0.58[*]	−0.26
Phenolics	0.68[***]	−0.31
CMT	−0.19	−0.08
Flower length	−0.37	−0.19
Flower dry weight	0.77[***]	−0.16
Pollen viability	0.22	−0.02
Pod number plant^{-1}	0.65[***]	0.42
Seed weight plant^{-1}	0.41[*]	0.62[**]
Individual seed weight	0.03	0.55[**]
Seed number pod^{-1}	−0.43	0.67[***]
Shelling percentage	−0.38	0.49[*]
C-TSRI[a]	0.85[***]	0.18

[***], [**] and [*] represent $P \leqslant 0.001$, $P \leqslant 0.01$ and $P \leqslant 0.05$, respectively.
The data for 18 plant attributes were the same as used in the PC analysis obtained from the six cowpea genotypes using ultraviolet response index (UVRI) of three levels of UV-B treatments (5, 10 and 15 kJ m^{-2} d^{-1}) against control (0 kJ m^{-2} d^{-1}).
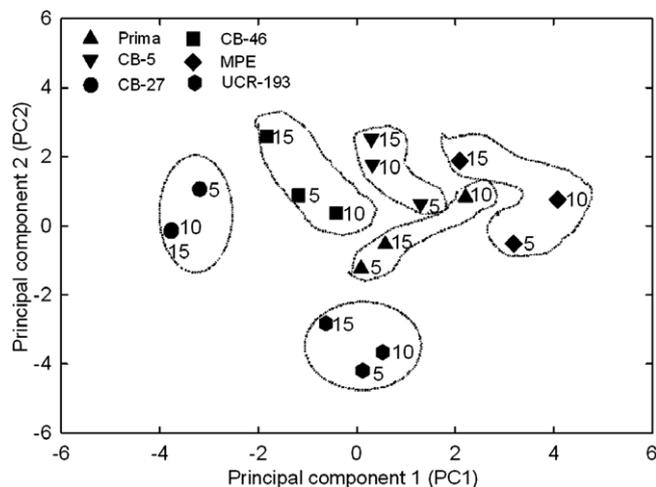[a] C-TSRI is the combined total stress response index as in Table 1.

Fig. 4. Principal component analysis (PCA) of 18 UV-B response variables (RVs) in six cowpea genotypes. The UVRI calculated for the three UV-B levels (5, 10 and 15 kJ m^{-2} d^{-1}) against control (0 kJ m^{-2} d^{-1}), were used. The biplot of first two principal component (PC) scores; PC1 and PC2 are shown. The numbers 5, 10 and 15 associated with symbols represent UV-B radiation treatments of 5, 10 and 15 kJ m^{-2} d^{-1}, respectively.

and UCR-193 (the lowest scores for PC1 and PC2, respectively; also UV-B sensitiveness) positioned on the right, left and middle bottom coordinates, respectively, as shown in Fig. 4. However, other genotypes appeared to be clustered in the center of the plot.

3.8. Cumulative ultraviolet response index

The CUVRI representing the overall effect of UV-B radiation of individual parameters against the control showed varying degree of sensitivity of cowpea genotypes. For the plant vegetative attributes, the lowest CUVRI was recorded for the DM production (−127, CB-27) followed by PH (−121, UCR-193). The highest positive CUVRI was recorded for SLA (+162, CB-27) followed by phenolic concentrations (+152, Prima) (Table 1). V-TSRI, a measure of overall genotypic responsiveness to UV-B radiation for vegetative growth, varied from +329 (MPE) to −295 (CB-27). In contrast to the vegetative and physiological traits, the reproductive plant attributes responded negatively showing the highest negative CUVRI for Fl Dwt (−188, CB-27) followed by Seed Wt (−143, UCR-193). R-TSRI, a measure of overall genotypic responsiveness to UV-B radiation for reproductive growth, of all the genotypes was negative indicating the most damaging effects of UV-B was on the reproductive growth and yield components of cowpea (Table 1). The R-TSRI varied from -78 (MPE) to -405 (CB-27). There was no significant correlation between V-TSRI and R-TSRI ($R^2 = 0.32$; $P = 0.24$). The C-TSRI which combines UV-B responsiveness of all the vegetative (V-TSRI) and reproductive (R-TSRI) plant attributes showed a greater magnitude of genotypic variability ranging from +251 (MPE) to −700 (CB-27). To understand the contribution of individual plant attributes to the over all UV-B treatments, the CUVRI of each of the 18 variables were correlated with C-TSRI. Only four variables, DM ($r = 0.75$, $P = 0.05$), SLA ($r = −0.87$, $P = 0.02$), Fl Dwt ($r = 0.78$, $P = 0.06$) and Pod no. ($r = 0.8$, $P = 0.05$) showed a reasonable correlation with C-TSRI. Genotypes were classified based on C-TSRI representing the total response over UV-B treatments as tolerant (C-TSRI > −74; MPE), intermediate (C-TSRI −74 to −387; CB-5, CB-46 and UCR-193) and sensitive (C-TSRI < −387; CB-27 and Prima,). The C-TSRI were strongly correlated ($r = 0.85$; $P < 0.001$) with PC1.

4. Discussion

4.1. Vegetative performance

Among the C$_3$ species, leguminous crops, particularly grown in the tropical regions, have been reported to be heavily influenced by both ambient [13,43] and elevated [9,24,26,28] UV-B radiation because of the thinner O$_3$ column and acute angle of the sun at these regions [4,24]. Reduction in over all plant size and changes in leaf morphology such as upward leaf cupping and development of chlorotic regions on the leaves of leguminous crops are common characteristic features caused by UV-B radiation [11,27]. Substantial reduction in PH and DM production caused by elevated UV-B reported in several tropical legumes are similar to the current study [9,10,24]. Pal et al. [13] reported 37% shorter plants with 27% reduced dry weight in a 65-day period for *Vigna radiata* plants. The alteration in PH, leaf thickness and morphology observed in our study may be partially due to the photo-oxidation of indole 3-acetic acid (IAA), a growth hormone that absorbs UV-B and involved in cell division and cell elongation processes [13,27]. The thinner leaves observed at elevated UV-B might have allowed increased direct transmittance of UV-B radiation deep into the sensitive tissues over time making plants more vulnerable to UV-B [24,44].

4.2. Photosynthesis, pigment and UV-B absorbing compounds

Large uncertainties exist regarding the photosynthetic performance of plants exposed to UV-B radiation. In current study, UV-B-induced significant reduction of P$_{net}$ observed in sensitive genotypes (Prima and CB-27) is in accordance with the earlier reports in many leguminous species [9,27,45]. Similarly, significantly decreased ETR observed in the same genotypes, which accounted 8–10% larger reduction than that of P$_{net}$, is in accordance with a previous study with the same species [29]. Mackerness et al. [46] pointed that the involvement of reactive oxygen species (ROS) in UV-B signaling pathway may lead to the down-regulation of photosynthesis. However, a significant increase in P$_{net}$ in tolerant genotypes (MPE and UCR-193) contrasted these findings suggesting genotypic variability in cowpea. Also, an increased Fv'/Fm', on exposure to UV-B, clearly contrasts the results obtained by Lingakumar et al. [10]. This supports the view questioning the key role of PSII inhibition in response to UV-B [47,48]. Except Prima, genotypes such as MPE and UCR-193 with increased phenolic concentrations were more tolerant to the UV-B. Phenolic compounds have been reported to screen UV-B radiation [44,47]. Exposure of many tropical legumes to UV-B (simulating 15–25% O$_3$ depletion) has shown a 5–50% increase in UV-B absorbing compounds in leaves which generally is accompanied by 5–30% reduction in total chlorophyll content [9,17,27,28,44] similar to our results.

4.3. Reproductive performance

The delay in flowering at 15 kJ m^{-2} d^{-1} UV-B observed in the present and previous studies in other legume species [23,43,49,50] might be attributed to the impact of high UV-B on the gibberellins biosynthesis as reported by Saile-Mark and Tevini [23]. The severe effect of UV-B on Fl length and Fl Dwt is not uncommon. In a similar study on the soybean plants, Koti et al. [18] reported a drastic reduction in flower components and pollen germination. However, the smaller effect of UV-B on PV in MPE and UCR-193 observed in our study are in agreement with other studies, although precise mechanisms are not clearly understood [51]. The substantial (11–48%) reduction in seed yield which was more pronounced at the highest UV-B (15 kJ m^{-2} d^{-1}) treatment is in close agreement with other studies including *Phaseolus vulgaris*

(50%) [23], *Vigna radiata* (76%) and *Phaseolus mungo* (62%) [24,43]. Rajendiran and Ramanujam [50] also reported smaller and fewer seeds per pod along with reduced pod numbers (25%), seed weight (45%) and shelling percentage (7%) in *Vigna radiata* exposed to UV-B radiation. Compared to the highest dose in the current study (15 kJ m^{-2} d^{-1}), the UV-B doses used by Singh [24] and Rajendiran and Ramanujam [50] were lower (10.08 and 12.2 kJ m^{-2} d^{-1} simulating 15% and 20% O$_3$ depletion, respectively), but the damaging effect of UV-B on reproductive parameters was much greater than the reductions observed in this study. This could be explained by the fact that in the previous studies, UV-B was applied intensely over a 2-h period, each day, compared to the 8-h period in our and other studies [53]. It is apparent from the present study that the UV-B exposure caused more damage to the reproductive performance than vegetative structures in cowpea. This appeared to be due to smaller flowers with lower dry weight, and a noticeable decrease in pollen viability. Saile-Mark and Tevini [23] found that UV-B induced lower yield components were associated with fewer numbers of flowers produced along with lower pod set, and seed weight. In the present study, a gradual decrease in the Pod no. and Seed Wt was also observed in the genotypes, CB-5, CB-46 and UCR-193 as UV-B increased. The increase in allocation of carbon resources towards repair mechanisms and biosynthesis of UV-B absorbing compounds at the expense of the reproductive structures might also contribute for the reduction in flower characteristics and seed yield [18].

4.4. PCA: plant attributes and genotypes response to UV-B

The association of 14 measured plant attributes with the main UV-B responsive components of PC1 and PC2 supports the observed responsiveness of similar parameters to UV-B in other crops [23,24,28]. The plant biomass production and yield characteristics (e.g. Fl length, Fl Dwt, Pod no., Seed Wt, individual seed weight, and seed number per pod) were the most determining factors controlling the overall UV-B responsiveness in cowpea, as shown from the relatively higher significant correlation ($P < 0.01$) with either PC1 or PC2. Whereas photosynthetic parameters, CMT, PV and shelling percentage exhibited none or less significant ($P < 0.05$) correlation with PC scores indicating their lower contribution in determining UV-B responsiveness in cowpea. Additionally, the strong correlation of phenolic compounds with PC1 (Table 2) also supports previously observed defense role of phenolic compounds from UV-B damage [24]. The importance of SLA was reflected possibly due to its contribution to increased sensitivity caused by reduced leaf thickness [45]. The pronounced genotypic responses associated with UV-B doses could be observed from the biplot of the first two PCs. A negative trend was observed only in CB-27 and CB-5 in response to increasing UV-B radiation as seen along the axis of PC1 (Fig. 4). However, other genotypes did not show a distinct pattern over the range of UV-B. It is evident from Fig. 4 that the collective effects of three elevated UV-B on different genotypes were confined to a certain location in the plot reflecting genotypic variability. Regardless of the doses of UV-B radiation, it is evident (Fig. 4) that MPE with its location at the positive end of PC1 axis and towards the positive side of PC2 was the most tolerant whereas, CB-27 which is located at the negative end of the PC1 axis and its placement towards the negative side of PC2 axis is the most sensitive genotype to UV-B radiation.

4.5. Vegetative vs. reproductive performance and classification of genotypes

The TSRI used to assess the quantitative effects of UV-B radiation in the current study was equally effective as in other crops [22,23,52]. The high negative CUVRI values for DM, Pod no. and

Seed Wt seems to be the most highly affected plant attributes by UV-B radiation (Table 1). The genotype MPE performed well vegetatively (e.g. +329, V-TSRI) had the lowest reduction in the overall reproductive parameters (e.g. −78, R-TSRI). Similarly, the genotype CB-27 with the lowest V-TSRI (-295) was also the highly affected in the overall reproductive performance (−405, R-TSRI). This indicates that there is an association between vegetative parameters and reproductive parameters with regard to the relative impact of UV-B on some of the studied cowpea genotypes. However, there was no significant correlation ($R^2 = 0.32$; $P = 0.24$) between V-TSRI and R-TSRI, when all genotypes were included. A differential sensitivity of vegetative and reproductive responses to UV-B was observed in the current study as shown by highly negative values of R-TSRI compared to the positive and/or less negative values for V-TSRI. Similar differential response patterns were also observed in soybean exposed to UV-B [52]. C-TSRI which combined the response of both vegetative and reproductive plant attributes varied greatly among the genotypes in negative direction except for the genotype, MPE. Large intra-specific variabilities in responses to UV-B radiation have also been reported in bush bean [23], rice [22] and soybean [18]. The highly significant correlation ($r = 0.85$, $P < 0.001$) between C-TSRI and PC1 which clearly indicates the usefulness of C-TSRI as a mean for relative classification of genotypes in response to UV-B tolerance. Based on C-TSRI, MPE was considered as the most UV-B tolerant, whereas CB-27 was classified as the most UV-B sensitive genotypes.

Although, spatial and temporal differences for natural UV-B doses received on the earth surface exist for the regions where the genotypes were developed (US cultivars receiving comparatively lower than African or Indian cultivars) [4,8], genotypic tolerance to UV-B could not be traced to the site of origin. The overall positive response of MPE to UV-B may partially be explained by the semi erect nature of the plants, faster growth habit, higher yielding capacity and resistant to multiple diseases [34]. These traits might have resulted in comparatively less UV-B radiation interception and more tolerance nature resulting in a better performer across several UV-B doses. Studies have demonstrated that leaf broadness and angle of the leaves play important roles in determining the sensitivity of the crop to UV-B radiation [13,49]. A trait-based breeding strategy that incorporates superior traits such as leaf erectness, more synthesis of UV-B absorbing compounds and high yield potential present in the modern and wild relatives of crop species into development of a new variety is needed in order to cope with the current and projected UV-B radiation levels.

Examination of the effect of UV-B on the individual plant attributes (CUVRI) in correlation with C-TSRI did not show a discrete parameter that can exclusively be used for screening purpose. However, plant DM, Fl Dwt, Pod no. and SLA seems to have reasonable contribution in the overall UV-B responsiveness of cowpea genotypes. These are among the plant attributes that also showed a strong correlation with the main component of UV-B responsiveness; PC1 in the PCA analysis (Table 2). The high UV-B responsiveness of plant biomass production, flower characteristics and fruit set in soybean genotypes and bush bean have also been reported [18,22,36,52]. In the presence of stress, plants use more energy to produce DM, which might cause insufficient partition of carbon skeletons towards the flower and pod production. The role of increased SLA is difficult to explain other than that the reduced leaf thickness might have increased the plant sensitivity to UV-B. Increased phenolic compounds are one of the most widely occurring responses or defense mechanisms in plants upon UV-B exposure [16]. Perhaps, the association of phenolic concentration with PC1 indicates its role for early selection of UV-B tolerance in cowpea populations during selection process. However, there are no studies that have used phenolic accumulation in plants for screening

purposes [52]. The results from this study suggest that the reproductive traits should be taken into consideration while cowpea genotypes are subjected to selection for UV-B tolerance.

The current study is conducted under ambient PAR conditions in SPAR units (transmit >95% solar irradiance) which transmitted 12% of UV-A (315–400 nm) radiation, and plants grown in the control unit did not receive UV-B. Also, cowpea plants were kept free from any bacterial symbiotic relationship to avoid any unwanted biotic interaction in this study. Caldwell et al. [19] reported that at ambient PAR, UV-A did not appear to be required for UV-B damage mitigation in soybean. In our study, plants were received less than 15 MJ m^{-2} d^{-1} on only six days and in on most days, the daily PAR was above 1000 µmol m^{-2} s^{-1}. The reports from previously published studies indicate that UV-B did not cause significant alteration in the symbiotic function of other legumes including cowpea [26,53]. Therefore, it is inferred that the data obtained from the current study should represent only the effects of UV-B radiation on cowpea. The negative plant response observed under elevated UV-B in this study may not be related to the balance of UV-B and PAR ratio similar to the observation in bean plants under high radiation levels [45]. However, square-wave UV-B delivery system that we used may exacerbate the damage that is typically observed in plants grown in growth chambers with much lower PAR than we typically see in the nature or as in our experiments [19,47]. In the view of the recognized limitations of controlled environmental studies, they have been widely used in UV-B experimentation and screening for UV-B responses across a wide range of plant species [18,19,21]. However, precautions are needed while extrapolating the results from this study due to the limited number of genotypes studied.

In conclusion, the current study revealed that most of the cowpea genotypes are sensitive to the current and projected UV-B radiation. UV-B exposure to the studied genotypes was greatly harmful to the plant reproductive growth in addition to the pronounced effects on DM production. The TSRI of vegetative and reproductive plant attributes tend to respond positively and negatively, respectively, indicating tolerance mechanisms in both processes operate differently. Therefore, it is possible that the selection based only on vegetative traits for UV-B tolerance may not confer the tolerance to reproductive traits. The differences in sensitivity among the cowpea genotypes imply the options for selecting or developing genotypes with tolerance to a niche environment based on current and projected UV-B radiation. Among the cowpea genotypes studied, MPE was classified as the most tolerant to UV-B due to its overall positive performance and CB-27 was considered as the most sensitive to UV-B because of the highest negative responses to UV-B radiation.

Acknowledgements

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