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Developing screening tools for abiotic stresses using cowpea [Vigna unguiculata (L.) Walp.] as a model crop

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DEVELOPING SCREENING TOOLS FOR ABIOTIC STRESSES USING COWPEA [*Vigna unguiculata* (L.) Walp.] AS A MODEL CROP

By

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Abiotic stresses cause extensive loss to agriculture production worldwide. Cowpea is an important legume crop grown widely in tropical and subtropical regions where high temperature, ultraviolet-B (UVB) radiation and drought are the common stress factors limiting production. Various vegetative, physiological, biochemical and reproductive plant attributes were assessed under a range of UVB radiation levels in Experiment I and in a combination with two doses of each carbon dioxide concentration [CO_2], temperature, and UVB radiation and their interactions in Experiment II by using six cowpea genotypes and sunlit plant growth chambers. The dynamics of photosynthesis and fluorescence processes were assessed in 15 cowpea genotypes under drought condition in Experiment III in pot-grown plants under sunlit conditions. A distinct response pattern was not observed in cowpea in response to UVB radiation form 0 to 15

kJ; however, plants grown under elevated UVB showed reduced photosynthesis resulting in shorter plants and produced smaller flowers and lower seed yield. Increased phenolic compounds appeared to be a defense response to UVB radiation. The growth enhancements observed by doubling of [CO_2] were not observed when plants were grown in combination with elevated UVB or temperature which also showed the most detrimental effects on plant growth and seed yield. Results form Experiment I and II revealed that cowpea reproductive traits were highly sensitive to abiotic stresses compared to the vegetative growth and development. A total stress response index (TSRI) technique, derived from all vegetative and reproductive parameters, was used to screen genotypes for their stress tolerance to UVB or combination of stresses. An increase in water use efficiency while maintaining higher rate of photosynthesis was an important drought tolerance mechanism in tolerant cowpea genotypes. Using principal component analysis technique, four groups of the genotypes were identified for their drought tolerance. Evaluating same genotypes across stress conditions revealed that no single genotype has the absolute tolerance characters to all stress conditions. The identified diversity for abiotic stress tolerance among cowpea genotypes and associated traits can be used to develop tolerant genotypes suitable for an agro-ecological niche though traditional breeding or genetic engineering methods.

DEDICATION

I dedicate this dissertation to my father Sri Haribansh Singh and mother Late Malti Singh. Without their love, affection, teachings, inspiration, encouragement and sacrifices this episode of my life was never possible.

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The author is reminded the words of Sir Isaac Newton (1642-1727), a British mathematician and physicist, “I was like a boy playing on the sea-shore, and diverting myself now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me”.

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CHAPTER I

INTRODUCTION

The world current population of approximately 6.7 billion (U.S. Census Bureau, 2008) has been projected to reach up to 10.75 billion by 2050 (U.N. Population Division, 2008). Agricultural output will have to double over the next 50 years just to keep pace with the rising population. World must develop the capacity to feed the rising human population in next 40 years, predominately in Asia and Africa (Evan, 1998). In addition, this vast increase in productivity must be achieved year after year with the help of technological knowledge and agricultural practices in hand , and in the face of changing climate, diminishing natural resources, and global conflict. This can only be achieved by providing a steady stream of new crop varieties that collectively must yield more than ever before and under harsher conditions that are unprecedented in agricultural history. The raw materials for these new crops are the genes that shape their form and behavior. The vast majority of those genes must be derived from existing plants, varieties and the wild relatives of crops. However, over the past centuries, these vital crop resources have been disappearing (FAO, 1993 and mentioned by Shand, 1997). The recent technological advancements help to evaluate and identify the tolerant genotypes and associated traits that can be used to develop new crop varieties which will confer better performance and stable yields across environments.

Abiotic stress factors are known to affect agricultural crops and in natural habitat crops may be exposed a combination of abiotic factors simultaneously. Of the various abiotic stress factors rising in atmospheric carbon dioxide concentration [CO_2], temperature, ultraviolet-B (UVB) radiation and drought are important factors influencing crop growth and development. Current CO_2 level of approximately 380 µmol mol^{-1} could reach anywhere between 730 and 1020 µmol mol^{-1} by the end of the 21st century (IPCC, 2007). As a consequences of increased [CO_2], the projected increase in global mean air temperature could reach from 2 to 4.5 °C (IPCC, 2007). Current global distribution of averaged erythemal daily dose of UVB radiation between the latitudes 40 °N and 40 °S during summer ranges between 2 and 9 kJ m^{-2} (McKenzie et al., 2007) which is about 3 kJ m^{-2} higher than the much earlier observation carried out in 1994 (Seckmeyer et al., 1995). The change in climate is always associated with changes in pattern and intensity of the precipitation (Giorgi et al., 1998). The interaction between these environmental factors may exacerbate rate and direction of individual climatic stress factors and their effects on terrestrial ecosystems. Increase in yields reported at elevated [CO_2] (Ainsworth and Long, 2005; Kimball et al., 2002) were not observed when plants are grown in combination with high temperature (Reddy et al., 1997; Prasad et al., 2003) or increased UVB radiation (Teramura and Sullivan, 1994; Sullivan, 1997; Zhao et al., 2003). Recently, Lobell and Asner (2003), evaluated the relationship between climatic variation and production of corn and soybean in United States from 1982 to 1998, and found that each degree centigrade increase in average growing season temperature, corn and soybean yield will be reduced by up to 17%.

Cowpea (*Vigna unguiculata* L. Walp.) plays an important role in the cropping system of tropical and subtropical regions of the world, especially in the sub-Saharan Africa, Asia, Central and South America (Singh et al., 1997). Western Africa alone accounts for more than 85% of World's 4.96 million Mt. of cowpea production which can be frequently subjected to high temperature and periods of drought due to dry region and short rainfall season (Singh et al., 1997; Singh, 2004; FAO, 2007). Although, cowpea is considered to be well adapted to high temperature and drought, heat and water deficits experienced at the critical stages can lead to substantial reduction in crop yield (Turk et al., 1980; Shouse et al., 1981; Hall, 2004a). Heat injury in legumes including cowpea is mostly associated with pollen infertility, anther indehiscence and lower pod set (Warrag and Hall, 1983; Singh, 1996; Thiaw and Hall, 2004;). Water stress during flowering can also cause more than 50% reduction in yield due to poor pod formation and seed set, probably caused by limited carbohydrate supply (Turk et al., 1980; Labanauskas et al., 1981). Anyia and Herzog (2004a; 2004b) and Souza et al. (2004) reported a drastic reduction in leaf photosynthesis, thus in dry matter production of cowpea subjected to water stress. Previous studies have reported that cowpea is highly sensitive to UVB radiation (Krupa, 1998; Musil et al., 2002a). Musil et al. (2002a) found that cowpea was exceptionally sensitive to UVB (15% O_3 depletion) among the evaluated 17 species native to or largely grown in South Africa.

The simultaneous occurrence of multiple abiotic stresses are common in natural habitat which causes most of the crop damage rather than the damage caused by single stress factor (Caldwell et al., 2007). The response of plants to multiple abiotic stresses is elusive and cannot be extrapolated from the response of plants to each of these different

stresses applied individually. And it has been emphasized that the effect of abiotic stress combinations should be addressed as if it is a new state of abiotic stress in plants and not simply the sum of two different stresses (Rizhsky et al., 2004; Mittler, 2006). The interaction studies will help to elucidate whether interaction between atmospheric [CO_2] and temperature can counteract the negative effects of UVB radiation and *vice versa*, or whether additive negative effect or greater-than-additive negative effect might occur (Caldwell et al., 2007). Premkumar and Kulandaivelu (2001) reported that enhanced UVB markedly alleviated the adverse effects of magnesium deficiency in cowpea whereas, interactive effects of elevated UVB and high temperature caused deleterious effect on soybean (*Glycine max* L.) growth and development (Koti et al., 2004). Although, there have been many studies on the effects of individual abiotic stress factors separately on crop performance including cowpea, interactive effects have received little attention.

The reduction in dry matter production and yield caused by high temperature, UVB radiation and drought could be due to the effect on both assimilation of CO_2 and on the reproductive development of plants. The mobilization and partitioning of carbohydrates towards the maintenance of vegetative structures under stress condition may have caused starvation and failure of reproductive processes (Warrag and Hall, 1984; Ahmed et al., 1993). Additionally, studies suggest that vegetative and reproductive processes of some plants may respond differently and independently to abiotic stresses (Reddy et al., 1997; Prasad et al., 2003; Koti et al., 2007). It implies that, even a cultivar which performs well vegetatively may not perform equally for reproductive traits under similar stress conditions. Studies evaluating the effects of a combination of abiotic

stresses on vegetative and reproductive growth simultaneously in crops including cowpea are limited.

It will be necessary to explore separately the vegetative and reproductive processes of cowpea plants subjected to abiotic stresses in order to understand the source-sink relationships. There are opportunities for genotypic variation that may be characterized by relatively vigorous growth and more dry matter production along with improved yield potential in the presence of abiotic stresses (Parry et al., 2005). Information is lacking about the interactive effects of multiple abiotic stresses on cowpea and how they affect the various vegetative and reproductive processes. Exposing same genotypes to multiple abiotic stresses provides an indirect approach to evaluate the inherited traits that may confer the tolerance characteristics at a range of environmental conditions. The underlying hypotheses are based on the assumption that the genotypic variability in cowpea may be linked to the adaptation at a range of abiotic stress conditions. We hypothesize that (1) the tolerant characteristics are present in cowpea with genotypic variability, (2) the vegetative and reproductive processes differ in their response to various abiotic stresses and their combination, and (3) genotypes respond dissimilarly to different abiotic stresses and their interactions. The objectives of the study were to (a) evaluate the vegetative and reproductive response of cowpea genotypes to multiple abiotic stresses singly or in combination, (b) develop screening techniques for cowpea tolerance to abiotic stresses, and (c) to determine the consistency of the tolerance for different abiotic stresses in cowpea genotypes.

CHAPTER II

LITERATURE REVIEW

Abiotic stresses and crop yield

Agriculture production and productivity are highly sensitive to changes in climate and weather conditions. Therefore, changes in regional and global climate, particularly the climatic variability, have been implicated to affect local as well as global food, fiber and forest production (Easterling et al., 2007). The atmospheric [CO_2], temperature, rainfall patterns, ozone and ultraviolet-B radiation have been changed since the dawn of Industrial Revolution and scientific community expects such trends to continue well into the future (Houghton et al., 2001; IPCC, 2007). While crop productivity may benefit from rising [CO_2], the increased potential for abiotic stresses such as increased incidence of drought, flooding, heat waves and higher doses of UVB radiation may pose challenges for farmers. Hence, the overall impact of climate change on agriculture will depend on the balance among these factors. These climate change factors have shown to cause reduction in the productivity of many crops on regional and global scale (Teramura, 1983; Lobell and Asner, 2003; Ciais et al., 2005; Lobell et al., 2008). A recent study suggests that due to climate change, Southern Africa could lose the production of approximately 30% of its main crop, maize, by 2030 and in South Asia the loss of many regional staples such as rice, millets and maize could be up to 10% by this period (Lobell et al., 2008). Similarly, Lobell and Asner (2003) estimated that each degree centigrade

increase in average growing season temperature will result in 17% reduction in soybean and corn production in USA. The studies indicate that climate change scenario that include a combination of factors such as heat stress, drought and flooding events reduce crop yields more than a change in a single factor alone (Easterling et al., 2007). Therefore, the abiotic stress factors are expected to interact with each other to influence the productivity of crops in future climates.

The genotype (thus the genetic background) of a plant defines the range of performance of the plant which is determined by a set of heritable traits (Hall, 2001). Consequently, the phenotype produced by particular genotype results from the interaction of these genotypic traits with the environment in which the plant is grown. Therefore, the crop yield is determined by genotypic effect, environmental effect and the effect attributed to the genotype × environment. In the natural habitat, crop plants are subjected to a combination of abiotic conditions that may include one or more stresses such as heat, UVB radiation and drought. The interactions among these factors elicit a variety of responses in plants depending upon the developmental stages in a species. In most of the cases, abiotic stress conditions cause reduction in crop performance and yield. One of the important strategies to cope with the abiotic stresses is to develop new cultivars with tolerance to the abiotic stress conditions that confers minimum yield loss or stable yield under multiple stress conditions. The selection of tolerant cultivars and genetic traits in a population is crucial to develop new cultivars that can adapt to a wide range of environmental conditions. This can only be obtained by subjecting the species of interest to different abiotic stress conditions and determining the responses of various growth and yield-related traits to these stressors. Studies utilizing both vegetative and reproductive

parameters simultaneously under realistic growth condition are limited. Therefore, our understanding of plant processes to a combination of stress factors and their relationships to one another is not well understood (Rizhsky et al., 2004; Koti et al., 2007; Tegelberg et al., 2008).

Crop response to atmospheric carbon dioxide

The projected increase in atmospheric [CO_2] is expected to enhance growth and production of agricultural plants (Easterling et al., 2007). Studies have also shown that the effect of elevated [CO_2] on plant growth and yield may depend on photosynthetic pathway, plant species, growth stage and management practices such as water and nitrogen applications (Jablonski et al., 2002; Kimball et al., 2002; Ainsworth and Long, 2005). Averaged across several species and under unstressed conditions, analysis shows that, compared to the current [CO_2], crop yield increase at 550 μmol mol^{-1} [CO_2] was 10-12% for C_3 crops and 0-10% for C_4 crops (Ainsworth et al., 2004; Gifford, 2004; Long et al., 2004). However, in a recent analysis of the FACE (free-air-carbon-dioxide enrichment) experimental results by Long et al. (2005; 2006) argued that crop responses to elevated [CO_2] might be lower than previously thought, because of overestimation of responses using crop models, while others have suggested that these new analyses are, in fact, consistent with previous findings from both FACE and other experimental settings (Tubiello et al., 2007). It is recognized that the models may overestimate the actual field-level responses due to many limiting factors including disease and insects, weeds, soil type, water and nutrients quality, which are neither well understood at large scales nor well implemented in the models (Easterling et al., 2007). In addition, the increase of

[CO_2] is subjected to a considerable interaction with other climatic factors, therefore, the rising [CO_2] can not be assumed to be a single factor because of the associated changes in the temperature and other climatic factors (Giorgi et al., 1998; Zoltán, 2005) which directly affects crop growth and development.

Growth enhancement under [CO_2] has also been observed in cowpea. Overdieck et al. (1988) used three [CO_2] ranging from pre-industrial era (270 µmol mol^{-1}) to the elevated (650 µmol mol^{-1}) [CO_2] and reported a linear increase in photosynthesis, dry matter production and specific leaf weight of cowpea in response to increasing [CO_2]. Experiments implementing elevated [CO_2] have also shown to increase water use efficiency, and leaf area resulting in more light interception and yield in many legumes including cowpea (Morison and Gifford, 1984). Positive responses of rate of development to [CO_2] in cowpea were also recorded in other studies (Ellis et al., 1995; Morison and Gifford, 1984). However, the elevated [CO_2] did not ameliorate the damaging effects of high night temperatures in cowpea (Ahmed et al., 1993). The availability of high [CO_2] and high temperature has complex interactive physiological effects, and both factors are likely to change in the years to come. Studies with long-term [CO_2] enrichment in association with other abiotic stress are limited in cowpea.

Crop response to ultraviolet-B radiation

Even though UVB (280–320 nm) represents a small fraction (0.5%) of total solar radiation, exposure to UVB at the current and projected levels is known to elicit a variety of responses to all living organisms including crop plants (Teramura, 1983; Runeckles and Krupa, 1994; Teramura and Sullivan, 1994; Caldwell et al., 1998; Kakani et al.,

2003). The changes in [CO_2] and temperature accompanied with emission of ozone depleting compounds such as chlorofluorocarbons (CFCs), methane and nitrous oxide caused by anthropogenic activities, reduces thickness and affects the distribution of stratospheric ozone column (IPCC, 2007). The increase in UVB radiation is closely associated with stratospheric ozone depletion as it absorbs the UVB radiation portion of the solar spectrum (Long, 1991). Relative to the 1970s, the midlatitudes O_3 column losses for the 2002-2005 period were approximately 3% in the Northern and 6% in the Southern hemisphere (WMO, 2007). Current global distribution of mean erythemal daily doses of UVB radiation during summer in most of the cowpea growing regions ranges from 2 to 9 kJ m^{-2} (McKenzie et al., 2007).

Previous reviews and published studies clearly demonstrate the extent of damage caused by both ambient (Teramura, 1983; Caldwell et al., 1989; Teramura and Sullivan, 1994) and elevated UVB radiation (Teramura, 1983; Rozema et al., 1997; Krupa, 1998; Searles et al., 2001; Kakani et al., 2003) on crop growth and yield which vary widely among the species and among the cultivars of the same species. Teramura et al. (1983) reported that more than 70% out of 130 species were significantly affected by elevated UVB in terms of the total biomass production showing a wide range of inter and intra-specific variability. In a statistical analysis of 77 crop species mostly based on the vegetative growth and few yield parameters, Krupa (1998) reported sensitivity of more than 50% crop species including several agriculturally important crops. In a recent review on 129 reports of 35 crop species including cereals, legumes, oil, sugar, fiber and tuber crops, enhanced UVB radiation has been shown to affect most of the crops growth directly through several first order effects (Kakani et al., 2003). These include

photosynthesis, production of defense compounds (UVB absorbing compounds and wax contents) and decrease in vegetative growth, leading to a myriad of secondary and tertiary effects including altered crop growth and development, which in turn, affects light interception that lowered canopy photosynthesis, reduced fruit production and retention, and finally yield.

There are uncertainties concerning realistic influence of UVB radiation on cowpea plants exposed to both above and below ambient levels of UVB radiation as shown in an analysis of the previously published papers. For instance, cowpea plants exhibited remarkable increases in growth parameters under enhanced UVB radiation simulating 15 to 25% O_3 depletion (Nedunchezhian and Kulandaivelu, 1997; Chimphango et al., 2003). Contrary to this, studies simulating 15 to 20% O_3 depletion caused pronounced decrease in biomass production and photosynthetic rates (Premkumar and Kulandaivelu, 1999; Musil et al., 2002a). In a study simulating an exclusion of ambient level of UVB, Lingakumar et al. (1999) found 30-60% increase in various growth parameters (Table 2.1). Cowpea has been reported as highly sensitive to UVB radiation (Krupa, 1998; Musil et al., 2002a). Musil et al. (2002a) found cowpea was exceptionally sensitive to UVB (15% O_3 depletion) among the evaluated 17 species native to or largely grown in South Africa.

UVB-mediated alterations in plant growth and yield were dependent upon species sensitivity and combined response to other abiotic and biotic stresses (Teramura and Sullivan, 1994). The inconsistencies may be explained by either genotypic differences in UVB sensitivity, different environmental conditions under which plants were grown, and/or the intensity of UVB supplementation (Musil et al., 2002b; Kakani et al., 2003). A

bulk of these studies conducted in growth chambers, greenhouses or in the field use different types of exposure systems which may be responsible for intra-specific differential sensitivity of cowpea crops (Runeckles and Krupa, 1994). None of the above experimental procedure included the yield response of cowpea exposed to different levels of the UVB.

Crop response to temperature

Temperature is the most important abiotic factor determining the plant adaptation to different climatic zones and season of the year. Most annual crops can be described as being adapted to either cool season or warm season (Hall, 2001; Cutforth et al., 2007) depending on their temperature range of survival ($T_{max} - T_{min}$; Reddy and Kakani, 2007). Temperature also play a very important role in the determining sowing dates of a crop species based on the seed germination and survival of the seedlings. The minimum threshold for seed germination differ among the crop species (soybean 10 °C, cowpea 18 °C, Upland cotton 16 °C and maize 14 °C; (Ismail and Hall, 1997; Hall, 2001; Cutforth et al., 2007). Similarly, the optimum temperatures depend upon the developmental stage of the plant and species. The optimum temperature for peanut growth and development is between 25 and 30 °C (Williams and Boote, 1995) whereas the optimum for pollen germination and tube growth ranges between 30-34 °C (Kakani et al., 2002). The cardinal temperature for growth and development of a crop species are process dependant (Kakani and Reddy, 2007).

A temperature stress could be anything below and/or above the optimum which influences the functionality and success of the biochemical pathways which may reduce

efficiency of the particular phase of development, resulting in loss of economic yield (Singh et al., 2008). Studies on cowpea and common bean have shown that heat stress during floral bud development can reduce fruit set due to damage to the pollen mother cells, resulting in poor anther dehiscence, reduced pollen number and pollen viability (Warrag and Hall, 1983; Warrag and Hall, 1984; Gross and Kigel, 1994). A negative association between increase in daily mean temperature and reduction in yield has been reported in many crops (Ismail and Hall, 1998; Walton et al., 1999). Lobell and Asner (2003) projected approximately 17% yield reduction in corn and soybean for each degree centigrade increase in average growing season temperature above the optimum in USA.

Many cowpea genotypes are susceptible to high temperature and an increased night temperature is more detrimental to cowpea reproduction compared to increase in day time temperature (Ismail and Hall, 1998). Earlier studies demonstrated that cowpea grain yield decreased linearly as minimum nighttime temperature increased from 15 °C, with 50% reduction occurring at 27 °C (Nielsen and Hall, 1985), whereas a 33 °C day temperature did not affect the pod set. Moreover, the pod set was reduced to zero at a combination of hot day and moderately high night temperatures (36/27 °C) in the heat sensitive genotypes (Warrag and Hall, 1983). The losses in cowpea yield due to high temperature are attributed to bud suppression, flower abortion, reduced pollen viability and pod set (Warrag and Hall, 1983; Warrag and Hall, 1984; Ismail and Hall, 1998). Decreased pollen production and pollen viability at elevated temperature were also found in sorghum (Prasad et al., 2006), soybean (Koti et al., 2005) and kidney bean (Prasad et al., 2002). The reduction in pollen production and pollen viability may be related to degradation of tapetum layer and limited carbohydrate supply to the reproductive

structure which influences the nourishment of pollen mother cell leading to infertile pollen (Warrag, 1994; Prasad et al., 2006).

The inferences from global circulation model simulations indicate that equilibrium Earth's mean surface air temperature (SAT) warming for a doubling of atmospheric [CO_2] is expected to increase by 2 - 4.5 °C (IPCC, 2007). Additionally, it is projected that heat waves will be more intense, more frequent and longer lasting in future warmer climate (Meehl and Tebaldi, 2004). The daily minimum temperatures are projected to increase faster (thus the night temperature) than daily maximum temperature (day time), leading to decrease in diurnal temperature trend (IPCC, 2007). Europe in summer 2003, for example, experienced such an extreme climate anomaly which caused July temperature up to 6 °C above the long-term mean resulting in about 30% reduction in terrestrial gross productivity over Europe (Ciais et al., 2005). The day/night temperature greater than 36/30 °C commonly occur during crop life cycle in most of the cowpea growing regions of the world where the daytime can reach occasionally up to 45 °C (Warrag and Hall, 1983, NCDC 2008; Ismail and Hall, 1998; Hall, 2004a). The projected global temperature increase will subject these locations to even higher temperature regime, particularly the night temperature (IPCC, 2007).

Crop response to drought

Water is one of the most important factors limiting crop production worldwide due the geographical limitation to the availability of irrigation water or occurrence of drought mainly caused by reduced rainfall. The demand for drought tolerant genotypes will increase due to diminishing water resources and alteration in the precipitation

patterns under the climate change scenarios (Longenberger et al., 2006; Christensen et al., 2007). Understanding the detrimental effects of drought on plant processes and identifying the tolerant mechanisms will be helpful for breeders to develop tolerant genotypes.

Difficulties in the past have been associated with the identification of physiological traits that could be used as indicators of drought tolerance (Longenberger et al., 2006). However, various plant characteristics such as water use efficiency (Condon et al., 2002), root characteristics (Basal et al., 2003), canopy temperature (Patel et al., 2001), leaf water potential and leaf relative water content (Chiulele and Agenbag, 2004) and stomatal conductance (Bota et al., 2001; Flexas et al., 2002; Medrano et al., 2002) have been used as possible indicators to assess drought tolerance in crop species. Understanding the mechanisms of drought tolerance in crop species, particularly those adapted to dry conditions will help to improve their agronomic performance by incorporating the superior traits into new species or cultivars (Clavel et al., 2005).

The annual rainfall could be less than 2.4 cm in some of major cowpea production zones such as Mali (NCDC, 2008). In the dry year of the cowpea growing rain-fed regions such as Sahelian zones, Senegal and Sudan the average rainfall is only about 17.5-20 cm. While the wetter regions of Sahelian zone experience approximately 38-58 cm rainfall (Hall, 2004b). Cowpea is inherently more drought tolerant crop than many other crops, but it also suffers from the scanty and irregular rainfall causing substantial reduction in seed yield as well as biomass production in major cowpea growing regions (Singh, 2004).

Previous studies have shown the detrimental effect of drought on vegetative growth and yield of many crops including cowpea (Thiaw et al., 1993; Anyia and Herzog, 2004a; Souza et al., 2004). Water stress during flowering causes extensive loss in yield due to poor pod formation and seed set probably caused by limited carbohydrate supply (Turk et al., 1980; Labanauskas et al., 1981). As an adaptive response, cowpea plants have shown dehydration avoidance by maintaining high leaf water status without substantial osmotic adjustment (Bates and Hall, 1981; Shackel and Hall, 1983; Souza et al., 2004). However, reduced leaf water status has also been reported in few studies (Anyia and Herzog, 2004a). Such water conservative nature of the plants has been described as 'isohydric', and has also been found in other crops such as maize, sugarcane and grapes (Jones, 1998; Tardieu and Simonneau, 1998; Medrano et al., 2002). Although, few studies have reported substantial reduction of leaf relative water content and/or leaf water potential in cowpea under drought conditions (Anyia and Herzog, 2004b; Chiulele and Agenbag, 2004), the response pattern of photosynthetic parameters in relation to the intensity of drought or stomatal conductance were similar indicating a leaf water stress independent, but soil water controlled stomatal regulation in cowpea (Souza et al., 2004). Crop adaptation to rainfed condition can be achieved by improved water use efficiency or by increasing water supply to plant through improved root system (Hall, 2004b). Intrinsic water use efficiency estimated as ratio of carbon assimilation to stomatal conductance has been well recognized as a measure of carbon gain per unit of potential water loss (Condon et al., 2002; Lefi et al., 2004). Large variability in water use efficiency has been reported among the species as well as cultivars of a species (Hall et al., 1990; Martin and Ruiz-Torres, 1992; Brodribb, 1996; Condon et al., 2002). Increase in water use efficiency

with stable or improved carbon assimilation rate during crop growth under water stress condition has been recognized as one of the best strategies to improve crop yield under drought conditions (Parry et al., 2005).

Changes in climate will also bring precipitation extremes and drought on regional scale causing flooding and drought in certain areas (Giorgi et al., 1998). Decrease in precipitation are predicted by most of the model simulations by the end of the 21st century in the subtropical regions (IPCC, 2007). Whereas increase in the precipitation extremes are also very likely in major agricultural production areas in Southern and Eastern Asia, East Australia and Northern Europe (Christensen et al., 2007). The 2003 summer drought in Europe caused severe reduction in corn yield in Eastern Europe (Ciais et al., 2005), and forest biomass productivity in Southern Europe (Gobron et al., 2005).

Crop response to multiple abiotic stress factors

In natural habitat, plants are routinely subjected to a combination of abiotic factors. Such as [CO_2], UVB radiation, temperature, water stress, etc. simultaneously and their performance can be assessed only when plants are grown under multiple abiotic stresses conditions. Many recent studies suggest that temperature and precipitation changes in future decades will modify, and often limit, the direct effect of [CO_2] enrichment on plants (Easterling et al., 2007). For instance, high temperature during flowering may lower CO_2 effects by reducing the potential sinks such as pod numbers, grain number per pod (Reddy et al., 1997; Baker, 2004; Caldwell et al., 2005). Increased temperatures may also reduce [CO_2] effects indirectly, by increasing water demand. Rainfed wheat grown at 450 µmol mol^{-1} [CO_2] demonstrated yield increases with

temperature increases of up to 0.8 °C, but declines with temperature increases beyond this point (Xiao et al., 2005). Future [CO_2] levels may favor C_3 over C_4 plants (Ziska; Ainsworth et al., 2004; Gifford, 2004; Long et al., 2004); however, the opposite is also expected because of coupled increase in temperature, UVB radiation and drought (Reddy et al., 1997; Xiao et al., 2005; Koti et al., 2007).

The experiments designed to explore the interaction among these factors is useful to determine the potential effects of these abiotic stresses on crop plants (Caldwell et al., 2007). In a modeling approach, Runeckles and Krupa (1994) suggested that there may be no interactions between these stress factors as a whole or to certain plant processes and the major variable will override the plant response. Otherwise, there may be an additive effect or may be greater-than-additive effect when the plant response is greater than the sum of responses to the individual factors. Additionally, there is a possibility of a less than additive interaction; for example if [CO_2] and/or temperature stimulate more plant dry matter production and repair processes in UVB sensitive plants as shown in the sunflower and maize seedling in one of the earliest interactive study that involves [CO_2], temperature and UVB radiation (Mark and Tevini, 1997).

In a recent study, Tegelberg et al. (2008) reported no significant interaction between elevated [CO_2], temperature and UVB for the activity of defensive enzymes, growth-regulating polyamines, photosynthetic pigments and soluble protein in silver birch. In contrast, there were significant interactions between these abiotic stresses for most of the vegetative and reproductive parameters in soybean (Koti et al., 2005; Koti et al., 2007). However, none of the studies have evaluated both the vegetative growth and the yield attributes simultaneously under multiple stress conditions. Because of the wide

range of climatic adaptation of cowpea, it will be very useful to study the relative response of vegetative and reproductive plant attributes under multiple environmental conditions projected in the future climatic conditions.

The interaction between abiotic stresses can drastically alter the response mechanisms in plants that may cause a positive or negative or even it can counteract (neutralize) each other's effect depending upon the species. Elevated temperature has shown to alleviate the damaging effect of UVB radiation on various growth parameters in sunflower and corn (Mark and Tevini, 1997) whereas high temperature in combination with UVB resulted in an increased reduction in growth of soybean (Koti et al., 2007). The response of plants to multiple abiotic stresses is unique and should be treated as a new state of abiotic stress rather than a combination of two or more stress factors (Mittler, 2006). One abiotic stress factor evokes a chain of complex metabolic processes in plants in the presence of other stress factor. Developing new crop genotypes of a species with enhanced tolerance to a given stress factor may fail to withstand in the presence of another abiotic stress. Therefore, this concern has been raised to consider the variable effects of possible climate change when developing breeding programs or transgenic plant for abiotic stress tolerance (Hall and Ziska, 2000; Mittler, 2006).

Screening for abiotic stress tolerance

The available genotypic variability of a species offers an opportunity for breeders to design and develop specific plant type to suit in different agro-ecological environments. The effectiveness of selection for a trait depends on magnitude of genetic and non-genetic cause in the expression of phenotypic differences among the genotypes

in a population and expressed as heritability of the trait (Thiaw and Hall, 2004). A thorough understanding of the physiological basis of the differences in stress tolerance could be used to select or create new cultivars of crops that have increased productivity under such conditions (Wentworth et al., 2006). The genetic association of a trait with higher level of physiological and/or developmental attributes facilitates the adaptation of a crop to stress condition and proved to be very useful for breeding purposes and to develop improved lines of a crop species (Singh and Sharma, 1996). Several screening methods such as cell membrane thermostability (CMT) in soybean (Martineau et al., 1979; Blum et al., 2001) and cowpea (Ismail and Hall, 1999), *in vitro* pollen germination in cotton (Kakani et al., 2005) and soybean (Koti et al., 2004), chlorophyll fluorescence in Arabidopsis (Barbagallo et al., 2003), photosynthesis and stomatal conductance in cotton (Lu et al., 1998) and intrinsic water use efficiency and associated gas exchange parameters in almond and wheat (Brodribb, 1996; Condon et al., 2002) have been used at field and laboratory scales to identify tolerant traits and genotypes to abiotic stresses.

Abiotic stresses adversely affect various cellular functions, but photosynthesis is particularly sensitive to heat and drought stress (Berry and Bjorkman, 1980; Brodribb, 1996; Haldimann and Feller, 2005). Fluorescence parameters have been shown to relate directly to the photosynthetic rates of leaves (Genty et al., 1990; Edwards and Baker, 1993) and have been widely used to study leaf photosynthetic performance (Maxwell and Johnson, 2000). Consequently, any small perturbation in photosynthetic metabolism significantly modifies fluorescence characteristics of plants. The sensitivity of chlorophyll fluorescence to the stress-induced perturbation in plants metabolism can make it potentially useful for screening tool for genotypes with differential response to

abiotic factors (Brodribb, 1996; Barbagallo et al., 2003). Previous studies suggest significant changes in the photochemical activities of cowpea leaves subjected to heat (Costa et al., 2003; Costa et al., 2004), UVB (Premkumar and Kulandaivelu, 1996; Lingakumar et al., 1999) and drought conditions (Lopez et al., 1987; Souza et al., 2004).

Advancement in cowpea breeding for dry and hot environments has been achieved by testing for yield of large collections over several locations and years (Hall et al., 1997). Robertson et al. (1985) has used herbicidal band screening techniques to screen cowpea lines with improved rooting for drought tolerance. Progress has been achieved with whole plant screening approach in cowpea for their growth, phenology and reproductive responses to heat (Dow el-Madina and Hall, 1986; Patel and Hall, 1990; Ehlers and Hall, 1996; Ehlers and Hall, 1998).

Hall (2004b) proposed yield component model that can be incorporated for selection of cowpea cultivars in the high temperature limited production zones. Four yield components (number of flowers per unit area, number of pods per flower, number of seed per pod and weight of individual seed) contributing to yield reduction were recognized. In a simple screening approach for heat tolerance, Ismail and Hall (1999) found an association between reproductive-stage heat tolerance and cell membrane thermostability measured as electrolyte leakage from leaves subjected to high temperature treatment. In an extremely hot field environment, negative correlations were observed between grain yield and electrolyte leakage ($r = -0.79$, $n = 9$), and pod set and electrolyte leakage ($r = - 0.89$, $n = 9$) among nine cowpea breeding lines. Whereas, Thiaw and Hall (2004) through genetic selection studies indicated that the heritability of leaf

electrolyte leakage was low and associations with pod set and grain yield under hot conditions were only moderate.

Under drought condition, selection for physiological and biochemical traits that confer adaptation to drought could complement a breeding program that is mainly based on selection for grain yield (Hall, 2004b). Delayed-leaf-senescence trait has been found to enhance adaptation of cowpea in dry condition by improving the capacity to survive and recover from mid-season drought through either greater extraction of soil moisture or drought avoidance by maintaining the leaf water status (Gwathmey and Hall, 1992; Gwathmey et al., 1992). The delayed-leaf-senescence trait is highly heritable and appeared to confer by a major gene (Ismail and Hall, 2000). Selection for delayed-leaf-senescence have not yet been fully developed, but they could be well adapted to the wetter parts of the Sahelian and the dry parts of the Savanna where rainfall is 38-58 cm and there is a high probability of midseason droughts.

Increased concern about abiotic stress effects on crop plants has prompted the screening of tolerance in crop population (Hall, 2001). Many crops have been screened by using various abiotic stress response indices derived from the different stages of plant growth in responses to single or multiple abiotic stresses (Dai et al., 1994; Saile-Mark and Tevini, 1997; Koti et al., 2004; Hubbard and Wu, 2005). Several crops including rice (Dai et al., 1994), wheat (Yuan et al., 2000), bush bean (Saile-Mark and Tevini, 1997), and corn (Hubbard and Wu, 2005) have been screened by using several UVB and drought response indices derived from plant growth responses under UVB or drought conditions. In addition, multivariate analysis such as principal component analysis (PCA) and factor analysis (FA) have efficiently been used for characterizing the stress responsiveness of a

population under study and associated plant attributes. (Hofmann et al., 2001; Kaspar et al., 2004).

The simultaneous occurrences of different abiotic stresses are common in natural plant habitat which greatly modifies the individual stress effect. This modification in the degree of response mechanisms could have been caused due to co-activation of different response pathway by simultaneous exposure of plants to different abiotic stresses leading to a synergistic or antagonistic effects (Mittler, 2006). To develop a crop plant with enhanced tolerance to a stress combination either by traditional breeding or genetic engineering requires an understanding of the complex cross-communication between different signaling pathways and their direct or indirect effects on plant growth and metabolism (Hall, 2004a; Mittler, 2006).

Hall and Ziska (2000) recommended that grain legume breeder should consider the possible climate change when developing a breeding strategy. The grain yield in cowpea can be enhanced by selection of greater reproductive sink under high temperature which will minimize the feedback effect that down regulates the photosynthetic mechanisms (Ahmed et al., 1993; Hall and Allen, 1993). However, yield has lesser importance at first hand in a trait-based breeding program particularly for heat and drought tolerance. The yield reduction caused by abiotic stresses are a consequence of several first order effects such as photosynthetic performance (photosynthesis and fluorescence reduced water use efficiency), morphogenesis (differentiation and developmental rate), production of defense compounds (phenolic compounds, and free amino acids and waxes) affecting over all vegetative growth and dry matter production. Therefore, survival capacity and the maintenance of their normal metabolic activity in the

presence of stress conditions are key features to sustain higher yield and should be considered as an important component of breeding programs.

The molecular genetic mapping of the plant genome have facilitated to the identification of biomarkers that are closely linked to known resistance genes, such that their isolation is clearly feasible in the future (Easterling et al., 2007 and references therein). The temperature and drought stress resistance are especially relevant to climate change. Earlier studies have demonstrated the genetic modifications to major crop species (e.g., maize and soybean) that increased their water deficit tolerance (Drennen et al., 1993; Kishor et al., 1995; Cheikh et al., 2000) although this may not extend to a wider range of crop plants. Little is known about how the desired traits achieved by genetic modification will perform under multiple abiotic stress conditions commonly occur in natural environment. The genomic approach offers new germplasm and understanding, but the emergent nature of yield from physiological processes demands that all components contributing to the yield be considered. It is important to understand the interactions of various regulatory pathways within plants, and between plants and environment in order to understand the key links between gene activity and crop yield (Sinclair and Purcell, 2005). Biotechnology is not expected to replace conventional agronomic breeding (Easterling et al., 2007); however, it will be a crucial adjunct to breeding because both will be needed to meet future environmental challenges, including climate change (Cheikh et al., 2000; FAO, 2004).

Cowpea production

The worldwide production and area of cowpea have increased radically by 280 and 150%, respectively since last 25 years (Fery, 1990; FAO, 2007). In 2006, West African countries, Burkina Faso, Mali, Niger, Nigeria and Senegal, contributed about 90 and 85% of the World's total area (10.7 million ha) and production (4.96 million Mt), respectively. Cowpea was a major agronomic crop in the US during the early part of the 20th century, with production peaking at 2.4 million ha in 1937 (Fery, 1990). In 1961, U.S produced 33500 Mt. cowpea on an area of 51000 ha. However, the introduction of newer types of forage crops and the availability of mechanized harvesting equipment for these newer crops resulted in cowpea production and area dropping to 7400 Mt. and 13500 ha, respectively in 2001 (Fery, 1990; FAO, 2007). The cowpea has long been valued in the southern US as a vegetable crop, and an extensive industry currently exists to supply fresh, canned, frozen, and dry-pack products that are marketed nationwide. Additionally, the cowpea has long been a popular item with home gardeners throughout the south (Fery, 2002).

Botanical classification

Cowpea is a *Dicotyledonea* which belongs to order *Fabales*, family *Fabaceae*, subfaminly *Faboideae*, tribe *Phaseoleae* and genus *Vigna* (Maréchal et al., 1978). *Vigna* is a pantropical genus with several species, whose exact number varies according to authors from 84 to 184 (Padulosi and Ng, 1997). The cutivated cowpeas are grouped under *Vigna unguiculata* subspecies unguiculata, which is subdivided into four cultigroups namely, Unguiculata, Bioflora, Sesquipedalis, and Textilis (Maréchal et al.,

1978; Ng and Maréchal, 1985). Cowpea is a diploid and possess 22 small chromosomes (Faris, 1965). Cowpea plants may be prostate, erect, or climbing to about 1 to 1.5 m. Cowpea germination is epigeal but may lose as much as 90% of their dry weight by the time seedlings emerge (Steele and Mehra, 1980). First leaves above cotyledons are simple and opposite; subsequent leaves are alternate and trifoliate with the terminal leaflet often bigger and longer than two asymmetrical leaflets. The leaves are 5-12.5 cm across and described as linear, lanceolate or narrowly ovate, rounded at the base and gradually tapering to a pointed tip (Duke, 1981). Flowers are born in multiple racemes on flower stalks (peduncles) that arise from the leaf axils. The inflorescence consists of two to eight whitish, yellowish, or violet flowers in pairs produced sequentially on the tip of a slender peduncle. The flower has a bent style, bearded on the inner curve immediately below the oblique stigma, and uniform anthers in two fused groups (diadelphous) around the style. The corolla is dull white, yellow or violet with standard 2-3 cm in diameter and keel is truncated forming a wing structure. The flower has a single ovary with eight to 20 ovules. Cowpea primarily is self pollinating however; flower is attractive to bumblebees and various other insects that forage upon both the nectar and pollen and may cause minor cases of cross pollination. The seeds are in slender pods 20-26 cm long with eight to 20 seeds; vary in size from 0.2 to 1.2 cm, weight from 5-30 g/100 seeds, shape from globular to kidney shaped, texture from smooth or wrinkled, and color from white, green, buff, red, brown, or black and are variously speckled, mottled, blotched, or eyed (Steele and Mehra, 1980).

Origin and domestication

The center of origin also referred to as a “center of diversity” that provides the valuable information regarding to the habitat and climatic adaptation of species and offers the broadest collection of genetic diversity commonly used for the development of new crop species. The center of origin and subsequent domestication of a species is mostly based on historical records, botanical and cytological proof, geographical distribution and presence of wild relatives (Faris, 1965; Steele and Mehra, 1980). Vavilov (1935) reported that India and Ethiopia are the primary countries of origin of cowpea. However, other studies indicated that cowpea might have been originated from West Africa (Rawal, 1975) or Western or Central Africa (Faris, 1965). Although, the regions for first domestication of cowpea is still under speculation, West Africa is considered to be the center of maximum genetic diversity and it has been mentioned in the ancient farming systems dating back to 4-5 thousand years ago (Ng and Marechal, 1985; Davis et al., 1991; Ba et al., 2004). According to the Ng (1995), the species *unguiculata* was first domesticated in West Africa as far back as 2000 B.C.

Cowpea is an annual grain legume widely grown in the tropical and subtropical regions covering a wide range of latitude 44 °N to 35 °S on the globe (Rachie, 1985; Davis et al., 1986; FAO, 2007). Cowpea is known by several names according to regions such as southern pea, black eye pea, and crowder pea (USA), lubia (South Asian countries and Middle East), coupe or frijol (South America), and yard long bean and asparagus bean (China) (Davis et al., 1991). Cowpea is a warm-season crop and adapted to heat and dry conditions. Cowpea is more drought resistant than common bean (Singh, 2004). Drought resistance is one reason that cowpea is such an important crop in many

underdeveloped parts of the world. Cowpea is an extremely resilient crop, and most of the cowpea in West and Central Africa is grown as an intercrop with millet and sorghum. The millet and sorghum provide staple food, fodder, fuel, and thatching materials for the family and cowpea provides cash income as well as protein supplement in the daily diets of people. Cowpea leaves, green pods, green peas, and dry grains are consumed as food and the green as well as dry haulms are fed to livestock, particularly in the dry season when animal feed is scarce.

The cowpea germplasm collection, evaluation and preservation have been one of the important priorities to improve cowpea cultivars across wide a range of environmental conditions. The national agricultural research program located in different parts of the world including Nigeria, Senegal, Tanzania, India and Unites States maintain substantial collections of cowpea germplasm (Singh, 2004). However, International Institute of Tropical Agriculture (IITA) had the largest collection of cowpea germplasm and holds the mandate for improvement of cowpea. Among all legumes, cowpea has the maximum diversity for plant type, growth habit, maturity, seed type and adapted to a wide range of environments where other legume may not produce well (Hall, 2004; Singh, 2004). Therefore, it offers a unique opportunity to develop specific plant type with desired traits that will suit targeted agro-ecological zones by tailoring through breeding and/or genetic manipulations (Hall et al., 2003; Singh, 2004).

CHAPTER III

ASSESSING GENOTYPIC VARIABILITY OF [*Vigna unguiculata* (L.) Walp.] TO CURRENT AND PROJECTED ULTRAVIOLET-B RADIATION

Abstract

The current and projected terrestrial ultraviolet-B (UVB) 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 UVB radiation due to a thinner ozone column in those regions. Better understanding of genotypic variability to UVB radiation is a prerequisite in developing genotypes tolerant to current and projected changes in UVB radiation. An experiment was conducted in sunlit, controlled environment chambers to evaluate the sensitivity of cowpea genotypes to a range of UVB 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 UVB 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 UVB responsiveness of 18 plant attributes measured. The magnitude of the sensitivity to UVB radiation also varied among cowpea genotypes. Plants from all genotypes grown in elevated UVB 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 UVB, whereas the reproductive parameters exhibited a negative response showing the importance of reproductive characters in determining tolerance of cultivars to UVB radiation. However, all cultivars, except MPE, behaved negatively to UVB when a combined response index was derived across parameters and UVB levels. Based on the combined total stress response index (C-TSRI) calculated as sum of individual vegetative, physiological and reproductive component responses over the UVB treatments, the genotypes were classified as tolerant (MPE), intermediate (CB-5, CB-46 and UCR-193) and sensitive (CB-27 and Prima) to UVB radiation. The differences in sensitivity among the cowpea genotypes emphasize the need for selecting or developing genotypes with tolerance to current and projected UVB radiation.

Introduction

Even though ultraviolet-B (UVB, 280–320 nm) represents a small fraction of total electromagnetic spectrum, exposure to UVB at the current and projected levels is known to elicit a variety of responses by all living organisms including crop plants (Caldwell et al., 1998; Kakani et al., 2003). The amount of UVB 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 (WMO, 2007). Current global distribution of mean erythemal daily doses of UVB radiation between the latitude 40 °N and 40 °S during summer ranges from 2 to 9 kJ m^{-2} (McKenzie et al.,

2007) which are comparatively higher than the earlier measurement of 2 to 6 kJ m^{-2} d^{-1} in 1994 (Seckmeyer et al., 1995). The three-dimensional Chemistry-Climate models estimates indicate that ground-level UVB 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 (WMO, 2007). 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 UVB radiation has and will continue to raise interest in understanding the deleterious effects of UVB 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 to 35 °S) on the globe (FAO, 2007; Singh, 1997b). The daily dose of UVB radiation in USA for the month of June-August, 2005 ranged between 0.02 to 8.75 kJ m^{-2} (USDA, 2005), however, on the global scale, maximum UVB radiation could reach up to 8-10 kJ m^{-2} d^{-1} in some cowpea growing regions (Singh, 1996).

Previous reviews and published studies clearly demonstrate the extent of damage caused by both ambient (Lingakumar et al., 1999; Pal et al., 1997; Teramura, 1983; Teramura and Sullivan, 1994) and elevated UVB radiation (Kakani et al., 2003; Krupa, 1998; Rozema et al., 1997; Searles et al., 2001; Teramura, 1983) 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. (2003) reported that enhanced UVB radiation affects most crop growth processes directly through several first order effects including reductions in

photosynthesis and vegetative growth, leading to lower yield. Moreover, UVB in combination with other abiotic stressors can drastically modify the magnitude and direction of plant responses (Krupa, 1998). Premkumar and Kulandaivelu (2001) reported that enhanced UVB, simulating 20% O_3 depletion, markedly alleviated the adverse effect of magnesium deficiency in cowpea, whereas, the impact of elevated UVB aggravated the negative effects of temperature on growth and development of soybean (Koti et al., 2004).

In general, plants may tolerate small increases in UVB by protective mechanisms such as reducing the transmittance of UVB through the epidermis by producing UVB absorbing compounds, scattering and reflecting light, quenching free radicals and photo-repair of sensitive systems such as nucleic acids (Premkumar and Kulandaivelu, 2001; Rozema et al., 1997; Teramura and Sullivan, 1994). Most defense mechanisms appeared to be light dependent such as photo-repair system for DNA and the biosynthesis of UVB absorbing compounds (Adamse et al., 1994; Caldwell et al., 1994; Rozema et al., 1997). Despite the known importance of photosynthetically active radiation (PAR), studies utilizing an unrealistic and unbalanced UVB and PAR ratio for plant growth are not uncommon resulting in unrealistic plant responses (Musil et al., 2002b). However, many species appeared to be more sensitive to the UVB radiation than others even under ambient PAR and such crop species may already be experiencing UVB stress (Lingakumar et al., 1999).

Crop economic yield is an important trait for selection of cultivar for a niche environment. Increased concern about the UVB radiation effects on crops has prompted developing screening tools and methods for tolerance in crop populations (Dai et al.,

1994; Kakani et al., 2003). The large differences among cultivar responses to UVB radiation offer a valuable tool for selection process in response to UVB radiation (Kakani et al., 2003). Many crops have been screened using various UVB response indices which were derived from short-term plant growth responses to UVB (Dai et al., 1994; Koti et al., 2004; Saile-Mark and Tevini, 1997). The reproductive growth and seed yield are important components of plant growth responses to UVB radiation (Koti et al., 2004), but have received little attention. Therefore, a season-long UVB exposure on crop plants is needed to understand the mechanisms and causes for crop yield losses.

Noticeable uncertainties exist concerning influence of UVB radiation on tropical legumes including cowpea plants exposed to both above and below ambient levels of UVB radiation (Chimphango et al., 2003; Lingakumar et al., 1999; Musil et al., 2002a; Nedunchezhian and Kulandaivelu, 1997; Pal et al., 1997; Premkumar and Kulandaivelu, 1999; Singh, 1995; Singh, 1996; Singh, 1997a). For instance, cowpea plants did not exhibit a significant change in plant height, leaf area and dry matter when grown under elevated UVB simulating 15 to 25% O_3 depletion (Chimphango et al., 2003; Nedunchezhian and Kulandaivelu, 1997). Contrary to this, studies simulating a similar O_3 depletion caused pronounced decrease in biomass production and photosynthesis (Lingakumar et al., 1999; Musil et al., 2002a; Premkumar and Kulandaivelu, 1999). These inconsistencies could be partially explained by genotypic differences, different growth environments, intensity and duration of UVB supplementation (Kakani et al., 2003; Musil et al., 2002b). The supplied UVB radiation in these studies represent very small addition 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 UVB radiation (Krupa, 1998; Musil et al., 2002a). Musil et al. (2002a) found that cowpea was exceptionally sensitive to UVB (15% O_3 depletion) among the evaluated 17 species native to or largely grown in South Africa. Earlier studies evaluating the UVB 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 (Chimphango et al., 2003; Musil et al., 2002a; Nedunchezhian and Kulandaivelu, 1997; Premkumar and Kulandaivelu, 1999; Premkumar and Kulandaivelu, 2001). To our knowledge, there are no reports on screening the responses of cowpea genotypes to UVB radiation based on both vegetative and reproductive growth processes. We hypothesized that UVB tolerant characteristics are present in cowpea with genotypic variability and when exposed to UVB, the vegetative traits respond dissimilarly compared 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 UVB radiation and to identify the genotypic variability using several plant attributes and statistical methods.

Materials and methods

Experimental facility

The experiment was conducted in four sunlit, controlled environment chambers known as Soil-Plant-Atmosphere-Research (SPAR, Plate 3.1) 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, UVB 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 UVB radiation and transmit 12% UV-A, and more than 95% incoming PAR (Zhao et al., 2003). 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. (2001).

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 (Fang et al., 2007; Hare, 1991; Warrag and Hall, 1983). The genotypes were seeded in 15 cm diameter and 15 cm deep plastic pots filled with fine sand on 26 July, 2005. After emergence, 7 days after sowing, thirty pots having healthy plants, 5 pots for each genotype and 3 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 μmol mol^{-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 optimum nutrient and water conditions for plant growth through an automated and computer-controlled drip irrigation system.

UVB radiation protocol

A UVB radiation (280–320 nm) treatment of 0 (control; no UVB) and three total daily doses of biologically effective UVB radiation intensities of 5, 10, and 15 kJ m^{-2} d^{-1} were imposed from 8 days after emergence (DAE) to maturity. The square-wave supplementation systems were used to provide desired UVB 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 (Plate 3.2) 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 UVB 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 UVB radiation levels 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 (Caldwell, 1971) as formulated by Green et al. (1974) which was normalized at 300 nm. The simulated O_3 depletion of the four UVB doses was 0, 6, 12, and 24%, respectively, at this location.

Vegetative growth parameters

One plant per pot, 5 pots per genotype, was cut at the soil surface 10 and 18 d after UVB 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

Plate 3.1 General view of the Soil-Plant-Atmosphere-Research (SPAR) Facility at Mississippi State University, Mississippi State, MS that were used in Experiment I and II.

Plate 3.2 Picture showing the measurement of UVB radiation in the SPAR chambers using UV-X meter during the experimental period.

(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.

Photosynthesis and chlorophyll fluorescence parameters

Eighteen days after treatment, leaf net photosynthesis (*A*), 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 µmol mol^{-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 by the equation $[(Fm'-Fs)/Fm'] \times fl\alpha_{leaf}$, 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).

Chlorophyll and UVB absorbing compounds (phenolics)

The total leaf chlorophyll (Chl) and UVB-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 (1987) were used to estimate the Chl concentration where as the phenolic concentration was estimated according to the Kakani et al. (2004) and expressed as equivalent of p-coumaric acid.

Cell membrane thermostability (CMT)

The leaf CMT in cowpea genotypes was assessed on 18 DAT according to the procedure described by Martineau et al. (1979) 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 3^{rd} or 4^{th} 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 minutes at 50°C in a temperature controlled-water bath, whilst the C-set of test tubes were left at room temperature (approx. 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 by using following equation, $\text{CMT\%} = \frac{1-(\text{TEC1}/\text{TEC2})}{1-(\text{CEC1}/\text{CEC2})} \times 100$, where, TEC and CEC are the measure of conductance in treated and controlled test tubes, respectively, at initial = 1 and final = 2 conductance measurements.

Reproductive parameters

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. (1964). 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.

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.

Data analysis and classification of genotypes

Analysis of variance (ANOVA)

To test the significance of UVB 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 (SAS Institute, 2004). 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 significant differences between genotypes means for the levels of UVB treatments for each parameter measured using PDIFF LINES option ($P = 0.05$).

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 UVB radiation among cowpea genotypes (Johnson, 1998). 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 (Johnson, 1998). PCA analysis was performed using PROC PRINCOMP procedure of SAS (SAS-Institute, 2004) on the correlation matrix (18 rows × 18 columns) of ultraviolet radiation response index (UVRI) data as described in next section. The UVRI were obtained from the three levels of elevated UVB radiation treatments (5, 10 and 15 kJ m^{-2} d^{-1}) versus control (0 UVB) 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 UVB 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 UVB responses in each dimension.

Cumulative UVB 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 UVB (5, 10 and 15 kJ m^{-2} d^{-1}) compared to the control (0 UVB) and is based on the response index concept reported in another UVB study (Dai et al., 1994) which was calculated as: $\text{UVRI} = \frac{\text{RV}_t - \text{RV}_c}{\text{RV}_c} \times 100$,

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 was 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:

$$\text{V}-\text{TSRI} = \left(\frac{\text{PH}_t - \text{PH}_c}{\text{PH}_c} + \frac{\text{DM}_t - \text{DM}_c}{\text{DM}_c} + \frac{\text{LA}_t - \text{LA}_c}{\text{LA}_c} + \frac{\text{SLA}_t - \text{SLA}_c}{\text{SLA}_c} + \frac{A_t - A_c}{A_c} + \frac{\text{ETR}_t - \text{ETR}_c}{\text{ETR}_c} + \frac{\text{Fv'/Fm'}_t - \text{Fv'/Fm'}_c}{\text{Fv'/Fm'}_c} + \frac{\text{Chl}_t - \text{Chl}_c}{\text{Chl}_c} + \frac{\text{Phe}_t - \text{Phe}_c}{\text{Phe}_c} + \frac{\text{CMT}_t - \text{CMT}_c}{\text{CMT}_c} \right) \times 100$$

$$R-TSRI=\left(\frac{Fl\,length_t-Fl\,length_c}{Fl\,length_c}+\frac{F\,Dwt_t-F\,Dwt_c}{F\,Dwt_c}+\frac{PV_t-PV_c}{PV_c}+\frac{Pod\,no_t-Pod\,no_c}{Pod\,no_c}+\frac{Seed\,Wt_t-Seed\,Wt_c}{Seed\,Wt_c}+\frac{g\,seed^{-1}{}_t-g\,seed^{-1}{}_c}{g\,seed^{-1}{}_c}+\frac{Seeds\,Pod^{-1}{}_t-Seeds\,Pod^{-1}{}_c}{Seeds\,Pod^{-1}{}_c}+\frac{Shelling_t-Shelling_c}{Shelling_c}\right)\times 100$$

where, PH = plant height, DM = dry matter of plant shoot, LA = leaf area, SLA = specific leaf area, *A* = 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 UVB radiation.

Results

Vegetative growth and dry matter production

The signature of the UVB 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 UVB radiation and genotypes (UVB × G) for PH and DM production accompanied with a significant

reduction in PH and DM at elevated UVB levels when averaged across genotypes (Table 3.1 and Fig. 3.1A, B). PH reduction was not significant in CB-5, CB-46 and MPE at 10 kJ m^{-2} d^{-1} or higher UVB levels. Genotypes also varied for DM production from no significant change (Prima) to significant increase (CB-5 and MPE) in response to UVB radiation. Compared with the control, averaged over UVB levels, UCR-193 and CB-27 produced 49 and 25% shorter plants, respectively (Fig. 3.1A). However, this reduction was less in CB-5 (13%) and MPE (2%). A similar trend was also recorded for DM (Fig. 3.1B). At any given UVB level, cowpea genotypes showed a significant UVB × G interaction with non significant increase in LA due to an increase in the leaf expansion per unit of leaf dry weight, SLA (Fig. 3.1C, D). SLA showed no UVB × G interaction and increased significantly in all genotypes at elevated UVB levels compared to control. However, there were no significant differences for SLA among the three elevated UVB levels. The CUVRI, representing the overall UVB responsiveness of individual traits (Table 3.1) clearly exhibited that all the genotypes responded negatively for PH and positively for SLA.

Photosynthesis and chlorophyll fluorescence

A and ETR of the photosystems exhibited considerable variability among cowpea genotypes and treatments showing highly significant UVB × G interaction (Table 3.1). Genotypes Prima and CB-27 recorded a significant reduction in *A* across all UVB treatments while MPE and UCR-193 showed significantly increased *A* under elevated UVB treatments (Fig. 3.2A). A similar pattern was also observed for ETR, however, the magnitude of reduction in ETR was greater than that of the reduction in *A* (Fig 3.2B). The

Table 3.1 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 (*A*), electron transport rate (ETR), chlorophyll fluorescence (Fv′/Fm′), total chlorophyll (Chl), phenolics (Phe), cell membrane thermostabilty (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). The CUVRI is the sum of UVRI (ultraviolet response index) which is the relative responses of treatments (3 UVB levels; 5, 10 and 15 kJ m^{-2} d^{-1}) in comparison to control (0 kJ m^{-2} d^{-1}) observed for individual vegetative, physiological and reproductive plant attributes. Analysis of variance (ANOVA) across the treatment of UVB radiation and genotypes on cowpea growth.

Genotype	CUVRI for vegetative and physiological attributes											CUVRI for reproductive attributes									
	PH	DM	LA	SLA	*A*	ETR	Fv′/Fm′	Chl	Phe	CMT	**V-TSRI**	Fl length	Fl Dwt	PV	Pod no.	Seed Wt	g seed^{-1}	Seeds pod^{-1}	Shelling	**R-TSRI**	**C-TSRI**
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	***	***	***	***	**	***	*	**	***	***	-	***	***	***	***	***	***	***	***	-	-
UVB	***	***	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.

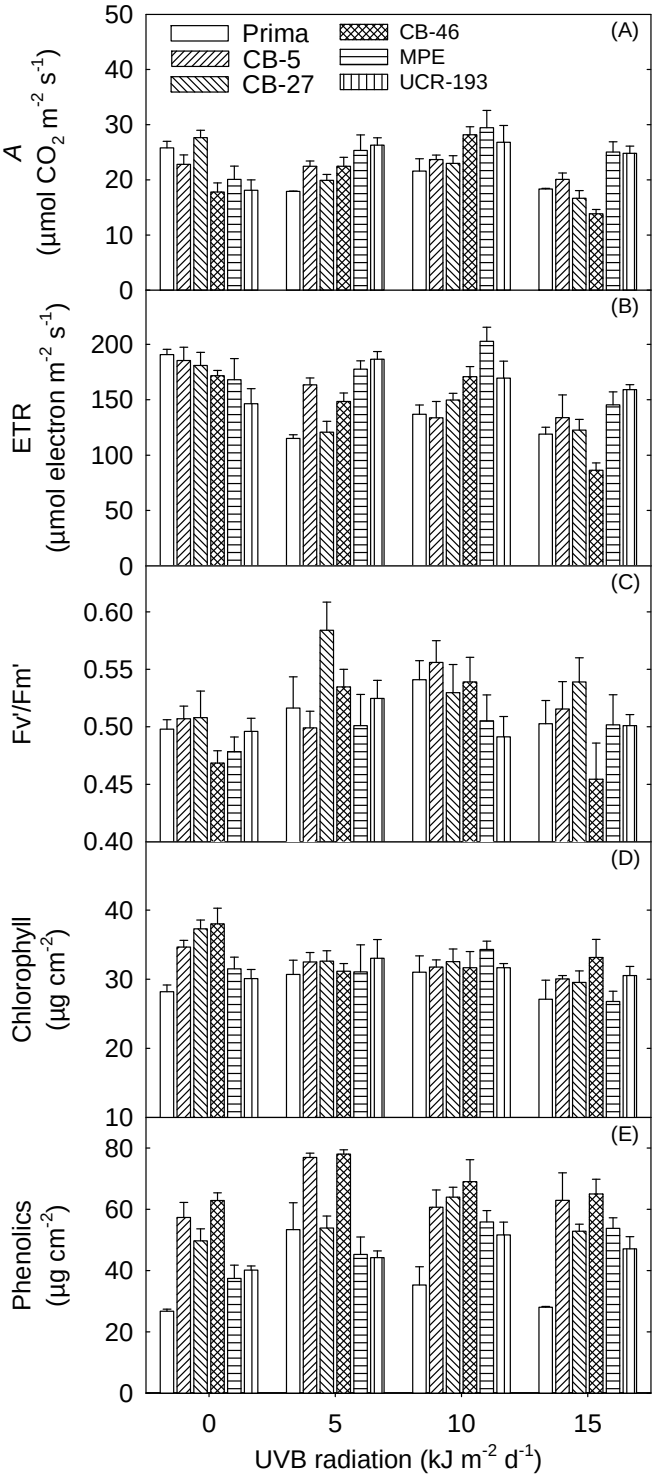

Figure 3.1 Influence of UVB radiation on (A) plant height, (B) plant dry matter, (C) leaf area and (D) specific leaf area of six cowpea genotypes. Error bars show standard deviation from 5 replicates.

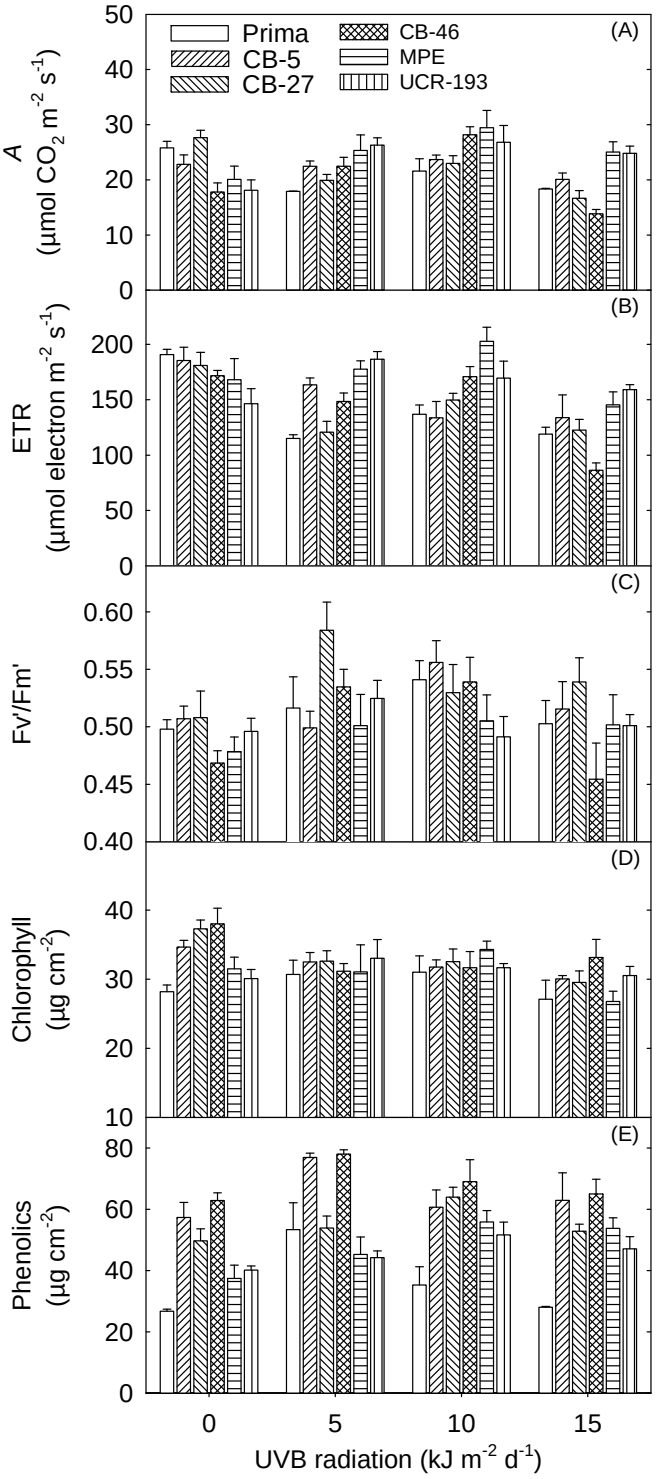

Figure 3.2 Influence of UVB radiation on the leaf (A) photosynthesis (*A*), (B) Electron transport rate (ETR), (C) Chlorophyll fluorescence (Fv′/Fm′), (D) chlorophyll and (E) phenolics concentrations of six cowpea genotypes. Error bars show standard deviation from 3 replicates.

Fv′/Fm′ values did not show a significant UVB × G interaction and were significantly higher for the plants exposed to 5 and 10 kJ m^{-2} UVB compared to the control plants (Fig. 3.2C). The values at 15 kJ m^{-2} however, were not significantly different from that of the control plants.

Leaf chlorophyll and UVB absorbing compounds (phenolics)

Total leaf chlorophyll and phenolic concentrations exhibited a significant UVB × G interaction (Table 3.1). Averaged over UVB levels, the Chl concentration showed significantly lower value in most of genotypes at 15 kJ m^{-2} d^{-1} UVB treatments (Fig. 3.2D). 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}) UVB radiation with a highly significant genotypic variation that ranged from 12% (UCR-193) to 45% (Prima), when averaged over UVB treatments (Table 3.1 and Fig. 3.2E). There was also a significant UVB × G interaction for phenolic concentration. The CUVRI for phenolic concentration also varied among cowpea genotypes and increased under UVB treatment (Table 3.1).

Cell membrane thermostability

Significant UVB × G interaction was observed in CMT (Table 3.1). The elevated UVB 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 UVB treatments.

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} UVB treatment delayed flower initiation by three days in CB-5 and CB-46 while it was three days earlier in CB-27 compared to the control. Variable degree of flower shedding was observed mostly in the plants grown under UVB treatments. In general, all genotypes under UVB treatments produced significantly smaller flowers that caused significant reduction in Fl Dwt across UVB radiation treatments (Table 3.1 and Fig. 3.3A). Flower length ranged from 22.5 mm (Prima) to 27.4 mm (CB-5) showing a significant UVB × G interaction (Table 3.1). PV also showed a significant UVB × G interaction causing a variable degree of responses among the genotypes (Fig. 3.3B). PV reduction was more pronounced in CB-27 at 15 kJ m^{-2} d^{-1} 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 3.1).

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 UVB × G. For CB-27, CB-46 and UCR-193, these traits exhibited a linear decrease as UVB increased from 5 to 15 kJ m^{-2} d^{-1} (Table 3.1 and Fig. 3.3C-D). The reductions in average Seed wt over UVB treatments varied from 11% (MPE) to 48% (UCR-193) among the genotypes. Individual seed weight (g $seed^{-1}$) showed a significant

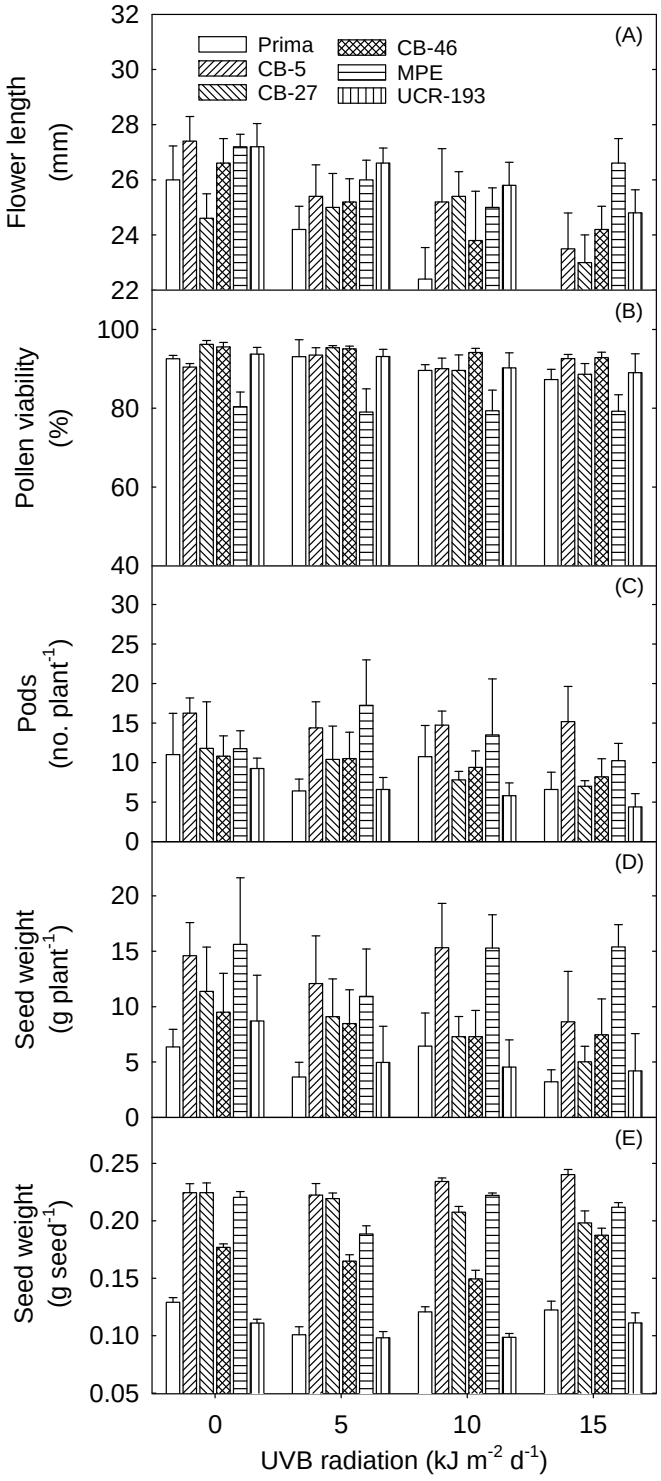

Figure 3.3 Influence of UVB radiation on (A) Flower length, (B) Pollen viability, (C) Pod number, (D) seed weight and (E) individual seed weight (g seed^{-1}) of six cowpea genotypes. Error bars show standard deviation from 5 replicates.

UVB × G interaction and genotypes Prima, CB-27 and UCR-193 exhibited significant reduction when averaged across UVB levels (Table 3.1 and Fig. 3.3E). The CUVRI for pod production and seed weight were highly negative for all genotypes except MPE (Table 3.1).

Principal Component Analysis (PCA)

Plant attributes response to UVB 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 UVB effect, PC1 and PC2 were considered the most important dimensions of UVB responsiveness. Therefore, PC1 and PC2 were regressed against plant attributes to find the contributing traits to the main response patterns of UVB radiation. Fourteen plant attributes were significantly ($P < 0.05$) correlated with either PC1 or PC2 (Table 3.2). The plant DM production, Fl Dwt, phenolic concentration and Pod no were strongly and significantly ($P > 0.001$) correlated with changes in PC1 scores indicating the contribution of these plant attributes in determining the responsiveness of cowpea genotypes to UVB 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, *A*, Chl concentration and individual seed weight (Table 3.1). Plant attributes such as Fv′/Fm′, CMT, Fl length and PV did not show any

Table 3.2 Pearson's correlation coefficient (r) for the 18 plant attributes representing a measure of UVB responsiveness to the first two principle components (PC 1 and PC2) and combined total stress response index (C-TSRI). 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 UVB treatments (5, 10 and 15 kJm^{-2} d^{-1}) against control (0 kJ m^{-2} d^{-1}).

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
A	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†	0.85***	0.18

***, ** and * represent $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, respectively.
†C-TSRI is the combined total stress response index as in the Table 3.1.

significant correlation with either of the first two PC scores.

Genotype response to UVB radiation

The PCA also accounted for genotypic variability at all three levels of elevated UVB treatments separately. The biplot of PC1 vs. PC2 clearly displayed the response patterns between the genotypes and doses of UVB radiation (Fig. 3.4). 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

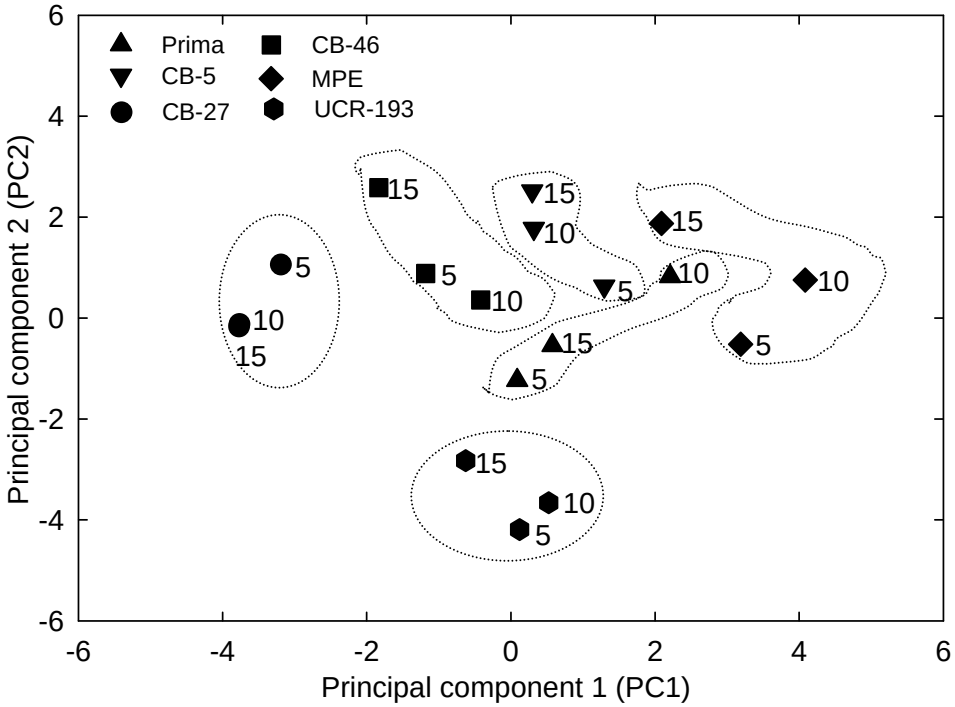

Figure 3.4 Principal component analysis (PCA) of 18 UVB response variables (RVs) in six cowpea genotypes. The UVRI calculated for the three UVB levels (5, 10 and 15 kJ m^{-2} d^{-1}) against control (0 kJ m^{-2} d^{-1}) were used. The biplot of first two principal component (PC) scores; PC1 and PC2 are shown. The numbers 5, 10 and 15 associated with symbols represent UVB radiation treatments of 5, 10 and 15 kJ m^{-2} d^{-1}, respectively.

UVB radiation did not show any distinct patterns of reduction among the genotypes studied. However, the collective effect of elevated UVB radiation appeared to be uniform on each genotype, as deduced from the separation of some genotypes form others (Fig. 3.4). For instance, pronounced UVB responsiveness could be observed for MPE (highest scores for PC1; also relatively higher UVB tolerance), CB-27 and UCR-193 (the lowest

scores for PC1 and PC2, respectively; also UVB sensitiveness) positioned on the right, left and middle bottom coordinates, respectively, as shown in Fig. 3.4. However, other genotypes appeared to be clustered in the center of the plot.

Cumulative ultraviolet response index

The CUVRI representing the overall effect of UVB 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 3.1). V-TSRI, a measure of overall genotypic responsiveness to UVB 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 UVB radiation for reproductive growth, of all the genotypes was negative indicating the most damaging effects of UVB was on the reproductive growth and yield components of cowpea (Table 3.1). 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 UVB 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 UVB 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 UVB 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 was strongly correlated (r = 0.85, $P < 0.001$) with PC1.

Discussion

Vegetative performance

Among the C_3 species, leguminous crops, particularly grown in the tropical regions, have been reported to be highly sensitive to both ambient (Amudha et al., 2005; Pal et al., 1997) and elevated (Chimphango et al., 2003; Musil et al., 2002a; Singh, 1995; Singh, 1996) UVB radiation because of the thinner O_3 column and acute angle of the sun at these regions (McKenzie et al., 2007; Singh, 1995). 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 UVB radiation (Nedunchezhian and Kulandaivelu, 1997; Teramura, 1983). Substantial reduction in PH and DM production caused by elevated UVB reported in several tropical legumes are similar to the current study (Lingakumar et al., 1999; Singh, 1995; Singh, 1996). Pal et al. (1997) 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 UVB and involved in cell

division and cell elongation processes (Nedunchezhian and Kulandaivelu, 1997; Pal et al., 1997). The thinner leaves observed at elevated UVB might have allowed increased direct transmittance of UVB radiation deep into the sensitive tissues over time, making plants more vulnerable to UVB (Balakumar et al., 1993; Singh, 1995).

Photosynthesis, pigment and UVB absorbing compounds

Large uncertainties exist regarding the photosynthetic performance of plants exposed to UVB radiation. In current study, UVB-induced significant reduction of *A* observed in sensitive genotypes (Prima and CB-27) is in accordance with the earlier reports in many leguminous species (Cen and Bornman, 1990; Nedunchezhian and Kulandaivelu, 1997; Singh, 1996). Similarly, significantly decreased ETR observed in the same genotypes, which accounted 8-10% larger reduction than that of *A*, is in accordance with a previous study with the same species (Premkumar and Kulandaivelu, 1999). Mackerness et al. (1999) pointed that the involvement of reactive oxygen species (ROS) in UVB signaling pathway may lead to the down-regulation of photosynthesis. However, a significant increase in *A* in tolerant genotypes (MPE and UCR-193) contrasted these findings suggesting genotypic variability in cowpea. Also, an increased Fv′/Fm′, on exposure to UVB, clearly contrasts the results obtained by Lingakumar et al. (1999). This supports the view questioning the key role of PSII inhibition in response to UVB (Allen et al., 1998; Nogués and Baker, 1995). Except Prima, genotypes such as MPE and UCR-193 with increased phenolic concentrations were more tolerant to the UVB. Phenolic compounds have been reported to act as UVB radiation screening compounds (Allen et al., 1998; Balakumar et al., 1993). Exposure of many tropical

legumes to UVB (simulating 15-25% O_3 depletion) has shown a 5-50% increase in UVB absorbing compounds in leaves which generally are accompanied by 5-30% reduction in total chlorophyll content (Balakumar et al., 1993; Musil et al., 2002a; Nedunchezhian and Kulandaivelu, 1997; Premkumar and Kulandaivelu, 2001; Singh, 1996) similar to our results.

Reproductive performance

The delay in flowering at 15 kJ m^{-2} d^{-1} UVB observed in the present and previous studies in other legume species (Amudha et al., 2005; Basiouny et al., 1975; Rajendiran and Ramanujam, 2004; Saile-Mark and Tevini, 1997) might be attributed to the impact of high UVB on the gibberellins biosynthesis as reported by Saile-Mark and Tevini (1997). The severe effect of UVB on Fl length and Fl Dwt is not uncommon. In a similar study on the soybean plants, Koti et al. (2004) reported a drastic reduction in flower components and pollen germination. However, the smaller effect of UVB on PV in MPE and UCR-193 observed in our study are in agreement with other studies, although precise mechanisms are not clearly understood (Flint and Caldwell, 1983). The substantial (11 to 48%) reduction in seed yield which was more pronounced at the highest UVB (15 kJ m^{-2} d^{-1}) treatment is in close agreement with other studies including *Phaseolus vulgaris* (50%) (Saile-Mark and Tevini, 1997), *Vigna radiata* (76%) and *Phaseolus mungo* (62%) (Amudha et al., 2005; Singh, 1995). Rajendiran and Ramanujam (2004) 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 UVB radiation. Compared to the highest dose in the current study (15 kJ m^{-2} d^{-1}), the UVB doses used by

Singh (1995) and Rajendiran and Ramanujam (2004) 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 UVB on reproductive parameters was much greater than the reductions observed in this study. This could be explained by the fact that in those previous studies, UVB was applied intensely over a 2-h period, each day, compared to the 8-h period in our and other studies (Chimphango et al., 2004). It is apparent from the present study that the UVB 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 (1997) found that UVB induced lower yield was associated with fewer number of flowers 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 UVB increased. The increase in allocation of carbon resources towards repair mechanisms and biosynthesis of UVB absorbing compounds at the expense of the reproductive structures might also contribute for the reduction in flower characteristics and seed yield (Koti et al., 2004).

PCA: plant attributes and genotypes response to UVB

The association of 14 measured plant attributes with the main UVB responsive components of PC1 and PC2 supports the observed responsiveness of similar parameters to UVB in other crops (Musil et al., 2002a; Saile-Mark and Tevini, 1997; Singh, 1995). 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 UVB 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 UVB responsiveness in cowpea. Additionally, the strong correlation of phenolic compounds with PC1 (Table 3.2) also supports previously observed defense role of phenolic compounds on exposure to UVB (Singh, 1995). The importance of SLA was reflected possibly due to its contribution to increased sensitivity caused by reduced leaf thickness (Cen and Bornman, 1990). The pronounced genotypic responses associated with UVB 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 UVB radiation as seen along the axis of PC1 (Fig. 3.4). However, other genotypes did not show a distinct pattern over the range of UVB. It is evident from the Fig. 3.4 that the collective effect of three elevated UVB on different genotypes was confined to a certain location in the plot reflecting genotypic variability. Regardless of the doses of UVB radiation, it is evident (Fig. 3.4) 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 UVB radiation.

Vegetative vs. reproductive performance and classification of genotypes

The TSRI used to assess the quantitative effects of UVB radiation in the current study was equally effective as in other crops (Dai et al., 1994; Koti et al., 2007; Saile-Mark and Tevini, 1997). The high negative CUVRI values for DM, Pod no and Seed wt

seems to be the most highly affected plant attributes by UVB radiation (Table 3.1). 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 UVB 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 UVB 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 UVB (Koti et al., 2007). 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 response to UVB radiation have also been reported in bush bean (Saile-Mark and Tevini, 1997), rice (Dai et al., 1994) and soybean (Koti et al., 2004). 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 UVB tolerance. Based on C-TSRI, MPE was classified as UVB tolerant whereas CB-27 was classified as the most UVB sensitive genotypes.

Although, spatial and temporal differences for natural UVB doses received on the Earth's surface exist for the regions where the genotypes were developed (US cultivars receiving comparatively lower than African or Indian cultivars) (McKenzie et al., 2007;

USDA, 2005), genotypic tolerance to UVB could not be traced to the site of origin. The overall positive response of MPE to UVB may partially be explained by the semi erect nature of the plants, faster growth habit, and higher yielding capacity (Hare, 1991). These traits might have resulted in comparatively less UVB radiation interception and more tolerance nature resulting in a better performer across several UVB doses. Studies have demonstrated that leaf broadness and angle of the leaves play important roles in determining the sensitivity of the crop to UVB radiation (Basiouny et al., 1975; Pal et al., 1997). A trait-based breeding strategy that incorporates superior traits such as leaf erectness, more synthesis of UVB 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 UVB radiation levels.

Examination of the effect of UVB 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 UVB responsiveness of cowpea genotypes. These are among the plant attributes that also showed a strong correlation with the main component of UVB responsiveness; PC1 in the PCA analysis (Table 3.2). The high UVB responsiveness of plant biomass production, flower characteristics and fruit set in soybean genotypes and bush bean have also been reported (Dai et al., 1994; Green et al., 1974; Koti et al., 2004; Koti et al., 2007). 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

UVB. Increased phenolic compounds are one of the most widely occurring responses or defense mechanisms in plants upon UVB exposure (Searles et al., 2001). Perhaps, the association of phenolic concentration with PC1 indicates its role for early selection of UVB tolerance in cowpea populations during selection process. However, there are no studies that have used phenolic accumulation in plants for screening purposes. The results from this study suggest that the reproductive traits should be taken into consideration while cowpea genotypes are subjected to selection for UVB 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 UVB. Also, cowpea plants were kept free from any bacterial symbiotic relationship to avoid any unwanted biotic interaction in this study. Caldwell et al. (1994) reported that at ambient PAR, UV-A did not appear to be required for UVB 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 reached at midday above 1000 μmol m^{-2} s^{-1}. The reports from previously published studies indicate that UVB did not cause significant alteration in the symbiotic function of other legumes including cowpea (Chimphango et al., 2003; Chimphango et al., 2004). Therefore, it is inferred that the data obtained from the current study should represent only the effects of UVB radiation on cowpea. The negative plant response observed under elevated UVB in this study may not be related to the balance of UVB and PAR ratio similar to the observation in bean plants under high radiation levels (Cen and Bornman, 1990). However, square-wave UVB 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 (Allen et al., 1998; Caldwell et al., 1994). In view of the recognized limitations of controlled environmental studies, they have been widely used in UVB experimentation and screening for UVB responses across a wide range of plant species (Caldwell et al., 1994; Koti et al., 2004; Musil et al., 2002b). However, precaution is 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 UVB radiation. UVB 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 UVB 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 UVB radiation. Among the cowpea genotypes studied, MPE was classified as the most tolerant to UVB due to its overall positive performance and CB-27 was considered as the most sensitive to UVB because of the highest negative response to UVB radiation.

CHAPTER IV

SCREENING COWPEA [*Vigna unguiculata* (L.) Walp.] GENOTYPES TO MULTIPLE ABIOTIC STRESSES

Abstract

The carbon dioxide concentration [CO_2], temperature and ultraviolet-B (UVB) radiation are the concomitant factors influencing the global environment and their possible interactions are of significant interest to agriculture. The objectives of this study were to evaluate interactive effects of atmospheric [CO_2], temperature, and UVB radiation on growth, physiology and reproduction of cowpea genotypes and to identify genotypic tolerance to multiple stressors. Six cowpea (*Vigna unguiculata* [L.] Walp.) genotypes differing in their sites of origin were grown in sunlit, controlled environment chambers. The treatments consisted of two levels each of atmospheric [CO_2] (360 and 720 μmol mol^{-1}), UVB [0 and 10 kJ m^{-2} d^{-1}) and temperatures [30/22 and 38/30 °C] from eight days after emergence to maturity. In response to increased UVB and temperature, the ameliorative effect of elevated [CO_2] observed for most of the vegetative and photosynthetic traits in cowpea were not observed for pollen production, pollen viability and yield attributes. The combined stress response index (C-TSRI) derived from vegetative (V-TSRI) and reproductive (R-TSRI) parameters revealed that the genotypes responded negatively with varying magnitude of responses to the stressors. Additionally, in response to multiple abiotic stresses, the vegetative traits behaved dissimilarly with

that of reproductive traits, as deduced from the positive V-TSRI and negative R-TSRI observed in most of the genotypes and poor correlation between these two processes. The UVB in combination with increased temperature caused the greatest damage to cowpea vegetative growth and reproductive potential. The identified tolerant genotypes and groups of plant attributes could be used to develop genotypes with multiple abiotic stress tolerance.

Introduction

The atmospheric carbon dioxide concentration [CO_2] has increased globally by more than 100 μmol mol^{-1} (36%) over the last 250 years with the highest recorded average growth rate of 1.9 μmol mol^{-1} yr^{-1} over the last decade (IPCC, 2007). The current [CO_2] level of approximately 380 μmol mol^{-1} is estimated to reach between 730 and 1020 μmol mol^{-1} by 2100 (IPCC, 2007). Changes projected in [CO_2] and other greenhouse gases is expected to increase mean global air temperature by 2.5 to 4.5 °C during the same period (IPCC, 2007). In addition to these changes in climate, current and projected increase in ground-level UVB radiation is closely associated with stratospheric ozone column depletion as it attenuates the incoming solar UVB (280-320 nm) radiation (Long, 1991; WMO, 2007). Relative to the 1970s, the midlatitudes O_3 column losses for the 2002-2005 period were approximately 3% in the Northern and 6% in the Southern hemisphere (WMO, 2007). Current global distribution of mean erythemal daily doses of UVB radiation between the latitude 40°N and 40°S during summer ranges from 2 to 9 kJ m^{-2} (McKenzie et al., 2007). The daily dose of UVB radiation in USA for the month of June-August, 2005 ranged between 0.02 and 8.75 kJ m^{-2} (USDA, 2005).

The interaction among the environmental stress factors such as [CO_2], temperature, and UVB evokes a variety of plant responses. An increase in the yield observed at elevated [CO_2] (Kimball et al., 2002) were not observed when plants are grown in combination with high temperature (Prasad et al., 2003; Reddy et al., 1997) or increased in UVB radiation (Qaderi and Reid, 2005; Teramura et al., 1990). Studies have shown that the projected changes in climate will drastically reduce crop yields when they coincide with the reproductive stage of plant growth (Hall and Ziska, 2000; Reddy et al., 1997). Therefore, the interaction among the environmental factors will severely modify the magnitude and direction of individual climatic stress factor effects on plants leading to cascading effects on terrestrial ecosystems (Lobell and Asner, 2003; Long et al., 2006; Mittler, 2006). Thus, an understanding of the effects of multiple environmental factors that simulate anticipated future climatic conditions will be useful to assess the growth and productivity of agronomic crops.

In nature, plants are routinely exposed to multiple abiotic stresses and recent studies demonstrate that plants response to a single factor are much different than the response under multiple stress conditions (Caldwell et al., 2007; Rizhsky et al., 2002; Rizhsky et al., 2004). Hall and Ziska (2000) recommended that crop breeders should consider the possible climate change while developing a breeding strategy for yield improvement. Ahmed et al. (1993) pointed that developing greater and sustained sink capacity will be needed for higher yields under stressful environments. However, to date, the effects of multiple stress factors on growth and reproductive potential in many plants are lacking under realistic radiation environment. The quality and quantity of light play a important role in the determining plant responsiveness to a given environment (Allen et

al., 1998; Goto, 2003; Summerfield et al., 1976). Low light conditions have been shown to reduce yield (Summerfield et al., 1976). Moreover, UVB defense mechanisms such as photo-repair system for DNA (Lois and Buchanan, 1994) and biosynthesis of UVB absorbing compounds require high light conditions similar to natural solar radiation regimes (Adamse et al., 1994; Caldwell et al., 1994). Many of the recent studies evaluating the influence of combination of the abiotic stresses have been carried out under lower solar radiation regimes (Tegelberg et al., 2008) or unrealistically lower artificial light conditions (<300 μmol m^{-2} s^{-1} PAR) (Qaderi and Reid, 2005; Qaderi et al., 2007; Rizhsky et al., 2002; Rizhsky et al., 2004; Kant et al., 2008) compared to natural settings. The inferences derived from these studies may not be reflective of actual effect of those abiotic stress factors in natural environment and hence limiting the portability of the results to field conditions.

In multiple abiotic stress scenario, the interaction studies will help to elucidate whether interactions between atmospheric [CO_2] and temperature can counteract the negative effect of UVB radiation and vice versa (Caldwell et al., 2007; Runeckles and Krupa, 1994). Premkumar and Kulandaivelu (2001) reported that enhanced UVB markedly alleviated the adverse effects of magnesium deficiency in cowpea whereas, interactive effects of elevated UVB and high temperature caused deleterious effects on soybean (*Glycine max* L.) growth and development (Koti et al., 2005, 2007). Although, few studies have investigated the interactive effects of CO_2 and temperature on crop plants including cowpea (Ahmed et al., 1993), the studies are limited that evaluated the effects of a combination of [CO_2], temperature, and UVB radiation and their interactions on crop growth and development, particularly on reproductive parameters (Koti et al.,

2007; Mark and Tevini, 1997; Tegelberg et al., 2008). Because of the extreme genetic diversity and wide range of climatic adaptation of cowpea (Singh, 2004), it will be intuitive to study the relative responses of this species on vegetative and reproductive plant attributes in accordance with the changing climate.

Recent studies dealing with multiple environmental factors on various plant processes from genes to canopies concluded crop tolerance in many crops is needed to cope with changes projected in climate (Caldwell et al., 2007; Hall, 2004; Mittler, 2006). Few genotypes have been screened by using various abiotic stress response indices derived from the different stages of plant growth in response to single or multiple abiotic stresses (Dai et al., 1994; Koti et al., 2005; Saile-Mark and Tevini, 1997). The earlier studies evaluating the responsiveness of cowpea to abiotic stresses represented smaller set of plant attributes usually measured either from part of plant organ and/or growth stage involving limited number of genotypes (Ahmed et al., 1993; Musil et al., 2002; Premkumar and Kulandaivelu, 2001; Warrag and Hall, 1983). In this study we present the results of an experiment designed to explore the extent to which most commonly investigated plant attributes including vegetative and reproductive processes affected by a combination of multiple abiotic stress factors such as high [CO_2], UVB radiation and temperature.

We hypothesized that the tolerant characteristics to abiotic stresses are present in cowpea with genotypic variability and when exposed to multiple abiotic stresses, the vegetative traits will respond dissimilarly to that of the reproductive characteristics, and the rate and direction of the genotypes response to each of these abiotic stressors will be modified under combination of multiple stress conditions. The objectives of this study

were to determine whether doubling of [CO_2] will counteract the negative effects of UVB and temperature, and to evaluate interactive effects of [CO_2], temperature, and UVB radiation on growth, physiology and reproduction of cowpea genotypes and to identify genotypic tolerance to multiple abiotic stressors.

Materials and methods

Research Facility and plant material

Eight sunlit, soil-plant-atmosphere-research (SPAR) units located at the R.R. Foil Plant Science Research Center (33° 28′ N, 88° 47′ W), Mississippi State, Mississippi, USA, were used to conduct the current study. Each SPAR growth chamber has the capability to precisely control the atmospheric [CO_2], temperature, UVB radiation, and desired nutrient and irrigation regimes at determined set points under near ambient levels of photosynthetically active radiation (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 plant canopy with sufficient velocity (4.7 km h^{-1}) to cause leaf flutter, mimicking field conditions. Variable density shade cloths, designed to simulate canopy spectral properties, placed around the edges of the plant canopy, were adjusted regularly to match canopy height and to eliminate the need for border plants. The Plexiglas chambers are completely opaque to solar UVB radiation, but transmits 12% UV-A and >95% incoming PAR (wavelength 400–700 nm; Zhao et al., 2003). During this experiment, the incoming daily 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 SPAR units supported by an environmental monitoring and control systems are networked to provide automatic acquisition and storage of the data, monitored every 10 s throughout the day and night. Many details of the operations and controls of SPAR chambers have been described by Reddy et al. (2001). The relative humidity (RH) of each chamber were monitored with a humidity and temperature sensor (HMV 70Y, Vaisala Inc., San Jose, CA, USA) installed in the returning path of airline ducts. The vapor pressure deficits (VPD) in the units were estimated from these measurements as per Murray (1967).

Six contrasting genotypes of cowpea [*Vigna unguiculata* (L.) Walp.] representing differential sensitivity/tolerance to heat and diverse sites of origin, California blackeye (CB)-5 and CB-46 (both heat sensitive, University of California, Davis, USA), CB-27 (heat tolerant, University of California, Riverside, USA), Mississippi Pinkeye (MPE, heat sensitivity is not known, Mississippi State University, Mississippi, USA), Prima (heat tolerant, Nigeria), and UCR-193 (heat tolerant, India) (Fang et al., 2007; Hall et al., 2003; Hare, 1991; Warrag and Hall, 1983), were evaluated in the present study. The genotypes were seeded in 15 cm diameter and 15 cm deep plastic pots filled with fine sand on 26 July, 2005. After emergence (7 days after sowing), thirty pots having healthy plants (5 pots for each genotype and 3 plants in each pot) were transferred and arranged randomly in each SPAR chamber. Plants were irrigated three times a day with full-strength Hoagland's nutrient solution delivered at 8:00, 12:00, and 17:00 h to ensure optimum nutrient and water conditions for plant growth through an automated and computer-

controlled drip irrigation system. The excess solution was drained through the holes in the bottom of the pots and SPAR soil bins.

Treatments

Eight treatments consisting of two levels of each of three environmental factors: CO_2 [360 and 720 μmol mol^{-1} (+ CO_2)], temperature [(30/22 and 38/30 °C (+T)] and UVB (280-320 nm) radiation intensities [0 and 10 (+UVB) kJ m^{-2} d^{-1}] were imposed from eight days after emergence (DAE) to plant maturity. The control treatment consisted of 360 μmol mol^{-1} CO_2, 30/22 °C temperature and 0 kJ m^{-2} d^{-1} UVB and all SPAR chambers were maintained at this condition until 8 DAE. The UVB dose of 10 kJ m^{-2} d^{-1} was designated to simulate 12% ozone depletion at the experimental site. The seasonal data for daily mean temperatures and daytime [CO_2] are presented in Table 4.1. The quality control of CO_2 and temperature in SPAR chambers are described in detail by Reddy et al. (2001).

The square-wave supplementation systems were used to provide desired UVB radiation doses which were delivered from 0.5 m above the plant canopy for 8 h, each day, from 8:00 to 16:00 h by eight fluorescent UVB-313 lamps (Q-Panel Company, Cleveland, OH, USA) horizontally mounted on a metal frame inside each SPAR chamber, driven by 40 W dimming ballasts. The UVB radiation delivered at the top of the plant canopy was monitored at 10 different locations in each SPAR chamber 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 lamp output was adjusted, as needed,

Table 4.1 The set treatments, atmospheric [CO_2], UVB and day/night temperature (T) conditions, and measured chamber [CO_2] from a typical day, daily mean UVB radiation dosage, mean temperature, and daytime vapor pressure deficit (VPD) during the experimental period for each treatment.

Treatment			Measured variables			
CO_2 (μmol mol^{-1})	UVB (kJ m^{-2} d^{-1})	T (°C)	CO_2 (μmol mol^{-1})	UVB (kJ m^{-2} d^{-1})	Mean T (°C)	VPD (kPa)
360	0	30/22	362.01 ± 0.30	0.00 ± 0.00	25.97 ± 0.06	2.18 ± 0.01
	0	38/30	361.32 ± 0.30	0.00 ± 0.00	33.73 ± 0.04	3.40 ± 0.02
	10	30/22	360.56 ± 0.29	9.14 ± 0.12	25.98 ± 0.06	1.93 ± 0.03
	10	38/30	360.11 ± 0.51	9.15 ± 0.09	33.69 ± 0.04	3.90 ± 0.01
720	0	30/22	722.28 ± 0.63	0.00 ± 0.00	25.81 ± 0.05	2.37 ± 0.01
	0	38/30	720.23 ± 0.54	0.00 ± 0.00	33.79 ± 0.03	3.21 ± 0.02
	10	30/22	721.61 ± 0.40	9.20 ± 0.11	26.08 ± 0.06	2.28 ± 0.01
	10	38/30	721.85 ± 0.68	9.10 ± 0.10	33.54 ± 0.04	3.54 ± 0.02

Each value represents the mean ± SE for one typical day for [CO_2], and 10 August to 28 October 2005 for UVB, temperature and VPD.

to maintain desired UVB level. 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 film was changed every 3 to 4-days to account for the degradation of CA properties. During the experiment, the weighted total biologically effective UVB radiation at the top of the plant canopy are presented in Table 4.1 which were calculated using generalized plant response spectrum (Caldwell, 1971) as formulated by Green et al. (1974), normalized at 300 nm.

Vegetative growth measurements

One plant per pot (5 plants per genotype) were harvested 10 and 18 days after treatment (DAT) to determine plant height (PH), leaf area (LA), leaf number (LN), and dry matter (DM) of the leaves and stems. Leaf area was measured using LI-3100 leaf area

meter (LI-Cor Inc., Lincoln, NE, USA), and specific leaf weight (SLW) was calculated as leaf weight per unit of leaf area (g cm^{-2}). The plant components were oven dried for 72 h at 70 °C to obtain dry weights. The final remaining one plant per pot was harvested at the maturity, 53 DAE.

Photosynthesis and chlorophyll fluorescence measurements

Eighteen days after treatment, leaf net photosynthesis (*A*), electron transport rate (ETR) and fluorescence (Fv′/Fm′) were measured between 9:00 to 14:00 h on 3rd or 4th leaf from the terminal, using an infrared gas analyzer built into a leaf cuvette in an open gas exchange system (LI-COR 6400) with an integrated fluorescence chamber head (LI-COR 6400-40 Leaf Chamber Fluorometer). The cuvette chamber conditions were set to provide photosynthetic photon flux density of 1500 µmol m^{-2} s^{-1} and cuvette block temperature was maintained at the respective treatment daytime temperature using a computer-controlled Peliter module mounted in the cuvette.

Leaf pigments, phenolics and cell membrane thermostability measurements

The total leaf chlorophyll, carotenoids and UVB-absorbing compounds were extracted and determined (18 DAT) on five 0.38 cm^{-2} leaf disks by placing them in a vial containing either 5 ml of dimethyl sulfoxide for pigments extraction or 10 ml of a mixture of methanol, distilled water and hydrochloric acid in 79:20:1 ratio for phenolics extraction and incubated in dark for 24 h. Thereafter, the concentration of the extract was determined at 648, 662 and 470 nm for estimation of total chlorophyll and carotenoids and at 320 nm for estimation of phenolic compounds by using Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). The equations of

Lichtenthaler (1987) were used to estimate the chlorophyll and carotenoids concentrations, whereas the phenolic concentration was estimated according to the Kakani et al. (2004) and expressed as equivalent of p-coumaric acid.

The leaf cell membrane thermostability (CMT) in cowpea genotypes was assessed on 18 DAT according to the procedure described by Martineau et al. (1979) with minor modifications. In brief, a sample for assay consist of a paired set namely; control (C) and treatment (T) set, of five leaf disks each 1.3 cm^{-2}, cut from five fully expanded 3^{rd} or 4^{th} leaves selected randomly from each treatment. 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 exchanges 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 deionized water and sealed with aluminum foil to avoid the evaporation. 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 (approx. 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) were made by using an electrical conductivity meter (Corning Checkmate II: Corning Inc., New York, USA) 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 final conductance (CEC2 and TEC2) measurements were recorded. The CMT was calculated by the equation, $\text{CMT\%} = \frac{1-(\text{TEC1}/\text{TEC2})}{1-(\text{CEC1}/\text{CEC2})} \times 100$, where, TEC and

CEC are the measure of conductance in treated and control test tubes, respectively, at initial (TEC1 and CEC1) and final (TEC2 and CEC2) conductance measurements.

Flower morphology, pollen production and pollen viability measurements

The day from sowing to the appearance of first open flower were recorded. We found that cowpea anthers dehisce between 05:00 and 08:00 h from a time-series observations (data not shown), and therefore all flower and pollen parameters were measured during this time frame. Flower length (Fl length), percentage pollen viability (PV) and flower dry weight (Fl Dwt) were determined on 10 flowers randomly collected 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% sucrose solution was found to be the best for cowpea pollen staining (data not shown). The pollen grains were dusted gently by tapping with an artist brush on the microscope glass slides containing a drop of TTC solution as described by Aslam et al. (1964). The preparations were stored at room temperature in dark, after 16 h, the total and stained pollen grains were counted in two microscopic fields of 2.4 mm^2 having >100 pollen grains from each field of view using a microscope (SMZ 800 microscope, Nikon Instruments, Kanagawa, Japan). Then, the same flowers were dried in an oven to measure flower dry weights.

Pod production and yield components

Cowpea plants were harvested when most of the pods were mature and dry (53 DAE). The yield components such as total number of pods $plant^{-1}$, number of seeds pod^{-1}, total seed wt $plant^{-1}$ and weight of individual seeds (g $seed^{-1}$, average of 100 seeds) were

determined on all plants in each genotype. Dry weights were measured after complete drying of pods and seed at room temperature. Shelling percentage was calculated as actual seed mass over pod mass multiplied by 100.

Analysis of variance (ANOVA)

The ANOVA was performed by using the general linear model "PROC GLIMMIX" procedures of SAS (SAS Institute Inc, 2004) to test the significance of atmospheric [CO_2], temperature, UVB radiation and genotypes, and their interactive effects on plant parameters studied. The least square means (LSMEANS) comparisons were used to determine significance differences between treatments for each parameter using PDIFF LINES option ($P = 0.05$).

Cumulative stress response index (CSRI) and total stress response index (TSRI)

CSRI was calculated as the sum of stress response index (SRI) of individual plant-attribute response at a given treatment compared to the control, and is based on the response index concept reported in the study of Dai et al. (1994) which was calculated as:

$\mathrm{SRI} = \frac{\mathrm{RV_t} - \mathrm{RV_c}}{\mathrm{RV_c}} \times 100$, where SRI = stress response index (that could be measured at any treatment), RV = individual response variable (that could be any of 21 measured plant responses) under t = treatment and c = controlled conditions. For PH, LA, LN, SLW, and DM, the average of two measurements (10 and 18 DAT) were used. All other growth parameters were from the final harvest date. The CSRIs for vegetative (V-CSRI)

and reproductive (R-CSRI) were calculated separately by the following equations:

$$V-CSRI=\left(\frac{PH_t-PH_c}{PH_c}+\frac{LA_t-LA_c}{LA_c}+\frac{LN_t-LN_c}{LN_c}+\frac{SLW_t-SLW_c}{SLW_c}+\frac{DM_t-DM_c}{DM_c}+\frac{A_t-A_c}{A_c}+\frac{ETR_t-ETR_c}{ETR_c}+\frac{Fv'/Fm'_t-Fv'/Fm'_c}{Fv'/Fm'_c}+\frac{Chl_t-Chl_c}{Chl_c}+\frac{Caro_t-Caro_c}{Caro_c}+\frac{Phe_t-Phe_c}{Phe_c}+\frac{CMT_t-CMT_c}{CMT_c}\right)\times 100$$

$$R-CSRI=\left(\frac{Fl\,length_t-Fl\,length_c}{Fl\,length_c}+\frac{F\,Dwt_t-F\,Dwt_c}{F\,Dwt_c}+\frac{PP_t-PP_c}{PP_c}+\frac{PV_t-PV_c}{PV_c}+\frac{Pod\,no_t-Pod\,no_c}{Pod\,no_c}+\frac{Seed\,Wt_t-Seed\,Wt_c}{Seed\,Wt_c}+\frac{g\,seed^{-1}{}_t-g\,seed^{-1}{}_c}{g\,seed^{-1}{}_c}+\frac{Seed\,Pod^{-1}{}_t-Seed\,Pod^{-1}{}_c}{Seed\,Pod^{-1}{}_c}+\frac{Shelling_t-Shelling_c}{Shelling_c}\right)\times 100$$

where, PH = plant height, LA = leaf area, LN = leaf number, SLW = specific leaf weight, DM = dry matter of plant shoot, *A* = net photosynthesis, ETR = electron transport rate, Fv′/Fm′ = the efficiency of energy harvesting by oxidized (open) PSII reaction centers in the light, Chl = total leaf chlorophyll, Caro = carotenoids, Phe = phenolics content, CMT = cell membrane thermostability, Fl length = flower length, Fl Dwt = flower dry weight, PP= pollen grains anther^{-1}, PV = pollen viability, Pod no= pods plant^{-1}, Seed Wt = seed weight plant^{-1}, g seed^{-1} = individual seed weight (g seed^{-1}), Seed Pod^{-1} = seed number pod^{-1}and Shelling = pod shelling percentage under, t = treatment and c = controlled conditions.

TSRI, sum of the CSRIs over all the treatments was evaluated for vegetative (V-TSRI) and reproductive (R-TSRI) responses separately and in combination (C-TSRI) for each genotype. Based on the 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 multiple environmental factors individually and in combination.

Factor analysis

The factor analysis (FA) was used to summarize large number of variables by identifying the relationships among the group of variables, which when examined may suggest an underlying common factor that explains why these variables are correlated (Johnson, 1998). Factor analysis was performed on the correlation matrix of 48 rows (6 genotypes, 8 treatments) and 21 columns (12 vegetative and 9 reproductive response variables) using principal factor method with an iterative procedure of PROC FACTOR (SAS Institute Inc., 2004). The factors were rotated orthogonally by verimax option and the numbers of underlying factors were determined by SBC (Schwarz's Bayesian Criterion).

Results

Vegetative growth

Cowpea genotypes were very responsive to all treatments and their interactions for vegetative growth (Table 4.2, Fig. 4.1). Leaves grown under +UVB conditions showed the earliest symptom (5 DAT) of minor yellowing of veinal and inter-veinal regions which developed into small chlorotic patches at a latter stage. The [CO_2] significantly interacted with UVB and temperature for PH and LA (Table 4.2) resulting in an increase in PH either +CO_2 condition alone (59%) or in combination with +UVB (17%) and +T (26%), averaged over genotypes. However, plants grown under +CO_2+UVB+T condition were 35% shorter compared to control, when averaged across

Table 4.2 Analysis of variance across the genotypes (G) and treatments of carbon dioxide [CO_2], temperature (T), ultraviolet-B (UVB) radiation and their interaction on cowpea vegetative and physiological attributes; plant height (PH), dry matter plant^{-1} (DM), leaf area (LA), leaf number plant^{-1} (LN), specific leaf weight (SLW), net photosynthesis (*A*), chlorophyll fluorescence (Fv′/Fm′), electron transport rate (ETR), total chlorophyll (Chl), carotenoid (Caro), phenolics (Phe), and cell membrane thermostabilty (CMT).

Source of Variation	PH	LA	LN	SLW	DM	*A*	ETR	Fv'/Fm'	Chl	Caro	Phe	CMT
G	***	***	**	***	***	***	***	**	***	***	***	**
CO_2	***	***	***	***	***	***	**	***	***	***	NS	***
UVB	***	*	NS	***	***	***	***	NS	***	NS	*	*
T	***	***	NS	***	***	***	***	*	***	***	*	***
G × CO_2	*	*	NS	***	***	*	NS	NS	***	***	**	NS
G × UVB	NS	NS	NS	***	*	***	***	NS	NS	*	**	NS
G × T	***	NS	NS	**	NS	***	*	NS	***	***	NS	**
CO_2 × UVB	***	*	NS	***	NS	NS	NS	*	NS	**	NS	NS
CO_2 × T	***	*	***	NS	**	***	NS	NS	NS	NS	NS	***
UVB × T	**	*	**	NS	NS	***	NS	**	NS	NS	NS	NS
G× CO_2 × UVB	NS	NS	NS	NS	NS	***	NS	NS	*	**	NS	***
G × CO_2 × T	**	NS	NS	NS	*	***	***	NS	***	***	NS	NS
G × UVB ×T	**	NS	*	NS	NS	**	NS	NS	***	***	***	NS
CO2 × UVB × T	NS	NS	NS	**	NS	***	*	NS	***	**	**	***
G × CO_2 × UVB × T	NS	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 genotypes. The damaging effects of individual stress factors (+UVB and +T) were less compared to the combined effects (+UVB+T) primarily due to significant negative interaction of UVB ×T. Among the genotypes, CB-27 and UCR-193 showed the greatest reduction in PH and LA across the treatments (Fig. 4.1A, B). SLW exhibited a significant CO_2×UVB×T interaction, and on average, increased in all treatments except +UVB and +UVB+CO_2 treatments (Fig. 4.1C). The main treatment effects on LN were significant only for CO_2 (Table 4.2). The LN varied from 6 (MPE) to 8 (CB-27) under control

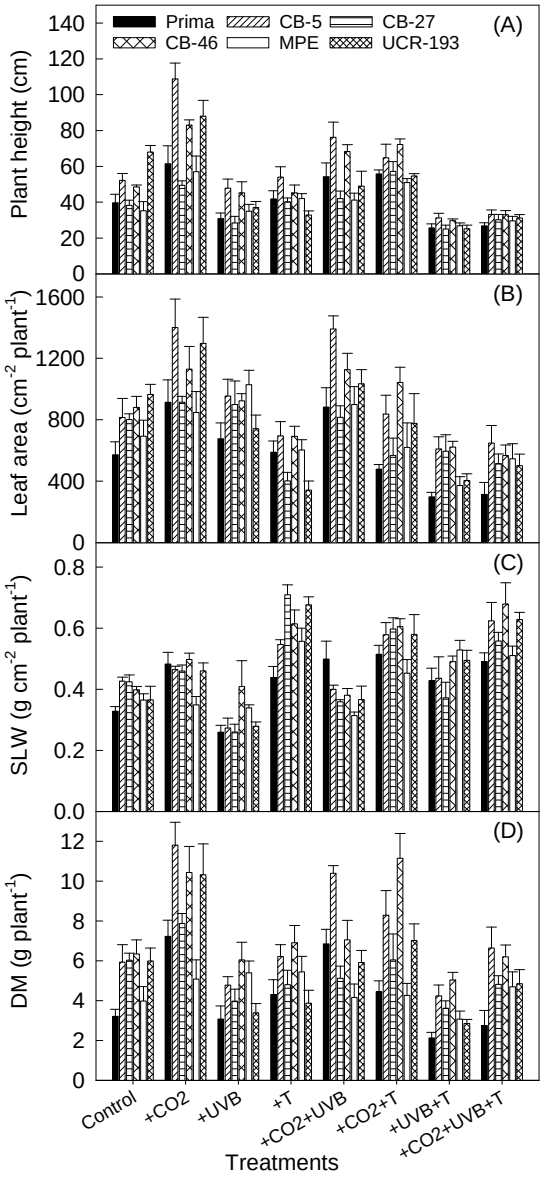

Figure 4.1 Influence of carbon dioxide concentration, temperature and UVB radiation either alone or in combination on (A) plant height, (B) leaf area, (C) specific leaf weight and (D) plant dry matter (DM) of six cowpea genotypes measured at eighteen days after treatment; control (360 µmol mol^{-1}, 30/22 °C and 0 kJ UVB), +CO_2 (760 µmol mol^{-1}, 30/22 °C and 0 kJ UVB), +UVB (10 kJ UVB, 360 µmol mol^{-1}, 30/22 °C), +T (38/30 °C, 360 µmol mol^{-1} and 0 kJ UVB), +CO_2+UVB (720 µmol mol^{-1}, and 10 kJ UVB and 30/22 °C), +CO_2+T (720 µmol mol^{-1}, 38/30 °C, and 0 kJ UVB), +UVB+T (10 kJ UVB, 38/30 °C, and 360 µmol mol^{-1}), and +CO_2+UVB+T (720 µmol mol^{-1}, and 10 kJ UVB and 38/30 °C). The error bars show the standard deviation from three replicates. The error bars show the standard deviation from five replicates.

condition and increased 1-2 leaves plant^{-1} under treatment conditions for all genotypes except CB-27 (data not shown).

The main effects of all treatments were highly significant for DM production (Table 4.2). Similar to the PH, +CO_2 alone increased the DM by 68% as compared to the control, averaged over genotypes. However, this increment was less under +CO_2+UVB and +CO_2+T conditions (Fig. 4.1D). In contrast, without CO_2 enrichment, temperature alone or in combination with +UVB significantly lowered the DM production. Compared to the control, the highest reduction in DM was observed in MPE (52%) at +UVB+T condition.

Leaf photosynthesis and chlorophyll fluorescence

Significant CO_2×UVB×T, CO_2×T and UVB×T interactions were observed for *A* (Table 4.2). Compared to the control, higher photosynthetic rates were observed in all treatments except +UVB+T, which showed 12% lower rates (Fig. 4.2A), when averaged across genotypes. Under +UVB condition, CB-27 showed a 17% reduction whereas under +UVB+T condition, the reduction in photosynthetic rate ranged from 11% (CB-27) to 25% (Prima) compared to the control. The electron transport rate also showed a significant CO_2×UVB×T interaction and decreased significantly under +UVB condition and in combination with either +CO_2 or +T conditions, when averaged over genotypes (Fig. 4.2B). However, ETR increased in other treatments, exhibiting a similar trend to that of *A*. The Fv′/Fm′ had significant UVB×T interaction and showed a value close to the control or even higher under studied stress conditions (Fig. 4.2C).

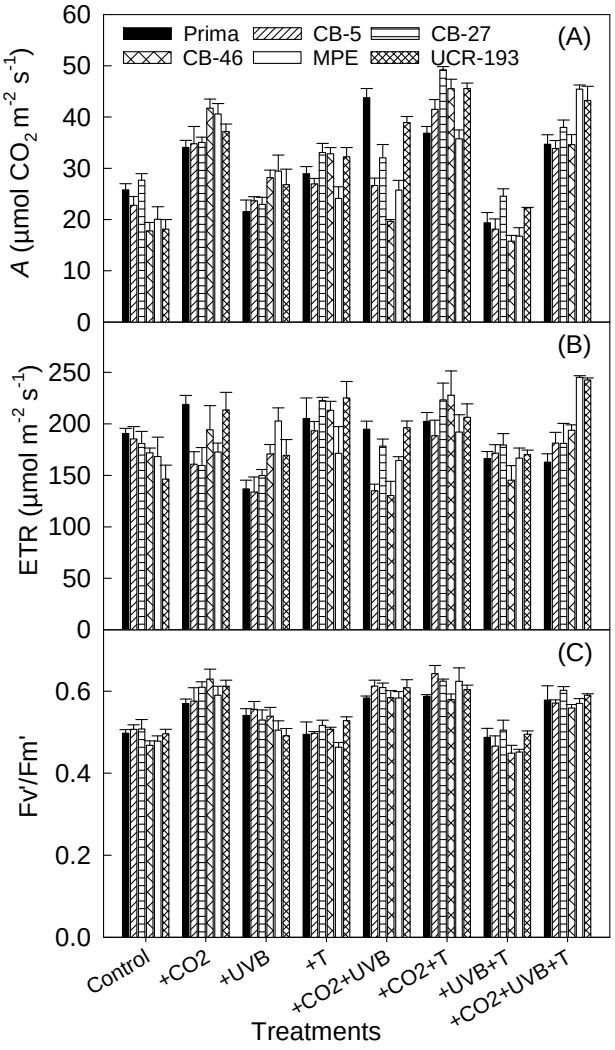

Figure 4.2 Influence of carbon dioxide concentration, temperature and UVB radiation either alone or in combination on (A) net photosynthesis (*A*), (B) electron transport rate (ETR), and (C) chlorophyll fluorescence (Fv′/Fm′) of six cowpea genotypes measured at eighteen days after treatment. The error bars show the standard deviation from three replicates. Other details are as in Fig.4.1.

Leaf pigments, phenolics and cell membrane thermostability

There was a CO_2×UVB×T interaction for both chlorophyll and carotenoid concentrations in cowpea leaves (Table 4.2). High temperature caused substantial

increase in chlorophyll and carotenoid concentrations in most of the genotypes (Fig. 4.3C, B). In contrast, elevated UVB caused a reduction in the concentration of leaf chlorophyll and carotenoid either alone or in combination with $+CO_2$ or +T. Compared to the control, the maximum chlorophyll reduction of 20% was observed in CB-46 at +UVB condition. The combined effect of $+CO_2$+UVB+T on chlorophyll and carotenoid was positive for most of the genotypes, with Prima exhibiting 26 and 29% higher rates, respectively.

Significant CO_2×UVB×T interaction was observed for phenolic concentrations in cowpea. Averaged over all the genotypes, UVB increased the leaf phenolics either alone (17%) or in combination with $+CO_2$ (27%), +T (2%) and with their interactions $+CO_2$+UVB+T (11%). Prima showed the highest increase across all treatments that ranged from 27% (+T) to 91% ($+CO_2$+UVB+T) condition. However, +UVB, +T showed a marked decrease in phenolic concentration with or without CO_2 enrichment in all genotypes except Prima and UCR-193 (Fig. 4.3C). Cell membrane thermostability was negatively affected for the plants grown only under +UVB condition with the maximum reduction observed in MPE (26%). Most of the genotypes exhibited improved CMT when grown under +T condition either alone or in combination with $+CO_2$ (Fig. 4.3D).

Flower morphology, pollen production and pollen viability

All genotypes produced flowers in all treatments; however, flowers that were open were seen in all treatments except +UVB+T condition. Days to flowering varied among treatments and genotypes (29-46 DAS). Most of the genotypes grown in $+CO_2$ and +T conditions flowered 1-3 d earlier. However, under $+CO_2$+T and +UVB

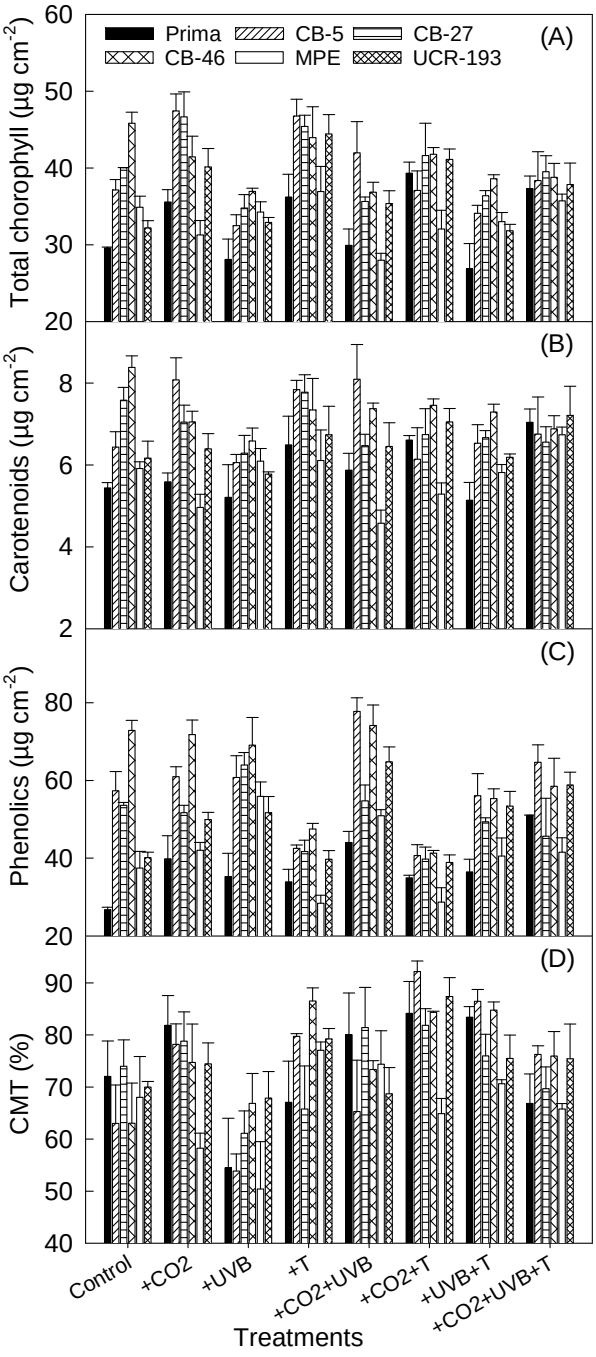

Figure 4.3 Influence of carbon dioxide concentration, temperature and UVB radiation either alone or in combination on (A) total chlorophyll, (B) carotenoid, (C) phenolic contents and (D) cell membrane thermostability (CMT) of six cowpea genotypes measured eighteen days after treatment. The error bars show the standard deviation from three replicates. Other details are as in Fig.4.1.

conditions, the time to flower was delayed by 1-3 d in all genotypes except, CB-27. The greatest delay in flowering was recorded under +CO_2+UVB (2-6 d) and +CO_2+UVB+T (5-10 d) across the genotypes except CB-27.

All the treatments interacted significantly for flower length and flower dry weight in cowpea (Table 4.3). The +CO_2 caused a small increase in flower length compared to the control. Temperature had no effect on flower length either alone or in combination with +CO_2 (Fig. 4.4A). The elevated CO_2 and temperature interacted negatively with UVB for flower length. The highest reduction was observed at +UVB+T condition that ranged from 69% (MPE) to 82% (CB-27). Averaged over genotypes, the flower dry weight was lower in all treatments compared to control with the highest reduction (79%) detected in +UVB+T condition (Fig. 4.4B). Addition of CO_2 reduced the negative influence of +T and +UVB+T on flower dry weight.

Pollen production and pollen viability were lower in all genotypes under all treatment conditions compared to the control (Fig. 4.4C, D), and significant interactions were observed among treatments (Table 4.3). High temperature caused significant reduction in pollen production either alone (31%) or in combination with +CO_2 (34%) and +UVB (25%), averaged over genotypes. The highest reduction in pollen production was observed in CB-27 (56%) followed by CB-5 (37%) at +CO_2+UVB+T condition (Fig. 4.4C). In the presence of +UVB and/or +T, pollen viability showed greater reduction when genotypes were grown under +CO_2 compared with ambient [CO_2] in the presence of the same stressors. None of the genotypes produced viable pollen grains under +UVB+T conditions (Fig. 4.4D).

Table 4.3 Analysis of variance across the genotypes (G) and treatments of carbon dioxide [CO_2], temperature (T), ultraviolet-B (UVB) radiation and their interaction on cowpea reproductive attributes; flower length (Fl length), flower dry weight (Fl Dwt), pollen production anther^{-1} (PP), % pollen viability (PV), 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}) and shelling percentage.

Source of Variation	Fl length	Fl Dwt	PP	PV	Pod no.	Seed Wt	Seeds pod^{-1}	g seed^{-1}	Shelling
G	***	***	***	***	***	***	***	***	***
CO_2	***	***	***	***	NS	NS	NS	NS	NS
UVB	***	***	**	***	**	***	***	**	*
T	***	***	***	***	***	***	***	***	***
G × CO_2	***	**	NS	***	***	*	NS	***	***
G × UVB	***	*	***	***	*	NS	NS	NS	NS
G × T	***	**	***	***	***	***	***	***	***
CO_2 × UVB	***	***	NS	***	NS	**	NS	NS	*
CO_2 × T	***	***	NS	***	NS	NS	NS	NS	NS
UVB × T	***	***	***	***	NS	***	**	**	NS
G× CO_2 × UVB	***	***	NS	***	NS	NS	NS	***	NS
G × CO_2 × T	***	NS	NS	***	***	*	NS	***	***
G × UVB ×T	***	NS	**	***	NS	NS	NS	NS	NS
CO2 × UVB × T	***	***	*	***	*	**	NS	NS	*
G × CO_2 × UVB × T	***	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.

Pod production and yield components

Cowpea genotypes were highly influenced by high temperature treatments and failed to set pods under four treatments involving +T conditions (Fig. 4.5). Therefore, the comparative statements in this section do not include temperature and its interaction with other environmental factors. Only +CO_2 had small beneficial effect on pod number and yield components when averaged over all the genotypes (Fig. 4.5A-D). Significant CO_2×UVB×T interaction for pod production and seed weight plant^{-1} were observed in cowpea genotypes (Table 4.3). For instance, compared to the control, higher pod numbers

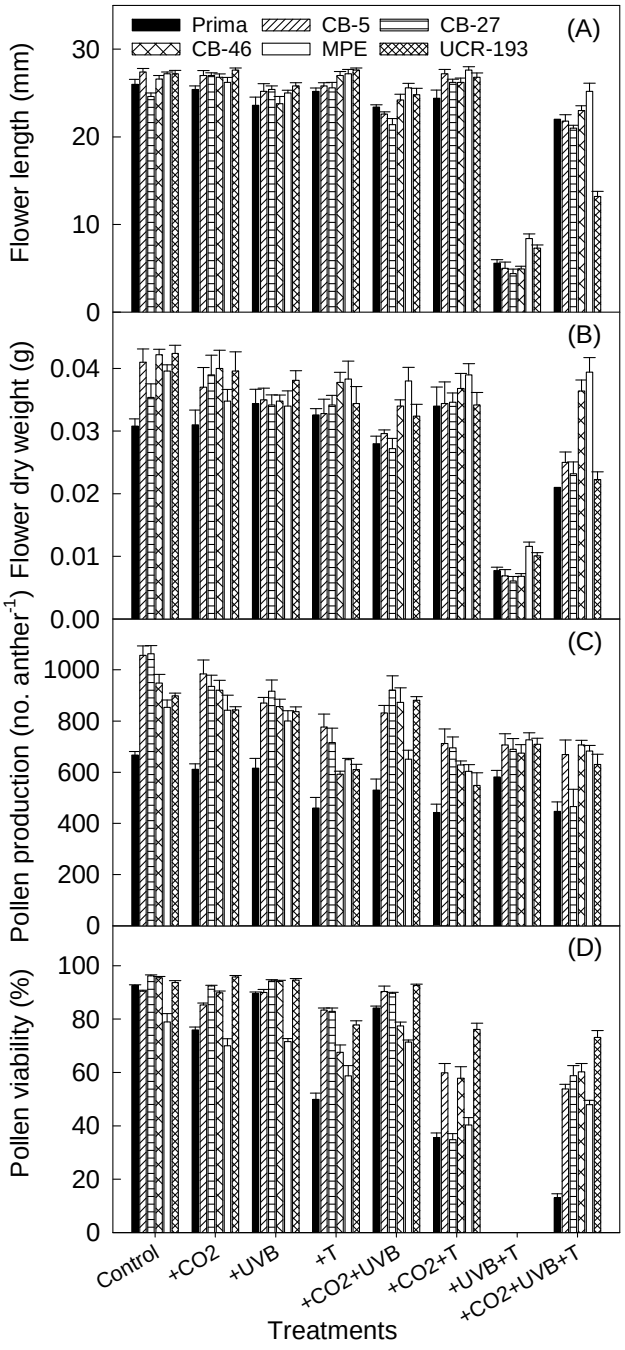

Figure 4.4 Influence of carbon dioxide concentration, temperature and UVB radiation either alone or in combination on (A) flower length, (B) flower dry weight, (C) pollen production and (D) pollen viability of six cowpea genotypes measured between 30 to 40 days after emergence. The error bars show the standard deviation from ten flower length and pollen viability, and five (pollen production) replicates. Other details are as in Fig. 4.1.

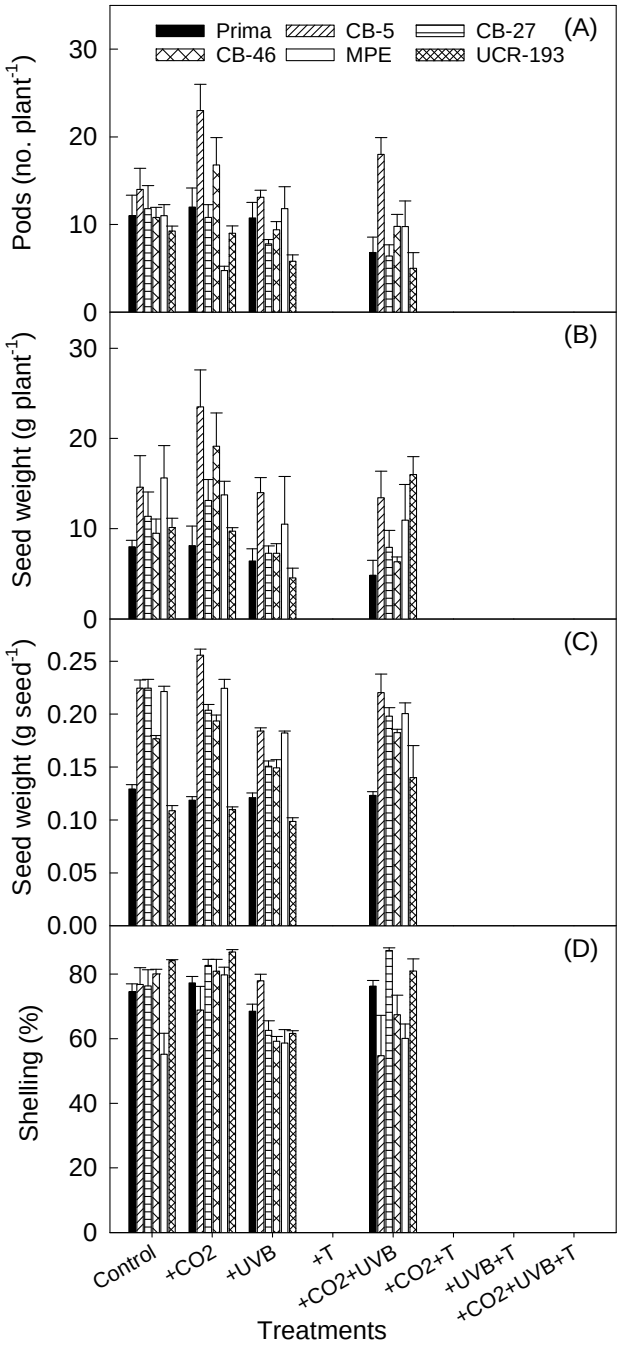

Figure 4.5 Influence of carbon dioxide concentration, temperature and UVB radiation either alone or in combination on (A) pod number plant^{-1}, (B) total seed weight plant^{-1}, (C) individual seed weight and (D) shelling percentage of six cowpea genotypes measured at 53 days after emergence. The error bars show the standard deviation from five replicates. Other details are as in Fig.4.1.

(13%), seed weight (26%) and seeds pod^{-1} (10%) observed in the plants grown under +CO_2 condition were not observed in the plants grown under +CO_2+UVB condition. Moreover, the addition of CO_2 exacerbated the deleterious effect of +UVB on pod production (Fig. 4.5A). The greatest reduction in pod number was observed in CB-27 (47%) followed by UCR-193 (46%) under +CO_2+UVB condition when compared to control. Whereas, the seed weight was highly influenced by +UVB alone which produced the lowest seed weight in UCR-193 (55%) followed by CB-27 (36%) (Fig. 4.5B). Similar to the seed weight, the seeds pod^{-1} was also substantially reduced in UCR-193 (17%) and CB-27 (12%) at +UVB condition.

The individual seed weight (g seed^{-1}) increased 10-14% in CB-5 and CB-46 at elevated [CO_2], while it decreased by 8-9% in CB-27 and Prima (Fig. 4.5C). Compared to the control, the +UVB condition caused the highest reduction (6-30%) in the individual seed weight, averaged over genotypes. At +CO_2 condition, the shelling percentage increased across the genotypes with highest increase in MPE (40%). Among the cowpea genotypes, the +UVB lowered the shelling percentage by 20-30% whereas this reduction was less at +CO_2+UVB condition (Fig. 4.5D).

Stress response index

The cumulative stress response index (CSRI) representing the overall stress response of plant attributes for a given treatment as compared to control showed varying degree of sensitivity of cowpea genotypes to different stress conditions (Table 4.4). Most of the genotypes exhibited positive CSRI for vegetative parameters (V-CSRI, Table 4.4). Only one negative V-CSRI was evident for Prima, MPE and UCR-193 whereas, CB-27

Table 4.4. Cumulative stress response index (CSRI), sum of relative individual plant attribute stress responses index (SRI) at a given treatment; and total stress response index (TSRI), sum of CSRI over all the treatments of six cowpea genotypes in response to elevated carbon dioxide (720 μmol mol^{-1}, $+CO_2$, high temperature (38/30 °C, +T, and increased UVB radiation (10 kJ m^{-2} d^{-1}, +UVB) and their interactions. TSRI were separated in vegetative (V-TSRI), reproductive (R-TSRI) and added together to obtain combined TSRI† (C-TSRI). CSRI is the sum of relative responses with treatments in comparison to control i.e. 360 μmol mol^{-1} (CO_2), 30/22 °C temperature (T) and 0 kJ m^{-2} d^{-1} (UVB) observed for vegetative (V-CSRI: plant height, leaf area, leaf number, specific leaf weight, dry matter, net photosynthesis, Fv′/Fm′, ETR, chlorophyll, carotenoid, phenolics, CMT) and reproductive (R-CSRI: flower length, flower dry weight, pollen production, pollen viability, pod number $plant^{-1}$, seed weight $plant^{-1}$, individual seed weight, number of seeds pod^{-1}, shelling percentage) parameters studied. A combined CSRI (C-CSRI) is the sum of V-CSRI and R-CSRI. ESRI (environmental stress response index) indicates the damaging effect of a given stress over all cowpea performance, ranks are in parentheses. ESRI were also calculated separately for vegetative (V-ESRI) and reproductive (R-ESRI) and combined (C-ESRI) parameters.

Stressor	Genotypes						
	Prima	CB-5	CB-27	CB-46	MPE	UCR-193	
	Vegetative cumulative stress response index (V-CSRI)						**V-ESRI**
$+CO_2$	+419	+358	+111	+356	+235	+429	+1908 (7)
+UVB	+19	−72	−221	−45	+168	−18	−170 (2)
+T	+114	+170	+49	+131	+147	+197	+809 (3)
$+CO_2$+UVB	+353	+157	−1	+98	+92	+279	+978 (4)
$+CO_2$+T	+247	+330	+157	+340	+212	+409	+1696 (6)
+UVB+T	−120	−44	−104	−89	−33	+ 12	−378 (1)
$+CO_2$+UVB+T	+79	+176	−9	+170	+260	+311	+987 (5)
V-TSRI†	+1111	+1075	−18	+961	+1081	+1619	-
	Reproductive cumulative stress response index (R-CSRI)						**R-ESRI**
$+CO_2$	+12	+169	+32	+191	+1	−12	+393 (7)
+UVB	−32	−20	−96	−86	+104	−136	−266 (6)
+T	−574	−559	−520	−571	−491	−556	−3271 (4)
$+CO_2$+UVB	−115	−62	−120	−87	−49	−3	−437 (5)
$+CO_2$+T	−591	−583	−594	−587	−544	−579	−3478 (3)
+UVB+T	−766	−798	−800	−794	−755	−770	−4684 (1)
$+CO_2$+UVB+T	−680	−637	−644	−590	−526	−651	−3728 (2)
R-TSRI†	−2746	−2490	−2742	−2524	−2260	−2706	-
	Combined cumulative stress response index (C-CSRI)						**C-ESRI**
$+CO_2$	+431	+527	+142	+547	+236	+418	+2302 (7)
+UVB	−13	−91	−317	−132	+272	−154	−436 (5)
+T	−460	−389	−471	−440	−344	−359	−2462 (3)
$+CO_2$+UVB	+238	+95	−121	+10	+43	+275	+541 (6)
$+CO_2$+T	−344	−253	−437	−247	−332	−170	−1782 (4)
+UVB+T	−886	−842	−904	−883	−787	−759	−5062 (1)
$+CO_2$+UVB+T	−602	−461	−653	−420	−266	−340	−2741 (2)
C-TSRI†	−1636	−1414	−2761	−1565	−1178	−1088	-

showed the highest numbers of negative V-CSRIs. The negative V-CSRI was mostly associated with +UVB and +UVB+T conditions with the highest negative value of –221 (CB-27) at +UVB. The V-TSRI, sum of V-CSRI over all the treatment conditions, varied greatly from –18 (CB-27) to +1619 (UCR-193).

In contrast to V-CSRI, the R-CSRI representing the cumulative responses of reproductive parameters for a given treatment condition were mostly negative in all the genotypes, from –2260 (MPE) to –2746 (CB-27) (Table 4.4). The positive R-CSRI was only observed under $+CO_2$ condition for all genotypes except in UCR-193. MPE exhibited positive CSRI for both vegetative and reproductive parameters under UV-B condition. The highest negative values were observed in +UVB+T condition across all genotypes and environments. There was no significant correlation ($r^2 = 0.04$, $P > 0.05$) between V-TSRI and R-TSRI.

The combined cumulative stress response index (C-CSRI), representing the combined stress responses over vegetative and reproductive plant attributes (V-CSRI + R-CSRI), were mostly negative and highly varied among the genotypes. However, positive C-CSRIs were observed under $+CO_2$ and $+CO_2$+UVB conditions in all the genotypes except CB-27. The highest negative C-CSRI was recoded at +UVB+T condition for all genotypes. The C-TSRI, representing the sum of C-CSRI over all treatment conditions, was all negative and varied from –1088 (UCR-193) to –2761 (CB-27) (Table 4.4).

The environmental stress response index (ESRI) representing the damaging effect of a given environmental factor either alone or in combination with other factors, on overall performance of cowpea, was calculated separately for vegetative (V-ESRI) and

reproductive (R-ESRI) parameters (Table 4.4). The ESRIs were ranked from 1-7 (1 being the most negative and 7 being the positive or least negative). Similar to the CSRIs, the V-ESRI was mostly positive whereas, R-ESRI and C-ESRI were mostly negative. The +UVB+T was ranked 1 and the +CO_2 as 7 in all the cases.

Factor analysis: Grouping the plant attributes

The factor analysis revealed that the 21 measured variables can be grouped into four groups and thus underlying factors influencing cowpea responsiveness to multiple environmental conditions. The marked patterns in the loadings of variables under each factor helped to propose the common underlying group (Table 4.5). The first factor had the largest eigenvalue and higher communalities for most of the variables. The plant attributes largely loaded on the Factor 1 are pollen production, pollen viability, pod number, total and individual seed weights, seed number and shelling percentage. These are the traits that are known to contribute for crop yield. Therefore, this group was named as the underlying factor "Yield attributes". The second factor had the higher loading for the traits contributing to vegetative traits are CMT and photosynthesis; therefore it was named as "Growth attributes". The third factor consists of higher loadings of SLW, chlorophyll, carotenoid and phenolics and grouped as an underlying factor "Leaf attributes". The two variables highly loaded in the fourth factor are flower length and flower dry weight suggesting an underlying factor "Flower attributes".

Table 4.5 Rotated factor loadings of 21 measured plant attributes representing group-wise responsiveness of cowpea to multiple abiotic stresses.

Response variable	Factor 1 (Yield attribute)	Factor 2 (Growth attribute)	Factor 3 (Leaf attribute)	Factor 4 (Flower attribute)	Communality
Plant height	0.31	0.80*	0.07	0.29	0.82
Leaf area	0.76	0.75*	−0.05	0.16	0.94
Leaf number	0.01	0.64*	0.21	−0.01	0.46
SLW	−0.58	0.32	0.66*	0.04	0.87
Dry matter	0.37	0.84*	0.36	0.14	0.99
A	−0.31	0.53*	0.17	0.31	0.50
Fv′/Fm′	0.00	0.57*	0.00	0.26	0.39
ETR	−0.51	0.22	0.26	0.13	0.39
Chlorophyll	−0.19	0.24	0.92*	0.13	0.95
Carotenoid	−0.11	0.18	0.91*	−0.04	0.87
Phenolic	0.50	0.29	0.59*	−0.15	0.52
CMT	−0.42	0.51*	0.28	−0.33	0.55
Flower length	0.15	0.27	0.03	0.95*	1.00
Flower dry weight	0.22	0.24	0.02	0.89*	0.90
Pollen production	0.82*	0.00	0.10	0.09	0.69
Pollen viability	0.72*	0.19	0.04	0.55	0.72
Pod number	0.86*	0.17	−0.11	0.20	0.82
Seed weight	0.88*	0.12	−0.12	0.22	0.85
gram seed^{-1}	0.90*	0.00	−0.23	0.26	0.94
Seed number pod^{-1}	0.82*	0.06	−0.36	0.30	0.89
Shelling %	0.81*	0.06	−0.35	0.29	0.87
Eigenvalues†	182	82	33	15	–

* indicates the variables with large factor loadings in the corresponding column.
†Indicates the eigenvalues of the correlation matrix.

Discussion

Cowpea genotypes varied significantly in their vegetative and reproductive performance under multiple abiotic stress conditions. The co-existence of two or more climatic factors, [CO_2], UVB and temperature modified the magnitude and direction of individual stress factor response thus supporting our hypothesis. For instance, the +CO_2 compensated the negative effects of +UVB and +T singly or in combination for most of

the vegetative and physiological traits including plant height, leaf area, net photosynthesis and dry matter production. However, the negative effects of +UVB and +T treatments on pollen viability, pod and seed set were not ameliorated by [CO_2] enrichment, suggesting that these processes are carbon independent. This was supported by mostly positive responses of vegetative whereas negative response of reproductive parameters under multiple stress conditions. The current study also revealed that the vegetative and reproductive processes operate differently under multiple abiotic stress conditions, as deduced from the opposite response and lack of correlation between these two processes. Compared to other treatments, under +UV-B+T condition the flower development was severely inhibited, but substantial number of pollen production was observed. Although, these pollen grains were not viable, it indicates that pollen production was not the main cause limiting the reproductive performance under multiple abiotic stress condition.

Substantial reductions in PH, LA and DM observed in the current study have also been reported in several tropical legumes exposed to UVB (Singh, 1996) or temperature (Prasad et al., 2002; Singh, 1996). The SLW, a measure of leaf thickness, increased in most of the treatments similar to earlier finding of Qaderi et al. (2006). The alteration in PH, leaf thickness and morphology may be partially attributed to the photo-oxidation of indole 3-acetic acid (IAA), a growth hormone that absorbs UVB and is involved in cell division and cell elongation processes (Nedunchezhian and Kulandaivelu, 1997; Pal et al., 1997). Qaderi et al. (2006) have also shown that increased temperature reduces the level of IAA in canola (*Brassica napus* L.) plants, there by reduction in plant growth and lower dry matter.

Stimulation of photosynthesis in cowpea caused by $+CO_2$ alone or in combination with either +UVB or +T in the current study is in agreement with the observed response in other C_3 crops (Long, 1991; Mark and Tevini, 1997; Qaderi et al., 2006). However, it contrasted with the results obtained in a previous study with cowpea (Ahmed et al., 1993). This dissimilarity might have been caused due to the temperature treatment differences, as only night time temperature varied in that study. Interestingly, compared to the control, the average photosynthetic rate was much higher under $+CO_2$+T (92%) condition than in either $+CO_2$ (69%) or +T (35%) condition. The lower photosynthetic rate observed for single factors (e.g. $+CO_2$ or +T) compared to their interaction might be explained by the feedback inhibition of photosynthesis due to faster accumulation of starch in leaves under $+CO_2$ condition whereas limited supply of carbohydrate under +T condition due to increase in photorespiration (Ahmed et al., 1993; Long, 1991; Ro et al., 2001). Conversely, higher [CO_2] reduces the photorespiration and the high temperature tends to decrease leaf starch by increasing sucrose synthesis which facilitates the recycling of inorganic phosphate to the chloroplast, hence enhanced rate of photosynthesis under $+CO_2$+T condition (Long, 1991; Ro et al., 2001). Therefore, the highest photosynthesis rate observed in this study under $+CO_2$+T condition is the manifestation of both the suppression of photorespiration and increased turnover rate of soluble sugars between chloroplast and cytoplasm.

In contrast, significant reduction in photosynthesis rate was observed under +UVB+T condition compared to the control. However, addition of [CO_2] ($+CO_2$+UVB+T condition) compensated the negative effect of +UVB+T. One of the primary causes proposed for photosynthesis inhibition under high temperature is reduced

capacity of RuBP regeneration that could be caused by down regulation of Rubisco due to either starch accumulation and/or Rubisco deactivation which is mediated by the enzyme Rubisco activase (Allen et al., 1998; Kubien and Sage, 2008). The Rubisco activase is a temperature sensitive enzyme that can be denatured and thus becomes non-functional at high temperature conditions (Kubien and Sage, 2008). However, under +UVB+T condition, none of these evidences appeared to be limiting photosynthesis rate, disclosing the mobilization of photosynthates and stability of Rubisco activase even at the 38 °C. The reduction in photosynthesis rate observed at +UVB+T condition might be attributed to increased photorespiration due to high temperature along with the decreased efficiency of photosystems. UVB and temperature have been reported as to damage thylakoid membranes, and negative interactions between these two factors might have caused increased photon leakage across the thylakoid membranes (Kubien and Sage, 2008; Nedunchezhian and Kulandaivelu, 1997). This was also supported in the current study showing a pattern similar to the photosynthesis for ETR and Fv′/Fm′ under +UVB+T and +CO_2+UVB+T conditions, respectively. Qaderi et al. (2006) also reported an increase in maximum quantum efficiency of PSII in canola plants grown under elevated [CO_2] and high temperature conditions.

The leaf pigments (chlorophyll and carotenoids) followed the same trend as that of photosynthesis in response to different stressors and increased similar to the earlier findings (Qaderi et al., 2006; Ro et al., 2001). Qaderi et al. (2006) also found a similar increase in leaf pigments under high temperature condition which was mostly caused by increased SLW. Among the genotypes, varying degrees of UVB and temperature induced stimulation in the synthesis of carotenoids and phenolic compounds are in

accordance with the previous studies and considered as a protective response against to these stress conditions (Nedunchezhian and Kulandaivelu, 1997; Premkumar and Kulandaivelu, 2001; Qaderi et al., 2006). Previously known relatively heat tolerant genotypes, Prima, CB-27, and UCR-193 along with MPE, were more responsive to UVB radiation for production of phenolic compounds. However, +T alone or in combination with $+CO_2$ caused marked reduction in phenolic compounds. Koti et al. (2007) also observed similar reductions in phenolic contents in soybean under higher temperature. CMT did not show a distinct pattern among the genotypes which contrasted to the results of Ismail and Hall (1999) where they found that heat tolerant genotypes exhibited greater CMT compared to the heat sensitive genotypes. This contradiction was probably due to the different temperature treatments used for plant growth and CMT assay.

Similar to the current study, the decrease in flowering time in response to elevated [CO_2] and temperature was also observed in previous studies (Ellis et al., 1995; Ismail and Hall, 1998; Ohler and Mitchell, 1995). Ismail and Hall (1998) demonstrated a temperature dependant linear decrease in days from sowing to flowering until a threshold of 30.9 °C (for heat tolerant genotypes) and 23.8 °C (for heat sensitive genotypes), above which the rate of progress towards flowering did not change or decrease as temperature increased. In contrast, the substantial delay in flowering observed in this study under all the possible combinations of UVB is in accordance with previous studies with other legumes (Pal et al., 1997; Rajendiran and Ramanujam, 2004; Saile-Mark and Tevini, 1997, Singh et al., 2008a). This delay in flowering caused by UVB and the negative interaction of UVB with either $+CO_2$ or +T conditions might be attributed to alteration of gibberellins biosynthesis and suppression of floral bud development (Ismail and Hall,

1998; Saile-Mark and Tevini, 1997). Developing early maturing lines to escape seasonal drought is one of the important breeding strategies commonly used to ensure adaptation of crops under semi-arid environments (Grantz and Hall, 1982; Singh, 2004). The current study suggests that the substantial delay in flowering caused by UVB radiation in the presence of [CO_2] alone or in combination with elevated temperature may increase the crop duration.

Contrary to the trends in vegetative growth and photosynthesis, +CO_2 did not counteract the negative impact of UVB and temperature on plant reproductive processes. A slight increase in yield components observed at +CO_2 in this study is a common beneficial effect of [CO_2] enrichment of increasing carbon availability leading to greater yield when other conditions are normal (Kimball et al., 2002; Prasad et al., 2002; Reddy and Hodges, 2000). However, elevated [CO_2] failed to counteract the negative effects of UVB in most of the genotypes and even recorded lower pod numbers, seed weight, and shelling percentage. UVB caused reduction in seed yield has also been reported in other tropical legumes (Saile-Mark and Tevini, 1997). Rajendiran and Ramanujam (2004) reported smaller and fewer seeds per pod along with reduction in pod number (25%), seed weight (45%), and shelling percentage (7%) in *Vigna radiata* exposed to UVB radiation. This appeared to be due to decreased flower dry weight, reduced pollen viability and lower pod set. Additionally, the increase in the allocation of carbon resources towards the repair mechanisms and biosynthesis of UV-B absorbing compounds at the expense of reproductive structure might contribute for the reduction of flower characteristics and seed yield.

The substantial reduction in flower size, pollen production and pollen viability caused by UVB and/or temperature in the current study are in accordance with the previous studies including cowpea (Prasad et al., 2002; Prasad et al., 2003; Warrag and Hall, 1983; Warrag and Hall, 1984). Fully developed flowers were observed under all the treatment conditions except +UVB+T in which flowers produced were small and did not open as in other treatments. Surprisingly, the flowers produced under +UVB+T condition showed developed anthers with substantial amount of non viable pollen grains (Fig. 4.4C), indicating that pollen vitality (pollen germination and viability) are being affected by these stress conditions and pollen production is not the cause leading of lower seed yield.

The stress response indices (CSRI, TSRI and ESRI, Table 4.4) used to assess the quantitative effects of multiple abiotic stresses in the current study is equally effective as in other crops with high intra-specific variability (Dai et al., 1994; Koti et al., 2007; Saile-Mark and Tevini, 1997). Generally, positive values of vegetative parameters (V-CSRI and V-TSRI) compared to the negative values for reproductive attributes (R-CSRI and R-TSRI) clearly show high negative impact of abiotic stresses on cowpea reproductive potential. As expected, the data showed high degree of genotypic variation for both vegetative and reproductive traits and the over all stress effect was negative in all genotypes as deduced from the C-TSRI. However, the magnitudes of genotypic responses were highly modified by different stresses either alone or in combination. This modified degree of response mechanisms might have been caused due to the differences in co-activation of different response pathways by simultaneous exposure of plants to different abiotic stresses leading to a synergistic (for example most of the vegetative growth and

photosynthetic parameters of cowpea in this study) or antagonistic (reproductive processes and yield attributes) effects (Mittler, 2006). There was no significant ($r^2 = 0.04$, $P > 0.05$) correlation between V-TSRI and R-TSRI suggesting that the genotypes that performed well for vegetative parameters did not perform in the same way for reproductive growth in the presence of the same stress condition. Vast amount of energy and resources are required for plants to acclimate to abiotic stress conditions, hence, nutrient deprivation including carbon could pose a serious problem to plants attempting to cope with heat or UVB stress (Mittler, 2006). This increase in allocation of carbon and other resources towards repair mechanisms and biosynthesis of protective compounds such as carotenoids and/or phenolic compounds at the expense of reproductive structures might have caused high sensitivity of reproductive traits. The combined response of vegetative and reproductive traits to multiple abiotic stresses (C-TSRI) facilitated the relative classification of cowpea genotypes in to three groups, as tolerant (UCR-193, MPE and CB-5), intermediate (CB-46 and Prima) and sensitive (CB-27) to multiple abiotic stresses.

A distinct plant attribute could not be isolated that can be used as selection criteria in cowpea for multiple abiotic stress tolerance. Hence, factor analysis was used to identify underlying plant attributes that can be used as screening protocols. Four groups of the plant attributes were identified in this study. The first group was “yield attributes” that include pollen production and pollen viability which can be used in determining multiple abiotic stress tolerance and should be included while planning for a breeding strategy to incorporate yield under multiple abiotic stresses in cowpea. Hall (2004) proposed yield component model that can be incorporated for selection of cowpea

cultivars in the high temperature-limited production zones. This yield model includes four components namely, numbers of flowers, pods and seeds per pod and weight of individual seed that have also been recognized in the current study. Similarly, plant "growth attributes" including photosynthesis were the second in the proposition and exhibit the plant survival capacity under stress condition. The third group called as "leaf attributes" comprised of protective responses such as SLW, leaf pigments and phenolic compounds implying their use for trait-based breeding programs to enhance the protective response in new lines for multiple abiotic tolerance.

The magnitude of genotypic variability of a species offers an opportunity for a plant breeder to design and develop specific plant type to suit in the different agro-ecological environments. The effectiveness of selection for a trait depends on its genetic control under different environmental condition which is expressed as heritability of the trait (Hall and Ziska, 2000; Thiaw and Hall, 2004). The genetic association of a trait with higher level of physiological and/or developmental attributes that facilitate adaptation for a stress condition are very useful for plant breeding purposes and to develop improved lines of a crop species (Singh and Sharma, 1996). By categorizing the interactions across plant attributes, it is evident from the result of this study that the stress protective response "leaf attributes" identified by factor analysis exhibit parallel increasing or less decreasing response patterns along with "growth and yield attributes" for at least in three cowpea genotypes (Prima, MPE and UCR-193). Similarly, the inheritance studies have demonstrated that heat tolerance during reproductive development requires a higher heritable recessive gene for flower production (Thiaw and Hall, 2004). In the current study, the appearance of flower and comparable pollen productions observed even under

+UVB+T condition have remarkable potential for trait-based selection criterion that may be used in other species to enhance stress tolerance via genetic manipulation.

Plant adaptation to abiotic stresses dependent upon the activation of molecular networks involved in stress perception, signal transduction and expression of specific stress related gene and metabolites, which ultimately result in morphological and physiological development (Vinocur and Altman, 2005). The linkage between stress-associated molecular mechanisms and physiological response is still a major gap in our understanding of crop tolerance to different stress conditions (Sinclair and Purcell, 2005). Most of the current studies involving combination of stress factors have used either short-term stress treatments and/or low radiation conditions, rather than evaluating stress response over plant life cycle under reasonable radiation environment (Kant et al., 2008; Koti et al., 2007; Qaderi and Reid, 2005; Qaderi et al., 2007; Rizhsky et al., 2002, 2004; Tegelberg et al., 2008). Therefore, due to the emergent nature of yield from physiological processes, and the physiological processes are the outcome of various molecular networks in response to different stresses, the results from these studies may not be transferable under natural environment and will lack the association with actual crop yield. A comprehensive portfolio of molecular and physiological basis of stress tolerance that combines the traditional and molecular breeding (genetic engineering) will help to improve crop tolerance and yield across abiotic stresses.

In conclusion, the current study revealed that regardless of [CO_2] enrichment, a combined effect of UVB and temperature possibly will pose a serious problem for cowpea and most likely for many summer-grown crop production in future climates. All cowpea genotypes responded in the same direction while the magnitude of these

responses to multiple stress conditions varied widely among genotypes. Elevated [CO_2] did not negate the damaging effects of UVB and/or high temperature on reproductive traits. The identified tolerant cowpea genotypes and groups of plant attributes could be used for selection and development of genotypes tolerance to multiple abiotic stresses by trait-based plant breeding or genetic engineering programs. The cowpea vegetative and reproductive attributes in response to abiotic stresses were not correlated indicating the tolerance mechanisms in both these processes operate differently. In addition, cumulative environmental stress response indices (E-ESRI and R-ESRI) of vegetative and reproductive parameters yielded poor correlation indicating the factors that may positively contribute for vegetative traits may not go hand-in-hand with reproductive traits. Therefore, developing cultivars for the future climate is daunting challenge addressing many facets of crop growth and development under multiple environmental stress conditions.

CHAPTER V

IDENTIFYING COWPEA [*Vigna unguiculata* (L.) Walp.] GENOTYPES FOR DROUGHT STRESS TOLERANCE BASED ON PHOTOSYNTHESIS AND FLUORESCENCE MEASUREMENTS

Abstract

Drought is the major abiotic stress factor that causes extensive losses to agriculture production worldwide. Developing simple and accurate tools to identify genetic variability among cultivars for drought tolerance will be useful in crop breeding programs. The objective of this study was to evaluate the dynamics of photosynthetic parameters including rate of photosynthesis (*A*), stomatal conductance (g_s), transpiration (E), ratio of intercellular CO_2 to ambient CO_2 concentration (C_i/C_a), fluorescence (Fv′/Fm′) and electron transport rate (ETR) to drought stress conditions. An experiment was conducted using fifteen cowpea genotypes representing different sites of origin seeded in 12-L pots, filled with fine sand, and irrigated with full-strength Hoagland's nutrient solution from emergence to 30 days after sowing (DAS). Thereafter, one set of plants continued to receive optimum water and the other set received no water for another 20 days. The photosynthetic parameters, leaf relative water content (RWC) and soil water content (SWC) were measured daily during the experimental period. Cowpea genotypes showed stomatal regulated extreme drought avoidance by maintaining high RWC. The photosynthetic parameters exhibited strong association with decline in SWC. *A* and

Fv′/Fm′ declined linearly with decreasing SWC whereas intrinsic water-use efficiency (WUE; A/g_s) increased under drought stress. Stomatal regulation was the major limitation to photosynthesis under drought stress. However, under severe drought conditions, increase in C_i/C_a along with reduced WUE showed the role of non-stomatal limitation of photosynthesis. Maintenance of a constant ETR, higher ETR/*A* ratio (an estimate of photorespiration) and resistant nature of Fv′/Fm′ under drought appeared to be important protective mechanisms from photoinhibition in cowpea under drought stress conditions. Although, drought stress-induced reduction in total chlorophyll and carotenoids accompanied with an increase in proline and wax contents were observed, they were not correlated with photosynthetic parameters studied indicating their role as stress indicators. Cowpea genotypes differed significantly for maximum photosynthesis and Fv′/Fm′, slopes of *A* and Fv′/Fm′ in response to SWC, WUE and the $C_i/C_a min^{-1}$. Genotypes were classified as tolerant (UCR-193, MBE and TPP), intermediately tolerant (Prima, MPE, TWC, Melakh, ZC and TVu-4552), intermediately sensitive (BC, CB-46 and CB-27), and sensitive (CB-5, MS and MP) to drought stress using photosynthesis and florescence parameters and principal component analysis.

Introduction

Stomatal regulated reduction in transpiration is a common response of plants to drought stress which also provides an opportunity to increase plant water-use efficiency (Parry et al., 2005). Under moderate drought stress conditions, reduced stomatal conductance (g_s) is the primary cause of photosynthetic inhibition from a reduced supply of CO_2 to the chloroplasts (Lawlor, 2002). However, under drought stress conditions,

reduced rate of photosynthesis rate (*A*) promotes an energy imbalance in photosystems (PS) causing an over excitation of PSII reaction centers. This poses photo-inhibitory damage and an additional non-stomatal limitation to photosynthesis (Medrano et al., 2002). One of the most important protection mechanisms for photoinhibition under stress conditions in plants is non-photochemical quenching or transporting electrons (e^-) other than CO_2, most importantly to oxygen, leads to photorespiration and/or Mehler reaction (Flexas et al., 2002; Heber, 2002). The other well known process to avoiding photoinhibition is the non-radiative energy dissipation mechanisms in which a significant proportion of absorbed photons are lost as a thermal energy (Maxwell and Johnson, 2000; Souza et al., 2004). These processes may bring the electron transport capacity into balance; however, it results in lower quantum yield of PSII (Govindjee, 1999). Under field conditions; however, when drought is coincided with high solar radiation and temperature conditions, these processes might be insufficient to utilize and dissipate all the excitation energy and might lead to photoinhibition (Krause, 1988).

Cowpea is an important legume crop grown mostly in the arid and sub-arid zones of the world where production mostly depends upon rain as a sole source of water (Ehlers and Hall, 1997; Singh et al., 1997). West Africa alone accounts for >65% area under cowpea cultivation which is frequently subjected to periods of drought (Singh et al., 2003). Drought stress during flowering can cause >50% reduction in yield due to poor pod formation and seed set probably caused by limited carbohydrate supply (Turk et al., 1980; Labanauskas et al., 1981). As an adaptive response, cowpea plants have shown extreme dehydration avoidance by maintaining high leaf water status without substantial osmotic adjustment (Bates and Hall, 1981; Lopez et al., 1987). Such a water conservative

trait in plants has been described as 'isohydric', shared by maize, sugarcane, grapes and several other crops (Tardieu and Simonneau, 1998; Medrano et al., 2002; Jones, 2007). Few studies; however, have reported substantial reduction of leaf relative water content and/or leaf water potential in cowpea under drought stress conditions (Anyia and Herzog, 2004; Chiulele and Agenbag, 2004). Similar response patterns of photosynthetic parameters in relation to the intensity of drought or stomatal conductance (g_s) were observed. Studies have shown that stomata can respond to root or soil water status directly via root-shoot signaling without any detectable changes in leaf water potential which may involve plant stress hormone, abscisic acid (Jones, 1998; Medrano et al., 2002). Therefore, the importance of monitoring soil water content (SWC) and/or g_s while studying the responses of photosynthetic parameters under drought condition has gained importance in recent years (Flexas et al., 2002; Medrano et al., 2002; Parry et al., 2005; Jones, 2007).

Crop adaptation to rain-fed conditions can be achieved by improved water-use efficiency or by increasing water supply to the plant through improved root system (Hall, 2004). Intrinsic water-use efficiency (WUE) estimated as a ratio of A/g_s has been well recognized as a measure of carbon gain per unit of water-loss and found to be inversely proportional to the ratio of intercellular and ambient CO_2 concentration (C_i/C_a) (Martin and Ruiz-Torres, 1992; Brodribb, 1996; Lefi et al., 2004). Large variability in WUE has been reported among several species as well as cultivars of a species including cowpea (Hall et al., 1990; Martin and Ruiz-Torres, 1992; Condon et al., 2002). Because higher rates of leaf photosynthesis are often associated with faster crop growth rates, a combination of this trait with improved WUE may play a vital role for yield enhancement

of crops under drought stress conditions (Parry et al., 2005). Therefore, wheat breeding programs for drought tolerance have been initiated to improve crop production by incorporation of early vigorous growth and high WUE into new cultivars to exploit WUE in a wide range of environments (Condon et al., 2002).

Previous studies have shown that cowpea photosynthetic performance can recover considerably after releasing the drought stress (Turk et al., 1980; Lopez et al., 1987; Anyia and Herzog, 2004; Souza et al., 2004). These studies also demonstrated a close association between photosynthetic performance and stomatal conductance reflecting a transient stage of photoinhibition (Souza et al., 2004) or residual impairment of photosystems at very low stomatal conductance (Lopez et al., 1987). However, the protective mechanisms for maintaining the photosynthetic apparatus under drought stress condition are not well understood (Anyia and Herzog, 2004). Studies are needed to derive functional relationships between stomatal conductance and photosynthetic parameters in order to understand their co-regulations as soil water status changes. Given the importance of soil water status in regulating stomatal conductance, the extent of a stomatal limitation to various photosynthetic parameters can be assessed by simultaneous measurement of leaf gas exchange and chlorophyll fluorescence parameters under drought stress conditions (Medrano et al., 2002; Long and Bernacchi, 2003; Parry et al., 2005). The underlying hypothesis is based on the assumption that (a) cowpea maintains high water status during drought by lowering leaf conductance which is more influenced by soil water status than leaf relative water content, (b) cowpea plants maintain high rates of electron transport and fluorescence (quantum efficiency of PSII) that prevents photoinhibition, and (c) genotypic variability for high WUE that can be due to increased

photosynthesis or decreased g_s or to both of these mechanisms is present in cowpea. The objectives of the study were to (a) investigate the responses of leaf gas exchange and chlorophyll fluorescence characteristics of 15 cowpea genotypes under drought conditions, (b) determine stomatal limitation to various photosynthetic parameters, and (c) determine the mechanism of maintaining stability in photosynthesis processes during drought stress conditions.

Materials and methods

Plant material and experimental conditions

An out-door pot culture experiment was conducted in 2007 growing season at the R. R. Foil Plant Science Research Center, Mississippi State University, Mississippi State, MS, USA (33° 28'N,88° 47'W). Fifteen cowpea genotypes representing diverse sites of origin (Table 5.1) were seeded in 12-L pots, filled with fine sand on 2 August 2007. The pots were 0.65 m in height and 0.15 m in diameter with a small hole at the bottom to drain excess water. The study comprised of 600 with 40 pots per genotype in two complete sets (20 control and 20 stressed). The pots were arranged randomly in thirty rows, oriented in a east to west direction with 1-m spacing between rows. Seedlings were thinned two per pot seven days after emergence. All plants were irrigated with full-strength Hoagland's nutrient solution (Hewitt, 1952) three times a day, from emergence to 30 days after sowing (DAS). Thereafter, plants (control) continued to receive optimum water and the other set (drought stressed) received no water until the end of experiment (50 DAS). The pots in the drought-stressed treatments were covered with plastic sheeting at the base of the plants to shield from rain water getting into the pots.

Leaf and soil water content measurements

From 30 to 50 DAS, photosynthetic parameters, leaf relative water content (RWC) and soil water content (SWC) were measured daily in all treatments. Immediately after the photosynthetic measurements, the same leaves were detached to measure the leaf fresh, turgid and dry weights. The turgid weight of the leaves was determined keeping the leaves in moistened paper towels for 24 h in dark, and dry weight of the same leaves was obtained after drying in an oven at 70 °C for 48 h. Leaf relative water content was determined as follows: RWC = (fresh weight – dry weight)/turgid weight – dry weight). Also, immediately after the photosynthetic measurements, SWC of the upper 6-10 cm of soil was measured with soil moisture probe (Type ML2X attached to HH2 moisture meter, Delta-T Devices, Burwell, UK).

Gas exchange and fluorescence measurements

Gas exchange and chlorophyll fluorescence parameters were measured simultaneously using Li-COR 6400 Photosynthesis System (LICOR Inc., Lincoln, Nebraska, USA) with an integrated fluorescence chamber head (Li-COR 6400-40 mounted with Leaf Chamber Fluorometer; LCF) on the 3rd or 4th fully expanded attached leaves between 10:00 and 13:00 h over two cm^{-2} leaf area in each genotype. The measurements were taken at 1500 µmol photon m^{-2} s^{-1} photosynthetically active radiation, cuvete temperature set to 30 °C, 360 µmol mol^{-1} CO_2 and 50 ± 5% relative humidity. The quantum efficiency by oxidized (open) PSII reaction center in light was calculated as (Fv′/Fm′) = (Fm′-Fo′)/Fm′ (Genty et al., 1989), where Fm′ = maximal fluorescence of light adapted leaves, Fo′ = minimal fluorescence of a light adapted leaf that has

momentarily been darkened. The actual flux of photons driving photosystem II (PSII), i.e. electron transport rate (ETR), was computed according to the equation $[(Fm'-Fs)/Fm'] \times fl\alpha_{leaf}$, 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 ($\mu mol\ m^{-2}\ s^{-1}$) flux density, and α_{leaf} = leaf absorptance set to 0.85 in this study. Intrinsic water use efficiency (WUE) was estimated as the ratio of A/g_s (Martin and Ruiz-Torres, 1992). ETR/*A* was taken as the relative measure of electron transport to oxygen molecules (Flexas et al., 2002).

Pigments, proline and wax measurements

Total chlorophyll, carotenoid, proline and wax concentrations were measured from the 3rd or 4th leaf from the top at 45 DAS in the control and drought-stressed plants when SWC were 0.06 and .01 $m^3\ m^{-3}$, respectively. The pigments were extracted by placing five 0.38 cm^{-2} leaf disks in a vial containing 5 ml of dimethyl sulfoxide and incubated in dark for 24 h. Thereafter, the absorbance of the supernatant was measured at 648, 662 and 470 nm by using Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). The total chlorophyll and carotenoids were estimated by using the equations of Lichtenthaler (1987) and expressed on leaf area basis ($\mu g\ cm^{-2}$).

For proline extraction, three leaves from each genotype were collected at noon and 0.5 g of leaf tissue was immediately placed in a vial containing 10 ml of 3% aqueous sulfosalicylic acid and stored at -20 °C. For analysis, the mixture was homogenized after bringing to room temperature and the homogenate was filtered through Whatman no. 2 filter paper. Two ml of filtrate was reacted with 2 ml each of acid-ninhydrin reagent and

glacial acetic acid in a test tube by heating on a water-bath maintained at 100 °C for one hour and the reaction was terminated in an ice bath (Bates et al., 1973). The reaction mixture was extracted with 4 ml of toluene and the free proline was extracted as outlined by Bates (1973) and expressed as μmol g^{-1} using a proline standard (L-Proline, Sigma-Aldrich, Inc., MO USA).

The extraction and quantitative analysis of leaf epicuticular waxes were carried out as per the method of Ebercon et al. (1977) with minor modifications. Ten leaf discs constituting an area of 35.36 cm^{-2} from 3rd or 4th leaf from the top were cut from each genotype from five different plants for each replication. Leaf waxes were removed by stirring the leaf disk in 15 ml of chloroform (Sigma-Aldrich, Inc., MO USA) in a test tube for 20 s. The wax extract was evaporated on a water bath maintained at 80 °C, cooled to room temperature; 5 ml of dichromate reagent was added and further heated on a water bath maintained at 80 °C for 30 minutes. The reagent was prepared by dissolving 20 g $K_2Cr_2O_7$ in 40 ml of de-ionized water and the resulting slurry was mixed with 1 L of H_2SO_4 and heated below boiling until a clear solution was obtained. The samples were removed from water bath and cooled and then 12 ml of de-ionized water was added, allowed for 15 minutes, and the intensity of the color was measured at 590 nm using Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). The wax content was expressed on a leaf area basis (μg cm^{-2}) by using a standard curve developed from the wax obtained from the same species.

Statistical Analysis

Cowpea response to drought was assessed by combining the data for all genotypes together and also within a genotype. The relationships among the SWC, RWC, different gas exchange and fluorescence parameters were tested for linear, exponential and logarithmic fit and the best fit equations were selected. The relationship between g_s and SWC was analyzed by exponential three parameter regression equation [$Y = y0 + (a \times e^{bx})$] where, b represent the rate of stomatal closure in response to decreasing SWC. An exponential decay function [$Y = y0 + (a \times e^{-bx})$] was used to describe the relationship between WUE and SWC, where (y0 + a) is the maximum WUE (WUE_{max}). The exponential rise to maximum function [$Y = y0 + a \times (1 - e^{-bx})$] was used to obtain the relationships between g_s and A, Fv′/Fm′, C_i/C_a, ETR and E, where (y0 + a) provided the maximum photosynthesis (A_{max}) and fluorescence (Fv'/Fm'_{max}). The relationship between g_s and C_i/C_a was fit under the condition in which g_s was the primary factor controlling the observed decrease in photosynthesis, as described by Brodribb (1996). The minimum C_i/C_a (C_i/C_a*min*) and the corresponding g_s value were obtained from these functions. To determine the co-regulation of these parameters (A, Fv′/Fm′, C_i/C_a, ETR and E) as a function of g_s, all parameters were normalized to the g_s value of three mol m^2 s^{-1}, representative of the plants grown under saturated SWC (0.06 m^3 m^{-3}).

The regression analyses were carried out using SigmaPlot version10 (Systat Software Inc, 2006). A one-way ANOVA analysis (SAS Institute Inc, 2004) was used to assess the genotypes variability for total chlorophyll, carotenoid, proline and wax concentrations ($P < 0.05$). The difference between genotype means was tested by using Fisher's Least Significant Difference (LSD).

Principal component analysis (PCA) is a statistical technique for multivariate data and is quite useful in separating experimental units into subgroups (Johnson, 1998). The PCA was performed on the correlation matrix of fifteen genotypes and six response variables, i.e. regression slopes of *A* and Fv′/Fm′ response to SWC, A_{max}, Fv′/Fm′$_{max}$, WUE_{max} and C_i/C_a*min*$^{-1}$ using the PROC PRINCOMP procedure (SAS Institute Inc, 2004). The *A* and Fv′/Fm′ values were normalized at saturated SWC to obtain the slope. The PCA produced loadings for these response variables termed as eigenvectors, principal component (PC) scores for each genotypes, and eigenvalues for each PC. A superimposed biplot with the PC scores and the corresponding eigenvectors was developed with the same scale units along the abscissa and ordinates having the same physical length as illustrated by ter-Braak (1983). The eigenvectors derived from the PC analysis were used to identify the variables that tend to have a strong relationship (i.e. having elements larger in absolute value than the other elements in the same eigenvector) with a particular PC. This criterion was used to describe and group cowpea genotypes for their drought stress responsiveness.

Results

SWC, RWC, photosynthesis and transpiration

The combined analysis of all cowpea genotypes showed no relationship between RWC and SWC (Fig. 5.1A). However, photosynthesis (*A)* showed a linear relationship with SWC (Fig. 5.1B). The g_s exhibited an exponential relationship with SWC and decreased to zero under severe drought conditions (Fig. 5.1C). Similar to the *A*, the transpiration rate (E) also exhibited a linear relationship with SWC; however, the analysis

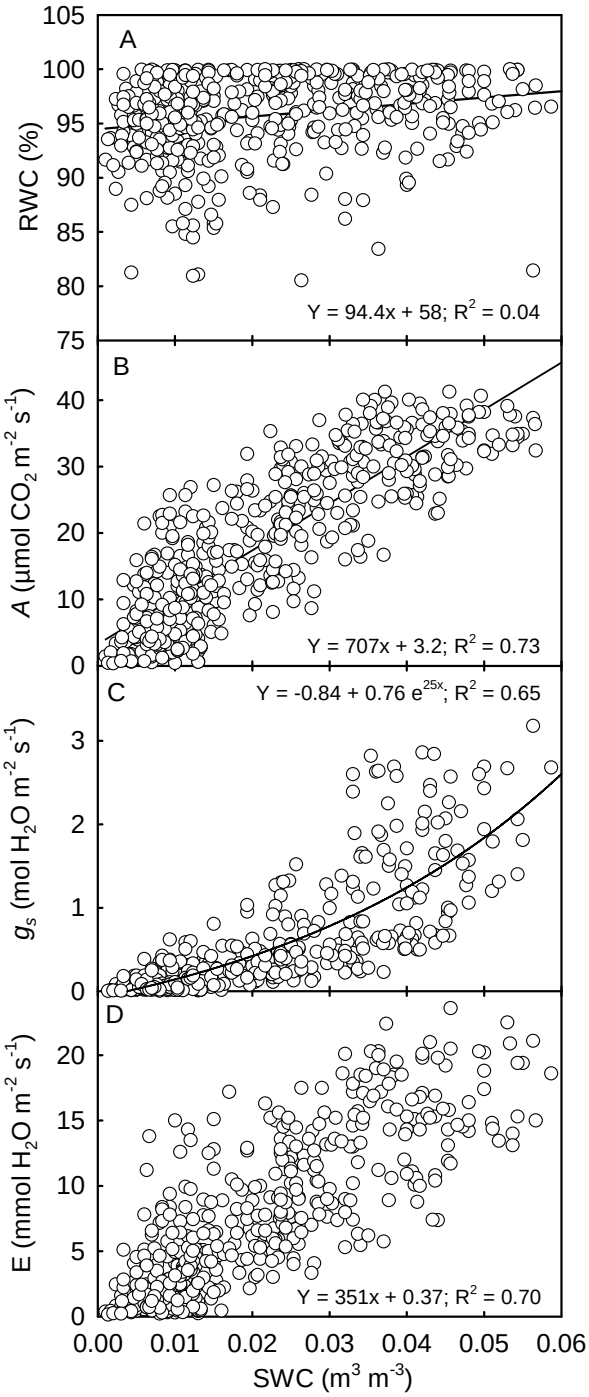

Figure 5.1 Relationships between soil water content (SWC) and (A) leaf relative water content (RWC), (B) photosynthesis (*A*), (C) stomatal conductance (g_s) and (D) transpiration rate (E). Data is from fifteen cowpea genotypes (P = >0.05 (RWC), >0.001 (*A* and g_s) and n = 512).

of the normalized data for both parameters revealed that the E decreased at a faster rate than *A* in response to drought stress (Fig. 5.1D).

Photosynthesis, intercellular CO_2 and WUE

Photosynthesis declined linearly as drought stress-induced C_i decreased to a minimum value of 95 μmol mol^{-1} and remained low even after an increase in C_i (Fig. 5.2A). In order to obtain a minimum C_i value, a linear regression was performed using the C_i values obtained above 0.04 mol H_2O m^{-2} s^{-1} g_s and *A*. The C_i value above 0.04 mol m^{-2} s^{-1} g_s were used because C_i started to increase when g_s decreased further. Based on the regression analysis, it is estimated that *A* reached zero at a C_i value of about 180 μmol mol^{-1}. The WUE, on the other hand, increased initially as g_s decreased and peaked roughly around the g_s value of 0.04 mol m^{-2} s^{-1}; after that it declined sharply with further decrease in g_s (Fig. 5.2B).

Drought induced stomatal regulation to photosynthetic parameters

Fig. 5.3 shows measured gas exchange (Fig. 5.3A-C) and fluorescence (Fig. 5.3D-F) parameters in response to stomatal conductance. All photosynthetic parameters (Fig. 5.3A-E) exhibited exponential response patterns with increased in stomatal conductance except ETR/*A* (Fig. 5.3F). Photosynthesis, C_i/C_a, ETR and Fv′/Fm′ appeared to saturate before the g_s value of 3 mol m^{-2} s^{-1}; whereas E continued to increase slightly beyond this point (Fig. 5.3B). The C_i/C_a decreased until a minimum value of C_i/C_a (predicted $C_i/C_a min$ = 0.41 and g_s = 0.002 mol m^{-2} s^{-1}) (Fig. 5.3C). However, an increase in C_i/C_a was observed near 0.04 mol m^{-2} s^{-1} g_s value. In contrast to *A*, fluorescence parameters decreased at much a slower rate. The ETR was maintained until very low

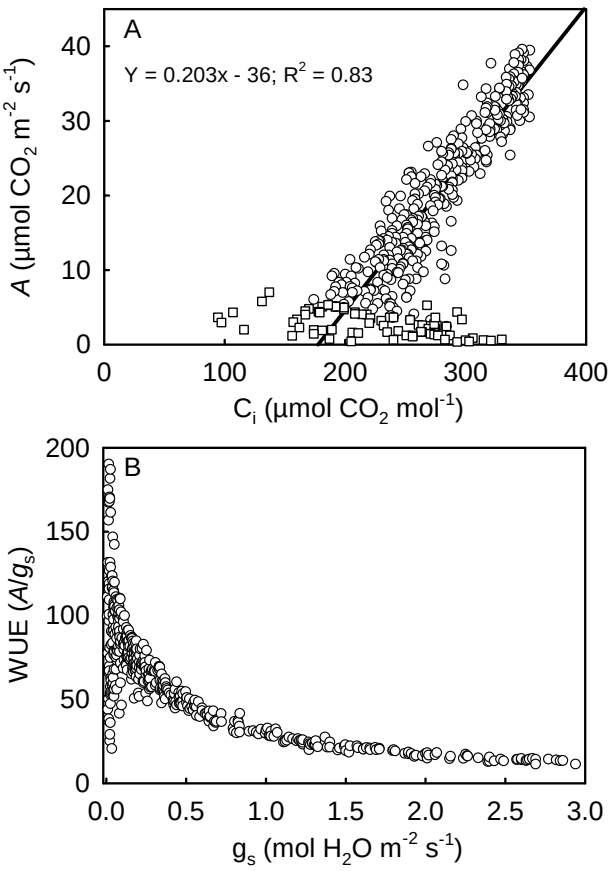

Figure 5.2 Relationships between (A) C_i and *A* and (B) between g_s and WUE in cowpea. Data is from fifteen cowpea genotypes (P = <0.001 and n = 512). The linear regression in figure *A* was only extended to the C_i value obtained above 0.04 mol m^{-2} s^{-1} g_s (circular symbols) thus, only 438 data was included.

values of g_s were reached; whereas, Fv′/Fm′ started to decrease at higher g_s than ETR and values remained higher at about 0.42 (Fig. 5.3D, E) under more severe stress. The ETR/*A* almost mirrored changes in ETR, and increased exponentially as g_s decreased, reaching a very high ratio up to (220 µmol e^- µmol CO_2^{-1}) at the lowest g_s (Fig. 5.3F).

Co-regulation of photosynthetic parameters

To understand the relative regulation of stomatal conductance, the data on gas exchange and fluorescence parameters were normalized (Fig. 5.4). The normalized plot

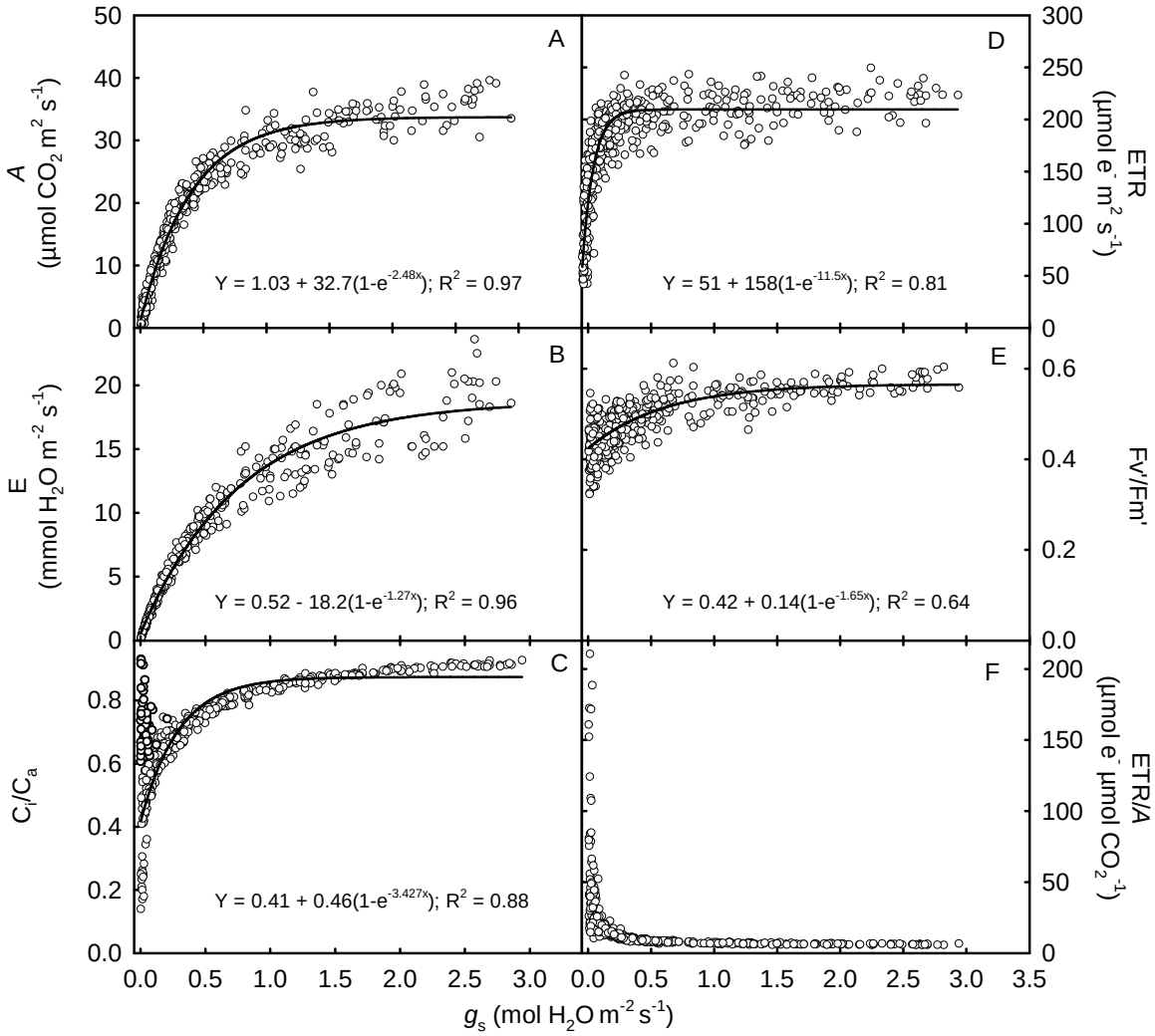

Figure 5.3 Relationships between stomatal conductance (g_s) and (A) photosynthesis (A), (B) transpiration rate (E), (C) C_i/C_a, (D) electron transport rate (ETR), (E) fluorescence (Fv′/Fm′) and (F) ETR/A for fifteen cowpea genotypes. P = <0.001 and n = 512 for all except C_i/C_a in which n = 438 and remaining 74 values were not included in the regression fit. The line in Fig. 5.3 C, represents the relationship between the g_s and C_i/C_a under condition in which g_s was the primary factor controlling decrease in photosynthesis (following Brodribb, 1996, Plant Physiol vol.111, p. 179-185).These line has been extended only to the g_s value at which C_i/C_a was minimal.

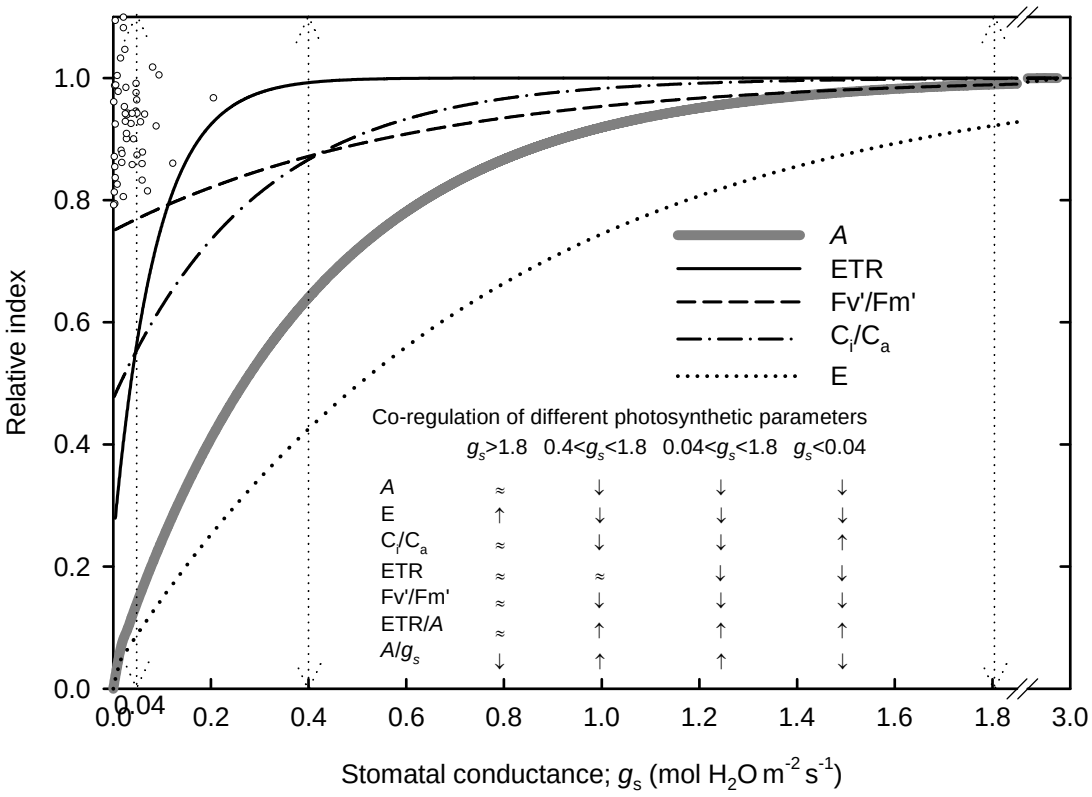

Figure 5.4 Analysis of the extent of the stomatal co-regulation to the different photosynthetic parameters in cowpea, using drought induced decrease in stomatal conductance (g_s) as a reference parameter. The normalized data of fifteen cowpea genotypes form the (Fig. 5.3) were used. The circular symbols at the top left corner of the figure indicate increase in C_i/C_a. The four stomatal conductance (g_s) regions are distinguished.

revealed four out of five parameters were saturated at the g_s value of 1.8 mol m^{-2} s^{-1}, with a very early saturation in ETR (approx. at g_s = 0.4 mol m^{-2} s^{-1}). The E was not saturated at 1.8 mol m^{-2} s^{-1} and values continued to increase beyond 3 mol m^{-2} s^{-1} of g_s.

Four well defined stomatal controlled regions (g_s>1.8, 0.4<g_s<1.8, 0.04<g_s<1.8 and g_s<0.04 mol m^{-2} s^{-1}) exhibiting the co-regulation of photosynthetic parameters were apparent from Fig. 5.4. In the first region (g_s>1.8), a stomatal conductance beyond 1.8 mol m^{-2} s^{-1} has no effect on *A*, ETR, Fv′/Fm′, C_i/C_a and ETR/*A* (ETR/*A* as in the Fig.

5.3F). Whereas, E continued to increase and was accompanied with reduction in A/g_s (A/g_s as in the Fig. 5.2B). In the second region ($0.4<g_s<1.8$ mol m^{-2} s^{-1}), as g_s decreased (77%) from 1.8 to 0.4 mol m^{-2} s^{-1}, A (36%), E (58%), Fv′/Fm′ (13%) and C_i/C_a (14%) were decreased continuously without any change in ETR. In contrast, at this g_s value, ETR/A showed about 22% increase. This was also the region when A/g_s began to increase (Fig. 5.2B).

The third region was apparent when g_s were reduced from 0.4 to 0.04 mol m^{-2} s^{-1} in drought stressed plants. The reductions accounted by different photosynthetic parameters were: 85% (A), >90% (E), 46% (C_i/C_a), 48% (ETR) and 23% (Fv′/Fm′); however, the ETR/A increased by >200% under these conditions (Fig. 5.3F). The A/g_s also continued to increase in this region. An additional decrease in g_s, signified the fourth region (<0.04 mol m^{-2} s^{-1} g_s) of stomatal conductance where A and E approached almost zero; whereas, ETR and Fv′/Fm′ were decreased by about 75% (51 µmol e^- m^{-2} s^{-1}) and 25% (0.42) of the maximum, respectively. In contrast to the previous region, a sudden drop in A/g_s was also observed (Fig. 5.2B). Though, the C_i/C_a also increased in some genotypes as much as its maximum value along with a continuous increase in ETR/A.

Genotypic variability for photosynthesis, fluorescence and WUE

The analysis of fifteen cowpea genotypes showed that photosynthesis and Fv′/Fm′ declined linearly with decreasing SWC (Table 5.1, and Fig. 5.5A, B). The genotypes differed significantly in their response to SWC and the slopes ranged from 614 in ZC to 1006 in CB-5 for A and from 2 in CB-27 to 5 in CB-5 for Fv′/Fm′ (Table 5.1). For clarity,

Table 5.1 Characteristics of the regression equations describing relationship of soil water content (SWC) with photosynthesis (*A*), fluorescence (Fv′/Fm′), intrinsic water use efficiency (WUE), estimated maximum WUE (WUE_{max}) and stomatal conductance (g_s) of fifteen cowpea genotypes. $P < 0.01$ and n varied from 30 to 36.

Genotype	Origin	*A*		R^2	Fv′/Fm′		R^2	g_s			R^2	WUE			R^2	WUE_{max}
		Coefficient			Coefficient			Coefficient				Coefficient				
		a	b		a	b		y0	a	b		y0	a	b		
Black Crowder (BC)	USA	4.04	624	0.66	0.43	2.91	0.43	–0.87	0.85	21.10	0.45	–26.5	115.4	15.8	0.45	89
CB-5	USA	–2.36	1006	0.87	0.37	5.06	0.72	–3.26	2.97	13.54	0.63	4.7	111.7	42.2	0.66	116
CB-27	USA	4.29	617	0.67	0.46	2.09	0.56	–1.30	1.24	16.29	0.77	–47.5	128.6	11.7	0.58	81
CB-46	USA	6.79	617	0.70	0.44	2.81	0.56	–0.72	0.71	28.49	0.69	18.9	103.1	65.4	0.60	122
Magnolia Blackeye (MBE)	USA	3.29	589	0.71	0.43	2.76	0.68	–0.10	0.10	51.33	0.83	53.2	141.9	166.4	0.61	195
Melakh	Senegal	5.09	768	0.70	0.45	3.10	0.62	–0.71	0.67	28.73	0.80	–3.5	118.8	35.9	0.51	115
Mississippi Pinkeye (MPE)	USA	1.80	753	0.82	0.43	3.01	0.73	–1.78	1.62	15.67	0.77	–31.9	138.4	20.9	0.75	107
Mississippi Shipper (MS)	USA	3.05	673	0.71	0.39	3.77	0.65	–0.27	0.25	45.78	0.61	–16.3	130.4	29.3	0.69	114
Mississippi Purple (MP)	USA	2.99	715	0.71	0.40	3.29	0.61	–0.68	0.65	20.15	0.70	33.2	93.4	73.6	0.65	127
Prima	Nigeria	2.66	766	0.73	0.42	3.10	0.64	–0.91	0.84	23.88	0.70	24.1	100.5	62.2	0.62	125
Tennessee White Crowder (TWC)	USA	4.04	690	0.78	0.41	3.24	0.63	–0.65	0.56	32.22	0.59	–7.4	124.0	32.1	0.72	117
Top Pick Pinkeye (TPP)	USA	1.89	735	0.75	0.46	2.63	0.63	–0.27	0.25	45.26	0.58	23.3	101.8	48.6	0.66	125
TVu-4552 (TVu)	Senegal	3.55	732	0.71	0.44	2.21	0.61	–0.26	0.25	41.00	0.69	19.5	98.5	49.6	0.55	118
UCR-193 (UCR)	India	0.90	808	0.87	0.45	2.51	0.63	–0.24	0.20	49.79	0.82	25.0	161.4	70.0	0.77	186
Zipper cream (ZC)	USA	4.51	614	0.76	0.43	2.34	0.60	–0.64	0.55	30.37	0.73	4.5	131.3	38.6	0.58	136

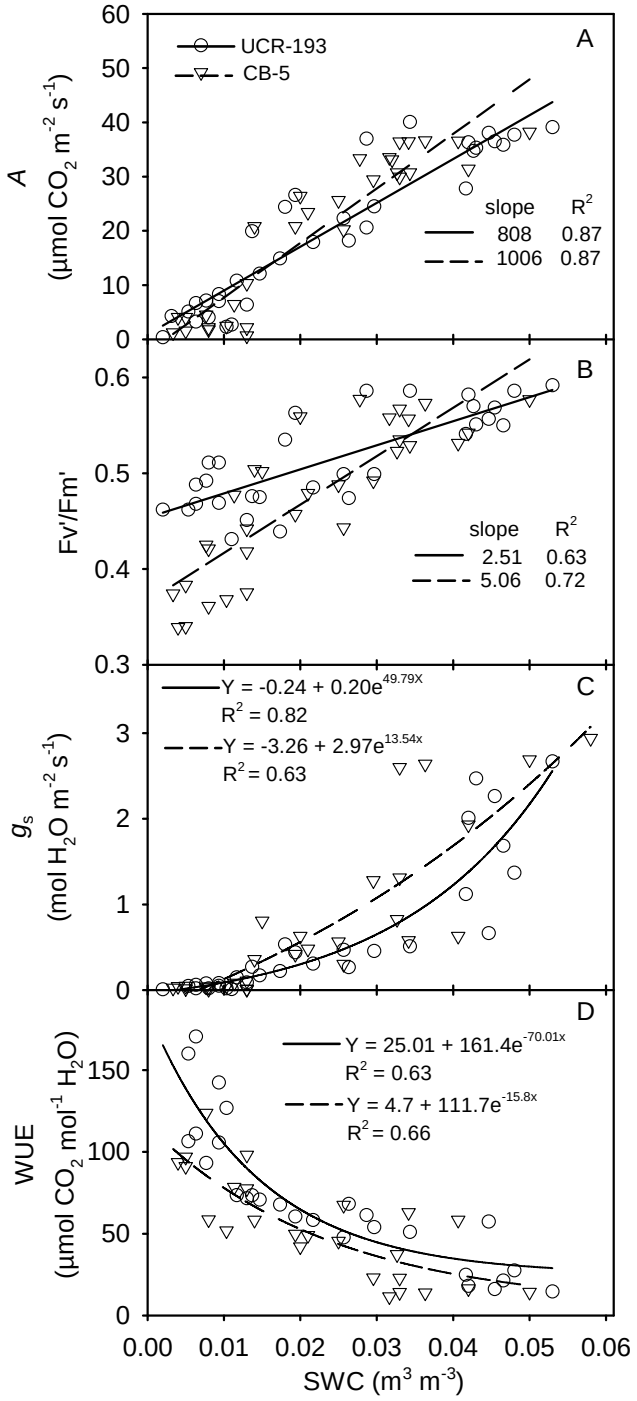

Figure 5.5 Relationships between soil water content (SWC) and (A) photosynthesis (*A*), (B) fluorescence (Fv′/Fm′), (C) stomatal conductance (g_s) and (D) intrinsic water use efficiency (WUE) in cowpea. Only two genotypes with their regression fits are shown. P = <0.001 for all curves, n = 32 (UCR-193) and 30 (for CB-5).

only data and response functions of two cowpea genotypes are shown in Fig. 5.5. Among the two parameters, changes in photosynthetic response to SWC were much greater than the changes in Fv′/Fm′, former approached to zero while the latter remained higher, across both cowpea genotypes under severe drought stress conditions (Fig. 5.5A, B). The g_s exhibited an exponential decrease in response to decrease in SWC (Fig. 5.5 C). The rate of stomatal closure in response to SWC expressed as slope of the relationship between g_s and SWC varied among genotypes, ranging from 13.5 in CB-5 to 51.3 in MBE (Table 5.1). In contrast to g_s, WUE increased exponentially as SWC decreased (Fig. 5.5D). The WUE_{max} varied from 81 in CB-27 to 186% in UCR-193 (Table 5.1) among the 15 genotypes.

The high correlation was observed between *A* and g_s and varied from 0.95 to 0.99 among genotypes (Table 5.2). At low g_s, the response of *A* was comparable, whereas at higher values, a similar increase in g_s yielded greater increase in *A* for many of the genotypes (Fig. 5.6A). A similar response was also observed between g_s and Fv′/Fm′ (Fig. 5.6B). The C_i/C_a exhibited biphasic response pattern over g_s, an initial stomatal regulated reduction phase followed by an increase, roughly below the g_s level of 0.048 (CB-5) and 0.002 mol m^{-2} s^{-1} in UCR-193 reflecting the onset of a non-stomatal limitation to photosynthesis (Fig. 5.6C). Around this g_s level, the Fv′/Fm′ also decreased sharply. Among cowpea genotypes, the A_{max} ranged from 30.7 in MS to 36.6 μmol m^{-2} s^{-1} in UCR-193 and the Fv'/Fm'_{max} ranged from 0.545 in MP to 0.630 in TPP (Table 5.2). The C_i/C_a*min* varied from 0.323 in UCR-193 to 0.592 in CB-27 with a corresponding g_s level of 0.002 and 0.048 mol m^{-2} s^{-1}, respectively (Table 5.2).

Table 5.2 Characteristics of the regression equations describing relationship of stomatal conductance (g_s) with photosynthesis (A), fluorescence (Fv′/Fm′), and ratio of intercellular CO_2 (C_i) to the ambient CO_2 (C_a) concentration (C_i/C_a) for fifteen cowpea genotypes. The estimated maximum A (A_{max}), maximum Fv′/Fm′ (Fv′/Fm′$_{max}$) and minimum C_i/C_a (C_i/C_a*min*) are also presented. The values in the parenthesis for C_i/C_a*min* column represent stomatal conductance at which the C_i/C_a*min* was found. $P < 0.001$ and n varied from 30 to 36.

Genotype	A			R^2	A_{max} (μmol CO_2 m^{-2} s^{-1})	Fv′/Fm′			R^2	Fv′/Fm′$_{max}$	C_i/C_a			R^2	C_i/C_a*min*
	Coefficients					Coefficients					Coefficients				
	y0	a	b			y0	a	b			y0	a	b		
BC	0.70	31.9	2.37	0.97	32.5	0.420	0.169	1.17	0.55	0.589	0.506	0.346	3.38	0.95	0.550 (0.041)
CB-5	0.52	33.3	2.48	0.98	33.8	0.381	0.178	1.92	0.84	0.559	0.464	0.443	2.24	0.90	0.477 (0.013)
CB-27	0.49	32.6	2.40	0.98	33.1	0.434	0.128	1.85	0.72	0.562	0.562	0.333	1.94	0.94	0.592 (0.048)
CB-46	0.11	31.5	2.91	0.95	31.6	0.414	0.144	2.31	0.71	0.558	0.370	0.508	3.63	0.89	0.388 (0.010)
MBE	0.35	33.0	2.75	0.98	33.4	0.419	0.159	2.28	0.71	0.578	0.266	0.540	6.83	0.91	0.302 (0.010)
Melakh	1.86	34.3	2.10	0.97	36.1	0.426	0.159	2.32	0.85	0.584	0.389	0.503	3.30	0.90	0.424 (0.022)
MPE	0.96	34.2	2.48	0.98	35.1	0.401	0.175	3.09	0.85	0.576	0.442	0.453	3.04	0.85	0.459 (0.013)
MS	0.38	30.3	3.15	0.96	30.7	0.386	0.168	2.35	0.76	0.553	0.408	0.466	3.27	0.85	0.430 (0.014)
MP	0.43	31.5	2.87	0.98	31.9	0.385	0.160	2.38	0.83	0.545	0.405	0.431	4.24	0.89	0.426 (0.012)
Prima	0.91	32.1	2.60	0.96	33.0	0.420	0.150	1.28	0.76	0.571	0.408	0.463	3.44	0.81	0.428 (0.013)
TWC	2.29	31.8	2.23	0.97	34.1	0.417	0.161	1.11	0.70	0.578	0.458	0.424	2.80	0.96	0.484 (0.023)
TPP	0.98	35.2	2.34	0.97	36.2	0.477	0.153	0.66	0.50	0.630	0.447	0.423	3.06	0.84	0.469 (0.018)
TVu	0.81	32.6	2.64	0.99	33.4	0.427	0.147	0.93	0.80	0.574	0.371	0.503	3.45	0.94	0.376 (0.003)
UCR	1.74	34.8	2.22	0.98	36.6	0.464	0.136	0.72	0.65	0.599	0.319	0.563	4.03	0.92	0.323 (0.002)
ZC	1.49	32.9	2.45	0.98	34.4	0.420	0.139	1.67	0.76	0.560	0.464	0.415	2.78	0.96	0.471 (0.007)

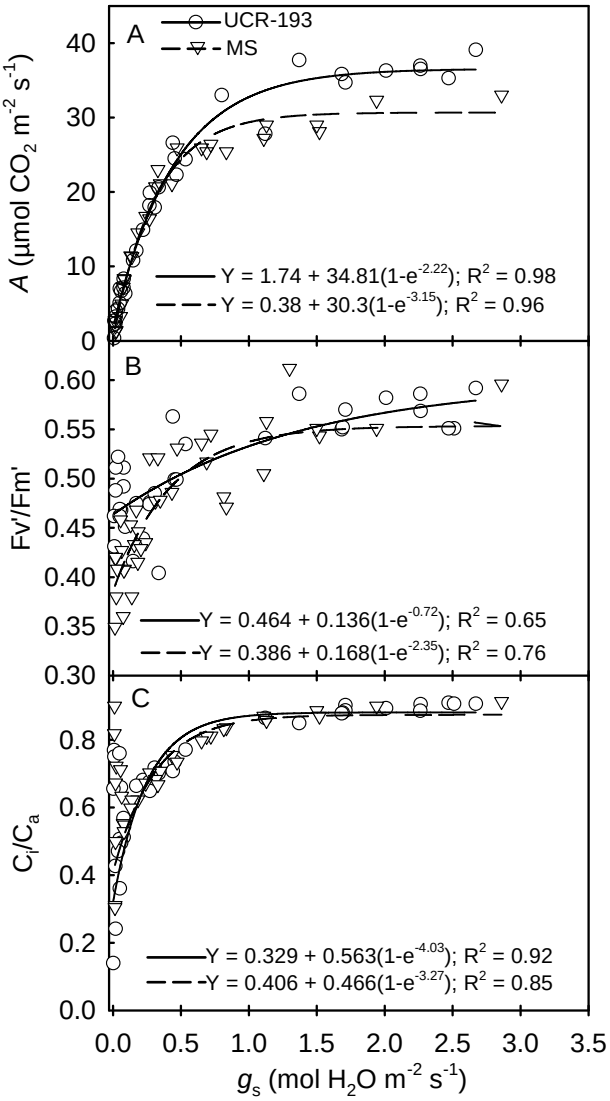

Figure 5.6 Relationships between stomatal conductance (g_s) and (A) photosynthesis (A), (B) fluorescence (Fv′/Fm′) and (C) C_i/C_a in cowpea. Only two genotypes with their regression fits are shown. P = <0.001, n = 32 (UCR-193) and 36 (MS), except the fit for C_i/C_a with g_s in which n = 27 (UCR-193) and 30 (MS) and the remaining values were not included in the regression fit.

Leaf pigments, proline and wax content

Table 5.3 shows changes in leaf pigments, proline and leaf epicuticular wax content in well-irrigated and drought-stressed plants. Cowpea genotypes varied

significantly for these parameters under both well-irrigated and drought-stressed conditions. The significance test between genotypes indicated that this variation was greater under irrigated condition compared to the drought-stressed plants for chlorophyll and carotenoids. Drought stress caused reduction in total chlorophyll and carotenoids concentrations with maximum decrease in MPE (53%) for both the pigments. Whereas proline and wax contents increased as maximum as 332 (PMP) and 46% (MPE), respectively.

PCA of drought tolerance

The differences and similarities in the response of cowpea genotypes to drought were assessed using PCA. The first two PC's chosen based on the scree plot explained about 67% total variations among cowpea genotypes for the six selected parameters. The eigenvectors for PC1 had high positive scores for A_{max}, Fv'/Fm'_{max}, WUE and $C_i/C_a min^{-1}$; whereas, the eigenvector of PC2 had high positive scores of A_{slope} and Fv'/Fm'_{slope} (Fig. 5.7). These slopes were also referred as drought sensitivity as higher the slope the more sensitive to drought because of the steep drop in the parameters due to increasing drought and vice versa. Therefore, genotypes with high PC scores should have high values for these parameters. For instance, in the biplot of PC1 and PC2 (Fig. 5.7), the genotype UCR-193 had the highest value for A_{max}, Fv'/Fm'_{max}, WUE and $C_i/C_a min^{-1}$ with lower score of A_{slope} and Fv'/Fm'_{slope} and was determined as tolerant to drought. Similarly, genotypes with relatively high scores for PC1 and low scores for PC2 were classified as drought tolerant (UCR-193, TPP, and MBE). Genotypes near the center of the plot have medium PC scores, reflecting their intermediate photosynthetic performance and medium

Table 5.3 Total chlorophyll, carotenoids, proline, and leaf epicuticular wax contents of cowpea genotypes under well irrigated and drought stress (SWC = 0.01 m3 water m-3 soil) conditions. Percent changes (%) form irrigated to drought-stressed plants are also shown. The results are mean ± SE for three replicates.

Genotype	Total chlorophyll (μg cm^{-2})			Carotenoids (μg cm^{-2})			Proline (μmol g^{-1})			Wax (μg cm^{-2})		
	Irrigated	Drought	%	Irrigated	Drought	%	Irrigated	Drought	%	Irrigated	Drought	%
BC	47.5 ± 2.49	33.6 ± 2.01	–29	10.80 ± 0.87	7.04 ± 0.31	–35	1.51 ± 0.09	3.54 ± 0.41	135	7.97 ± 0.73	10.80 ± 0.95	26
CB-5	62.1 ± 7.14	32.6 ± 2.17	–48	13.41 ± 1.15	7.70 ± 0.37	–43	1.12 ± 0.14	3.59 ± 0.25	221	9.70 ± 1.19	18.20 ± 1.49	47
CB-27	61.2 ± 3.27	33.5 ± 2.73	–45	13.13 ± 1.21	7.62 ± 0.44	–42	1.02 ± 0.16	2.80 ± 0.41	175	9.93 ± 4.28	17.26 ± 1.41	42
CB-46	67.1 ± 3.54	39.1 ± 3.99	–42	14.36 ± 0.88	8.63 ± 0.69	–40	0.71 ± 0.16	2.90 ± 0.72	311	12.74 ± 0.60	18.72 ± 1.14	32
MBE	63.5 ± 4.46	41.8 ± 2.03	–34	13.92 ± 0.86	8.76 ± 0.39	–37	0.52 ± 0.04	2.26 ± 0.23	332	13.16 ± 0.64	18.15 ± 2.70	27
Melakh	53.7 ± 6.29	33.1 ± 3.60	–38	11.15 ± 1.29	6.81 ± 0.95	–39	0.95 ± 0.05	3.90 ± 0.41	310	11.07 ± 1.08	17.26 ± 1.19	36
MPE	62.9 ± 4.86	29.7 ± 1.71	–53	13.75 ± 0.92	6.53 ± 0.20	–53	1.56 ± 0.16	3.07 ± 0.20	96	10.17 ± 0.50	18.67 ± 1.26	46
MS	52.9 ± 4.45	40.9 ± 2.49	–23	11.07 ± 1.06	8.69 ± 0.54	–21	1.75 ± 0.34	2.30 ± 0.14	31	6.61 ± 1.41	9.91 ± 1.13	33
MP	49.1 ± 3.16	32.4 ± 1.71	–34	10.85 ± 0.88	7.58 ± 0.44	–30	1.11 ± 0.12	3.61 ± 0.37	225	7.92 ± 0.21	13.85 ± 2.00	43
Prima	53.0 ± 2.24	29.6 ± 1.58	–44	11.60 ± 0.99	6.71 ± 0.27	–42	0.79 ± 0.11	1.74 ± 0.06	119	9.34 ± 1.30	13.43 ± 0.81	30
TWC	54.6 ± 5.82	33.4 ± 1.86	–39	11.01 ± 1.34	7.20 ± 0.51	–35	0.90 ± 0.19	1.43 ± 0.34	59	10.59 ± 0.66	17.05 ± 0.23	38
TPP	57.7 ± 6.65	39.6 ± 2.91	–31	11.46 ± 0.99	8.57 ± 0.42	–25	0.44 ± 0.11	0.77 ± 0.22	73	9.70 ± 0.32	13.53 ± 1.43	28
TVu	54.2 ± 2.97	36.0 ± 5.94	–34	12.00 ± 0.30	7.86 ± 0.85	–34	0.68 ± 0.11	0.94 ± 0.12	39	10.23 ± 0.64	13.27 ± 2.32	23
UCR	44.2 ± 2.75	37.1 ± 1.15	–16	10.29 ± 0.43	8.41 ± 0.40	–18	0.80 ± 0.08	1.10 ± 0.30	37	13.43 ± 0.43	15.47 ± 1.50	13
ZC	42.0 ± 3.30	30.0 ± 0.87	–29	7.89 ± 0.90	6.67 ± 0.34	–15	0.77 ± 0.20	1.31 ± 0.12	70	8.02 ± 0.78	8.86 ± 0.73	9
LSD†	13.0**	7.92*		2.82**	1.49*		0.447***	0.955***		3.93*	4.28***	

†LSD is the Fisher's Least Significant Difference for comparing the genotypes in the same column. Statistical significance of difference between the genotypes are given as: *($P<0.05$), **($P<0.01$) and ***($P<0.001$).

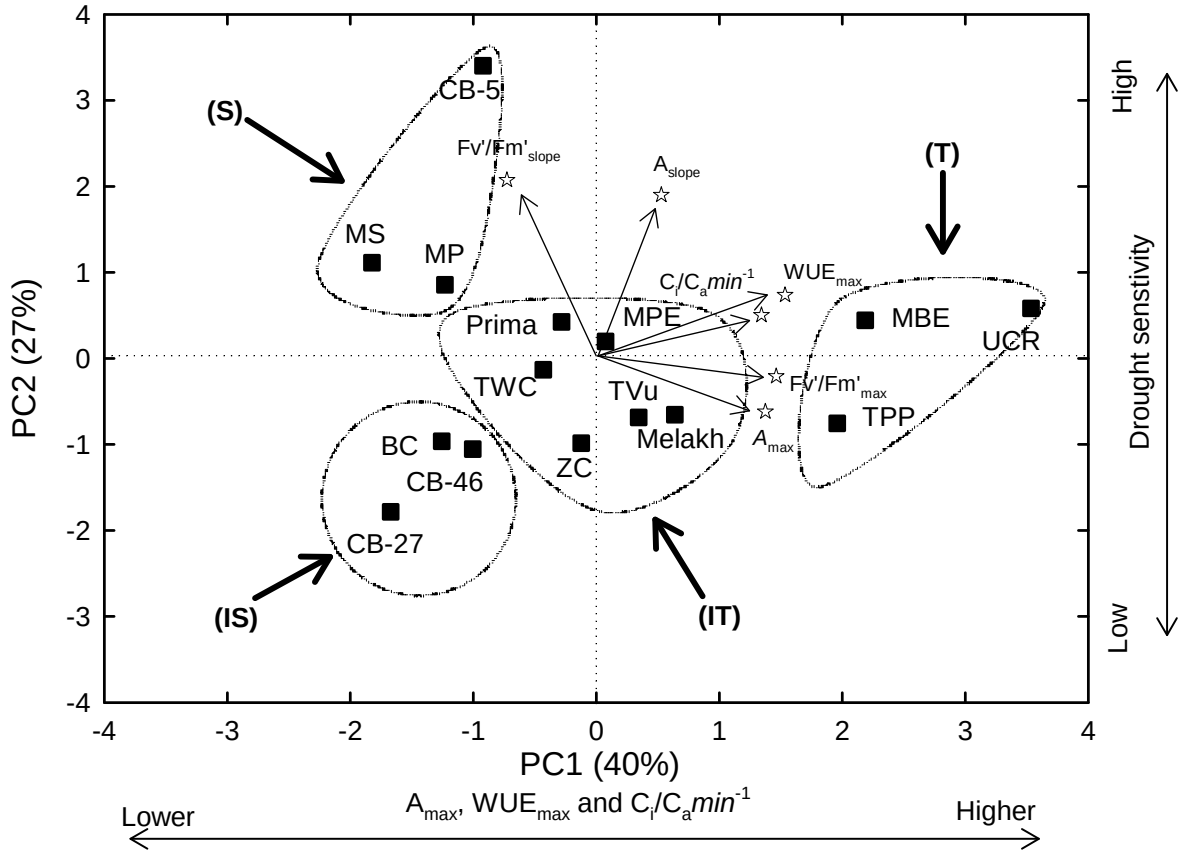

Figure 5.7 The biplot of principal components (PC) scores of PC1 vs. PC2 related to the classification of fifteen cowpea genotypes (solid diamond symbols) for their drought sensitivity. The eigenvectors (PC1 and PC2) for the photosynthetic parameters (solid stars) are superimposed with the PC biplot scores at the similar scale reflecting their contribution in determination of drought sensitivity. The arrows radiating from the center indicate the direction (angle) and magnitude (length) for the parameters. The eigenvectors were multiplied by four in order to obtain clear and superimposed figure. The arrow along the right y-axis and the bottom x-axis indicate the interpretation of the PCs. The genotypes are distinguished for their relative sensitivity to drought in the circumscribed area as tolerant (T), intermediately tolerant (IT), intermediately sensitive (IS), and sensitive (S) to drought stress condition.

drought sensitivity. These genotypes included Melakh, MPE, TVu, ZC, TWC and Prima. Due to high negative values for both PC scores, genotypes (BC, CB-46 and CB-27) were less drought sensitive with low A_{max}, Fv′/Fm′$_{max}$, WUE and $C_i/C_a min^{-1}$. They were,

therefore, classified as intermediate drought sensitive. Genotypes CB-5, MS and MP showing high negative scores for PC1 and high positive scores for PC2 reflected their low photosynthesis and WUE and high sensitivity to drought.

Since, PC1 and PC2 represented the main components of drought responsiveness, therefore PC1 and PC2 were correlated with the photosynthetic parameters to find the traits contributing to drought responsiveness (Table 5.4). The strong correlation of all the parameters with either PC1 or PC2 exhibited the importance of these parameters in determining drought sensitivity. A positive correlation between: A_{slope} and Fv'/Fm'_{slope} ($r = 0.55$, $P < 0.05$), A_{max} and Fv'/Fm'_{max} ($r = 0.72$, $P < 0.01$) and WUE and $C_i/C_a min^{-1}$ ($r = 0.90$, $P < 0.001$) were also observed.

Table 5.4 The Pearson's correlation (r) matrix showing the relationship between six photosynthetic parameters used in principal component analysis and their relationship with the first two principal component scores.

Variables	PC1	PC2	A_{slope}	A_{max}	Fv'/Fm'_{slope}	Fv'/Fm'_{max}	WUE_{max}
A_{slope}	0.27	0.79***					
A_{max}	0.76***	–0.08	0.35				
Fv'/Fm'_{slope}	–0.37	0.87***	0.55**	–0.29			
Fv'/Fm'_{max}	0.70***	–0.26	0.19	0.72**	–0.36		
WUE_{max}	0.79***	0.27	0.17	0.30	–0.11	0.21	
$C_i/C_a min^{-1}$	0.69***	0.21	0.05	0.13	–0.14	0.10	0.90***

Statistical significance of correlation are given as: **(P<0.01) and ***(P<0.001)

Discussion

Cowpea genotypes exhibited water-stress avoidance characteristics by maintaining a high leaf RWC, which confirms earlier findings of Bates and Hall (1981). In addition, RWC did not show any relationship with either SWC or photosynthetic parameters. Soil water status; however, affected the stomatal conductance and

photosynthetic parameters measured in leaves. The plant stress hormone, such as ABA, has long been known to be associated with changes in leaf conductance via root-shoot signal transduction mechanisms under drought condition (Davies and Zhang, 1991; Jones, 1998), but its role in cowpea has not been precisely determined.

Role of stomatal conductance under drought stress conditions

Leaf stomatal conductance is involved with different photochemical and biochemical processes related to photosynthesis under drought (Flexas et al., 2002; Medrano et al., 2002; Parry et al., 2005). A pattern of gradual response of photosynthetic parameters to the four regions of g_s was distinguished in cowpea. The first region (g_s>1.8) clearly indicated that extensive use of water by cowpea under well watered condition. In this region E increased continuously by decreasing WUE (A/g_s). It has been suggested that stomata control E more than *A*, as *A* levels off at high g_s, E continues to increase linearly (Condon et al., 2002). All other photosynthetic parameters remained constant in this region. One of the plausible implications of this region might be to improve WUE by increasing carboxylation efficiency in mesophyll cells which will ultimately increase *A* without affecting E or g_s (Farquhar and Sharkey, 1982). Hence maintaining, high photosynthesis capacity while stomata are partially open is an important strategy of crop tolerance to drought (Parry et al., 2005).

In the second region ($0.4<g_s<1.8$), stomatal limitation to photosynthesis appeared to be the main cause of *A* inhibition as deduced from a parallel decrease in C_i/C_a. The ETR was unaffected and ETR/*A* (a measure of photorespiration) increased by 22% which was about 6% higher than reduction in C_i/C_i. A similar response pattern at the initial

phase of stomatal closure was observed in grape (Flexas et al., 2002), and other studies suggest a large portion of excess electrons at reduced *A* might be used for photorespiration (Govindjee, 1999; Heber, 2002). We observed a constant ETR while both *A* and C_i/C_a decreased with a concomitant increase in ETR/*A*. However, the occurrence of other processes such as non-radiative energy dissipation in the form of heat might have also been involved, as inferred by a small decrease in Fv′/Fm′ (Maxwell and Johnson, 2000; Souza et al., 2004). The enhanced ETR/*A* and energy dissipation act as a photo-protective mechanism preventing photosystems from over excitations (Wu et al., 1991; Medrano et al., 2002; Lizana et al., 2006).

The further decrease in stomatal conductance in the third region ($0.04<g_s<0.4$) decreased in *A* and E by >80%, and ETR was reduced almost equal to the reduction in C_i/C_a (46-48%) whereas ETR/*A* increased drastically. The smaller reduction in ETR compared to *A* under water stress conditions indicates the relative increase in photorespiration (Wingler et al., 1999) which was in accordance with the continuous increase in ETR/*A* observed in this region. The stomatal-limitation still appeared to be the dominant cause of photosynthesis inhibition because the reduction in the C_i/C_a was still parraral to the reduction in *A*, and the Fv′/Fm′ was maintained relatively higher (73% of maximum). It was also supported by the fact that above this region of water-induced stomatal conductance, the percent reduction in g_s was always higher than the reduction of any other parameters. However, the presence of minor non-stomatal limitation to *A* might be possible because starch hydrolysis and accumulation of soluble sugar have been reported to occur at this region of g_s in cowpea that could cause a minimal non-stomatal

limited reduction in photosynthesis by feed back inhibition (Campos et al., 1999; Souza et al., 2004).

The appearance of non-stomatal limitation to photosynthesis was evident in the fourth region (g_s < 0.04 mol m^{-2} s^{-1}) as designated by increased C_i/C_a, drop in A/g_s, and the decreases in measured photosynthetic parameters that exceeded the percentage decline in stomatal conductance. A similar increase in C_i/C_a at very low g_s has been observed in other species under severe water stress conditions (Martin and Ruiz-Torres, 1992; Brodribb, 1996; Rouhi et al., 2007). The ETR/*A* continued to increase and Fv′/Fm′ remained higher (only 2% more reduction compared to the decline observed in the previous region) indicating that permanent photoinhibition was not the main cause of declining *A* under water-stressed conditions. Flexas et al. (1998) found that permanent photoinhibition determined by the photochemical activity (Fv/Fm) was rare even under severe water stress condition in grape. Maintenance of high Fv′/Fm′ has been suggested as a protective mechanism of the photosystem from photoinhibitory damage which may lead to the recovery of photosynthesis after water stress is released (Anyia and Herzog, 2004; Souza et al., 2004).

In this study, lowest estimated C_i value under severe water stress condition was ≈180 μmol CO_2 μmol mol^{-1}, which is not much different than ≈150 μmol mol^{-1} observed by Souza et al. (2004) in the same species. This C_i value corresponded to the g_s level of 0.04 mol m^{-2} s^{-1}, which was also the inflexion point of C_i/C_a in response g_s under water stress condition. This suggests that above this level of g_s (0.04) photosynthesis was predominantly controlled by stomata which limited the supply of CO_2 to leaf intercellular

space, and below this g_s level non-stomatal conductance to photosynthesis was evident as deduced from both an increase in C_i/C_a and the decrease in A/g_s.

The non-uniform stomatal distribution (or patchy stomatal closure) in some species is known to cause an over estimation of C_i, particularly under severe drought conditions, which may lead an erroneous conclusion of non-stomatal limitation to photosynthesis (Brodribb, 1996). Recently, Sekiya and Yano (2008) found that in cowpea subjected to various environmental conditions, soil water content had no significant effect on stomatal index and exhibited uniform stomatal index across environmental conditions including water stress. In this study, the uniformity of stomatal response to drought stress was assumed; however, precaution is recommended while using the results related to C_i.

Genotypic variability for photosynthesis and WUE

The slope of the linear reduction in *A* with concomitant decreased Fv′/Fm′ as SWC declined facilitated an indirect measure of drought sensitivity among cowpea genotypes. Genotypes with steeper slope (e.g. CB-5) would be more sensitive to drought and experience larger reduction in *A* per unit decrease in SWC, compared to genotypes (e.g. UCR-193) having lower slope (Fig. 5.5A). As mentioned in results and shown in Fig 5.6A, a similar increase in g_s, roughly around 0.4 mol m^{-2} s^{-1}, UCR-193 exhibited a very large increase in *A* as comparison to the genotype MS. Because the WUE is first derivatives of the curve (A/g_s), at a given g_s, moving vertically in the Fig. 5.6A, toward high *A*, will also confer higher WUE. Drought induced increase in intrinsic WUE have also been reported in other crops and found to represent water use by plants under field condition (Condon et al., 2002; Lefi et al., 2004; Rouhi et al., 2007). This aspect of

genotypic variation have been described as an important goal for crop breeding in order to induce drought tolerant and yield enhancement in dry environment (Parry et al., 2005).

WUE is a negative function of C_i/C_a hence $C_i/C_a min^{-1}$ represent the maximum WUE attainable during drought (Brodribb, 1996). Under drought stress g_s influences the supply of CO_2 to the leaf intercellular space; where as the capacity of *A* determine the demand of CO_2, therefore as shown in Fig. 5.6C and Table 5.2, lower $C_i/C_a min$ (e.g. UCR-193 compare to MS) obtained at similar or lower g_s values should increase WUE as consequence of higher capacity for *A* at a range of g_s. This was also supported by a strong correlation between WUE_{max} and $C_i/C_a min^{-1}$ observed in this study.

Leaf pigments, proline and wax content

Drought-induced reduction in leaf pigments is in accordance with other legume and might be attributed to a drought response mechanism in order to minimize the light absorption by cholorplast (Giardi et al., 1996). A substantial enhancement in proline biosynthesis was observed in all genotypes. Similar to the present study, Souza et al. (2004) also reported substantial increment in proline content in cowpea at extreme drought stress. Since no osmotic adjustment has been found in cowpea so far (Bates and Hall, 1981; Lopez et al., 1987), despite the known role of proline in osmotic adjustment, it has been considered as a symptom of injury in some plants including cowpea (Souza et al., 2004). Therefore, it can be considered as a response rather than a protective mechanism under water-stressed conditions. The observed enhancement of leaf surface wax content might contribute to reduction in cuticular transpiration as it is found in peanut (Samdur et al., 2003) and in several semiarid shrubs (Rao and Reddy, 1980).

However, no association between wax content and any of the photosynthetic parameters were observed in this study.

Classification of genotypes

The biplot (Fig. 5.7) is a scaled combination of PC scores representing genotypes and eigenvectors representing photosynthetic parameters that allowed the approximate similarities and differences of the genotypes to be displayed simultaneously and allow different photosynthetic parameters to be associated with genotypes (ter Braak, 1983; Singh et al., 2008b). All genotypes that have origin from the tropical countries and well adapted to dry and hot environments (Prima, TVu-4552, UCR-193 and Melakh; Table 5.1) (Hall, 2004a; Hall, 2004b) along with some genotypes grown in Southern region of USA (MPE, ZC, and TPP) (Hare, 1991; Arkansas News Letter, 2006) were classified as either tolerant or intermediately tolerant. Melakh has already been adapted to the dry conditions of the West African countries and has shown high tolerance during vegetative growth period (Hall, 2004a). One of the genotypes, CB-5, classified as physiologically drought sensitive is mostly grown in irrigated conditions in California regions of USA and has been considered as poor performer under drought conditions (Labanauskas et al., 1981; Hall et al., 2003). The identified tolerant genotypes exhibited lower drought sensitivity with relatively higher photosynthesis and improved WUE. The higher rate of photosynthesis during initial stages of drought confer plant survival by more dry matter accumulation (Parry et al., 2005). Plants with slow growth rate have very limited value for most of the agriculture condition because they can not utilize the available resources with full potential (Hall et al., 1990). The identified tolerant genotypes can be used in

trait-based plant breeding program to enhance early vigor with improved WUE under drought stress conditions.

Higher rate of g_s in response to drought stress and increased WUE in the identified tolerant genotypes compared to the sensitive genotypes give rise to the differences in susceptibility to drought. In fact, there was higher or stable *A* due to low drought responsiveness as assessed by the slope of the linear relationship between SWC and *A* as drought intensity increased in the identified tolerant genotypes. Thus, under water stress condition the stomatal limitation to *A* seems to be very important in tolerant genotypes and the higher WUE observed in these genotypes could be due to better functioning of carboxylation mechanism (Parry et al., 2005; Lizana et al., 2006). This was also supported by a smaller C_i/C_a*min* for tolerant genotypes and a strong correlation between WUE and $C_i/C_a min^{-1}$ (Table 5.4 and Fig. 5.7) observed in this study. The significant correlation between A_{slope} and Fv'/Fm'_{slope}, and A_{max} and Fv'/Fm'_{max} indicated that genotypes with comparatively more stable or high *A* under drought condition were also showed less photoinhibition by maintaining higher Fv′/Fm′. Lizana *et al.* (2006) demonstrated that bean cultivars showing large plasticity at biochemical and cellular level for g_s and *A* also exhibited resistant to photoinhibition.

In the conclusion, the current study confirms the stomatal regulated extreme drought avoidance behavior of cowpea by maintaining high leaf water status. Stomatal conductance is the major limitation to *A* under drought stress conditions in cowpea; however, a pronounced non-stomatal limitation can occur under severe drought stress that may also lead to impairment of photosynthetic activity. The less responsiveness of

Fv′/Fm′ and maintenance of high electron transport as SWC declined, and increased photorespiration under drought stress appeared to be an important protective mechanism from photoinhibition. Cowpea genotypes varied highly for their photosynthetic capacity and WUE under drought conditions. The faster decline in stomatal conductance to avoid water loss and the maintenance of comparatively higher *A* by better utilization of C_i was one of the important mechanisms identified in drought tolerant genotypes. The drought-induced reduction in leaf pigments and an increase in proline and wax contents were not associated with any measured photosynthetic parameters. Based on photosynthetic performance and water use efficiency, the cowpea genotypes were classified as tolerant (UCR-193, MBE and TPP), intermediately tolerant (Prima, MPE, TWC, Melakh, ZC and TVu-4552), intermediately sensitive (BC, CB-46 and CB-27), and sensitive (CB-5, MS and MP) to drought stress. The identified genotypes and photosynthetic parameters could be used by plant breeding programs to improve drought tolerance in cowpea. However, precaution is needed to extrapolate these results to field conditions as this study was conducted in large pots, and in real world situations, root growth dynamics also offer different mechanisms that provides variability in drought tolerance.

SUMMARY AND CONCLUSIONS

Three experiments were conducted using controlled environment chambers and pot-culture facility with the objectives to (a) evaluate the vegetative and reproductive response of cowpea genotypes to multiple abiotic stresses singly or in combination, (b) develop screening techniques for cowpea tolerance to abiotic stresses, and (c) determine the inheritance of the tolerance for various abiotic stresses in cowpea genotypes. Experiment I and II were conducted in naturally-lit, controlled environment chambers known as Soil-Plant-Atmosphere-Research (SPAR) units using six cowpea genotypes representing different sites of origin [Prima, California Blackeye (CB) -5, CB-27, CB-46, Mississippi Pinkeye (MPE) and UCR-193]. The objective of Experiment I was to evaluate sensitivity of cowpea cultivars to a range of UVB radiation. The objective of Experiment II was to evaluate interactive effects of [CO_2], temperature, and UVB radiation on growth, physiology and reproduction of cowpea and to identify genotypic tolerance to multiple stressors. Experiment III was conducted outdoors in large pots using fifteen cowpea genotypes that included the Experiment I and II. The objective of this study was to evaluate the dynamics of leaf photosynthetic, chlorophyll fluorescence and water use efficiency of cowpea cultivars for drought tolerance.

The current study revealed a significant genotypic variability with tolerance characteristics in response to different abiotic stresses in cowpea. Cowpea genotypes were sensitive to current and projected UVB radiation. Plants grown in elevated UVB radiation significantly decreased net photosynthesis, electron transport rates and caused

reductions in plant height, total dry matter, pollen viability and seed yield in most of the cowpea genotypes. However, these reductions were less in CB-5, CB-46 and UCR-193 and none in Mississippi Pinkeye. A significant increase in phenolics compounds in response to UVB radiation appeared to be one of the defense mechanisms against the UVB exposure in cowpea. The total stress response index for vegetative parameters (V-TSRI), a measure of overall genotypic responsiveness to UVB radiation for vegetative growth, was not correlated with total stress response index for reproductive parameters (R-TSRI), a measure of overall genotypic responsiveness to UVB radiation for reproductive growth, indicating that vegetative and reproductive parameters differ in their response to UVB. The high UVB responsiveness of plant biomass production, flower characteristics and fruit set appeared to be important traits for the selection of UVB tolerance in cowpea. Additionally, the increased leaf phenolic concentration indicates its role for early selection of UVB tolerance in cowpea populations. Based on the combined TSRI (C-TSRI) calculated as sum of individual vegetative and reproductive component responses over all the UVB radiation treatments, the cultivars were classified as tolerant (Mississippi Pinkeye), intermediate (CB-5, CB-46 and UCR-193) and sensitive (CB-27 and Prima) to UVB radiation.

The exposure of plants to a combination of UVB, temperature, and [CO_2] clearly indicated the negative impact of temperature and UVB stressors on cowpea growth and reproduction. Carbon dioxide enrichment substantially increased dry matter production and seed yield of all cowpea genotypes. Additionally, elevated [CO_2] alleviated the damaging effects of elevated UVB and high temperature on photosynthesis and vegetative growth parameters. On the contrary, reductions in the reproductive parameters

such as production, retention and size of flowers, pollen viability and seed yield were not ameliorated by elevated [CO_2] in the presence of enhanced UVB and/or temperature, suggesting that reproductive processes under high temperature and UVB either alone or in combination are carbon independent. This notion was further supported by mostly positive responses of vegetative while negative response of reproductive parameters under multiple stress conditions. A combination of UVB and temperature exhibited the most damaging effect on both vegetative and reproductive processes in cowpea as inferred form the environmental response index (ESRI). Compared to other treatments, under +UVB+T condition, flowers produced were smaller and did not open as in other treatments. However, the flowers produced under +UVB+T condition exhibited less developed anthers with substantial amount of non-viable pollen grains, indicating that pollen vitality (pollen germination and viability) is being affected by these stress conditions which led to lower seed yield. Phenolic compounds increased significantly only in the presence UVB radiation exhibiting a defensive mechanism against UVB, which is also a ubiquitous responses observed in the previous experiment. No correlation between V-TSRI and R-TSRI in response to multiple abiotic stresses reiterates the earlier conclusion that the vegetative and reproductive processes responded differently to these stress conditions. The identification of four groups of traits namely, yield attributes, growth attributes, leaf attributes and flower attributes implies the option for trait-based selection to confer multiple abiotic stress tolerance in cowpea. Based on the combined TSRI (C-TSRI), developed from sum of response indices of vegetative and reproductive parameters over multiple abiotic stresses, the genotypes were classified as tolerant (UCR-193, MPE and CB-5), intermediate (CB-46 and Prima) and sensitive (CB-27).

Drought-induced reduction on various gas exchange and fluorescence parameters significantly varied among cowpea genotypes. This study confirmed the extreme drought avoidance nature of cowpea by maintaining high leaf water status. Photosynthesis and fluorescence decreased linearly while water use efficiency increased exponentially in response to decreasing drought stress condition. This study also revealed that the stomatal regulation is the major limitation for photosynthesis under drought condition in cowpea and severe drought can cause damage to photosystems leading to an additional non-stomatal limitation to photosynthesis. Increase in water use efficiency while maintaining higher rates of photosynthesis is an important drought tolerance mechanism observed in tolerant cowpea genotypes. The chlorophyll fluorescence parameters appeared to be important for protection of photosystem from photoinhibitory damage under drought condition. Based on the photosynthetic performance and water use efficiency cowpea genotypes were classified as tolerant (UCR-193, MBE and TPP), intermediately tolerant (Prima, MPE, TWC, Melakh, ZC and TVu-4552), intermediately sensitive (BC, CB-46 and CB-27), and sensitive (CB-5, MS and MP) to drought. An association between identified drought tolerant genotypes and their sites of origin and adaptation were also observed.

In the conclusion, the current study showed that cowpea genotypes were highly responsive to abiotic factors and most of the abiotic stresses have greater influence on reproductive parameters compared to vegetative processes, suggesting that the genotypes that performed well for vegetative parameters did not perform in the same way for reproductive growth in the presence of the same stress condition. Studying same genotypes across stress conditions revealed that a single genotype possibly will not have

an absolute tolerance to all stress conditions; however, tolerance to more than one stress condition was observed. Mississippi Pinkeye, for example, exhibited tolerance to both UVB and multiple abiotic stresses. On the other hand, CB-27 exhibited an overall sensitivity to all stress conditions studied either alone or in combination. Phenolic compounds appeared to be useful for detection of early UVB tolerance in cowpea. However, a distinct parameter could not be detected that could be used as a screening tool for tolerance to multiple abiotic stresses. Therefore, both vegetative and reproductive traits are needed to develop a selection tool for cowpea tolerance to a combination of stresses. In drought study, photosynthesis and water use efficiency were the important parameters for selection of drought tolerance in cowpea. The variability among cowpea genotypes allow to choose for a specific or combination of traits based on their responsiveness to different abiotic stresses which may confer higher yield and/or stability under elevated UVB radiation, warmer temperature and increased drought conditions, most likely in future climatic conditions. The identified tolerant cowpea genotypes to a particular or a combination of stresses and the associated traits might be useful to develop tolerant genotypes suitable for an agro-ecological niche environment though traditional breeding or genetic engineering methods.

REFERENCES CITED

Adamse, P., S.J. Britz, and C.R. Caldwell. 1994. Amelioration of UV-B damage under high irradiance I: Role of blue light photoreceptors. Photochem. Photobiol. 60:110-115.

Ahmed, F.E., A.E. Hall, and M.A. Madore. 1993. Interactive effects of high temperature and elevated carbon dioxide concentration on cowpea [*Vigna unguiculata* (L.) Walp.]. Plant Cell Environ. 16:835-842.

Ainsworth, E.A., and S.P. Long. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol. 165:351-372.

Ainsworth, E.A., A. Rogers, R. Nelson, and S.P. Long. 2004. Testing the "source-sink" hypothesis of down-regulation of photosynthesis in elevated [CO_2] in the field with single gene substitutions in *Glycine max*. Agric. For. Meteorol. 122:85-94.

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

Amthor, J., and S. Pritchard. 2005. Crops and Environmental Change: An Introduction to Effects of Global Warming, Increasing Atmospheric CO_2 and O_3 Concentrations, and Soil Salinization on Crop Physiology and Yield. CRC Press, p.p. 221.

Amudha, P., M. Jayakumar, and G. Kulandaivelu. 2005. Impacts of ambient solar UV (280-400 nm) radiation on three tropical legumes. J. Plant Biol. 48:284-291.

Anyia, A.O., and H. Herzog. 2004a. Genotypic variability in drought performance and recovery in cowpea under controlled environment. J. Agron. Crop Sci. 190:151-159.

Anyia, A.O., and H. Herzog. 2004b. Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. Eur. J. Agron. 20:327-339.

Arkansas News Letter. 2006. Pea and Spinach Varieties Released by University of Arkansas, Vol. 10 (1), University of Arkansas, Fayetteville, AR 72701, Vol. 10, Fayetteville, AR, (Online access date July 10, 2008, http://hort.uark.edu/arkansas-horticulture-news/ark-hort-news-vol10-num1-2006.html)

Aslam, M., M.S. Brown, and R.J. Kohel. 1964. Evaluation of seven tetrazolium salts as vital pollen stains in cotton, *Gossypium hirsutum* L. Crop Sci. 4:508-510.

Baker, J.T. 2004. Yield responses of southern U.S. rice cultivars to CO_2 and temperature. Agric. For. Meteorol. 122:129-137.

Ba, F.S., R.S. Pasquet, and P. Gepts. 2004. Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. Crop Evol. 51:539-550.

Balakumar, T., V.H.B. Vincent, and K. Paliwal. 1993. On the interaction of UV-B radiation (280-315 nm) with water stress in crop plants. Physiol. Plant. 87:217-222.

Barbagallo, R.P., K. Oxborough, K.E. Pallett, and N.R. Baker. 2003. Rapid, noninvasive screening for perturbations of metabolism and plant growth using chlorophyll fluorescence imaging. Plant Physiol. 132:485.

Basal, H., P. Bebeli, C.W. Smith, and P. Thaxton. 2003. Root growth parameters of converted race stocks of upland cotton and two BC_2F_2 populations. Crop Sci. 43:1983-1988.

Basiouny, F.M., T.K. Van, and R.H. Biggs. 1975. Some morphological and biochemical characteristics of C_3 and C_4 plants irradiated with UV-B. Physiol. Plant. 34:177-181.

Bates, L.M., and A.E. Hall. 1981. Stomatal closure with soil water depletion not associated with changes in bulk leaf water status. Oecologia 50:62-65.

Bates, L.S., R.P. Waldren, and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39:205-207.

Berry, J., and O. Bjorkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. Ann. Rev. Plant Physiol. 31:491-543.

Blum, A., N. Klueva, and H.T. Nguyen. 2001. Wheat cellular thermotolerance is related to yield under heat stress. Euphytica 117:117-123.

Bota, J., J. Flexas, and H. Medrano. 2001. Genetic variability of photosynthesis and water use in Balearic grapevine cultivars. Ann. Appl. Biol. 138:353-361.

Brodribb, T. 1996. Dynamics of changing intercellular CO_2 concentration (C_i) during drought and determination of minimum functional C_i. Plant Physiol. 111:179-185.

Caldwell, C.R., S.J. Britz, and R.M. Mirecki. 2005. Effect of temperature, elevated carbon dioxide, and drought during seed development on the isoflavone content of dwarf soybean [*Glycine max* (L.) Merrill] grown in controlled environments. J. Agric. Food Chem. 53:1125-1129.

Caldwell, M.M. 1971. Solar ultraviolet radiation and the growth and development of higher plants, p. 131-177, *In* A.C. Giese, Ed. Photophysiology, Vol. 6. Academic Press, New York, NY.

Caldwell, M.M., A.H. Teramura, and M. Tevini. 1989. The changing solar ultraviolet climate and the ecological consequences for higher plants. Trends Ecol. Evol. 4:363-367.

Caldwell, M.M., S.D. Flint, and P.S. Searles. 1994. Spectral balance and UV-B sensitivity of soybean: A field experiment. Plant Cell Environ. 17:267-276.

Caldwell, M.M., J.F. Bornman, C.L. Ballaré, S.D. Flint, and G. Kulandaivelu. 2007. Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. Photochem. Photobiol. Sci. 6:252-266.

Caldwell, M.M., A.H. Teramura, M. Tevini, J.F. Bornman, L.O. Björn, and G. Kulandaivelu. 1998. Effects of increased solar ultraviolet radiation on terrestrial ecosystems. J. Photochem. Photobiol. B: Biol. 46:40-52.

Campos, P., J.C. Ramalho, J.A. Lauriano, M.J. Silva, and M. do Céu Matos. 1999. Effects of drought on photosynthetic performance and water relations of four *Vigna* genotypes. Photosynthetica 36:79-87.

Cen, Y.P., and J.F. Bornman. 1990. The response of bean plants to UV-B radiation under different irradiances of background visible light. J. Exp. Bot. 41:1489-1495.

Cheikh, N., P.W. Miller, and G. Kishore. 2000. Role of biotechnology in crop productivity in a changing environment, p. 425-436, *In* K.R. Reddy and H.F. Hodges, Eds. Climate Change and Global Crop Productivity. CAP International, Oxon, UK.

Chimphango, S.B.M., C.F. Musil, and F.D. Dekora. 2003. Effect of UV-B radiation on plant growth, symbiotic function and concentration of metabolites in three tropical grain legumes. Funct. Plant Biol. 30:309-318.

Chimphango, S.B.M., C.F. Musil, and F.D. Dakora. 2004. Impact of increased ultraviolet-B radiation stress due to stratospheric ozone depletion on N_2 fixation in traditional African commercial legumes. S. Afr. J. Bot. 70:790-796.

Chiulele, R.M., and G.A. Agenbag. 2004. Plant water relations and proline accumulation on two cowpea [*Vigna unguiculata* (L.) Walp.] cultivars as a response to water stress. S. Afr. J. Plant and Soil 21:109-113.

Christensen, J.H., B. Hewitson, A. Busuioc, A. Chen, X. Gao, I. Held, R. Jones, W.T. Kwon, and Coauthors. 2007. Regional climate projections, p. 847-940, *In* S. Solomon, D. Qin, and M. Manning, Eds. Climate Change 2007: Contribution of

Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Ciais, P., M. Reichstein, N. Viovy, A. Granier, J. Ogee, V. Allard, M. Aubinet, N. Buchmann, C. Bernhofer, A. Carrara, F. Chevallier, N. De Noblet, A.D. Friend, P. Friedlingstein, T. Grunwald, B. Heinesch, P. Keronen, A. Knohl, G. Krinner, D. Loustau, G. Manca, G. Matteucci, F. Miglietta, J.M. Ourcival, D. Papale, K. Pilegaard, S. Rambal, G. Seufert, J.F. Soussana, M.J. Sanz, E.D. Schulze, T. Vesala, and R. Valentini. 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437:529-533.

Clavel, D., N.K. Drame, H. Roy-Macauley, S. Braconnier, and D. Laffray. 2005. Analysis of early responses to drought associated with field drought adaptation in four Sahelian groundnut (*Arachis hypogaea* L.) cultivars. Environ. Exp. Bot. 54:219-230.

Condon, A.G., R.A. Richards, G.J. Rebetzke, and G.D. Farquhar. 2002. Improving intrinsic water-use efficiency and crop yield. Crop Sci. 42:122-131.

Costa, E.S., R. Bressan-Smith, J.G. Oliveira, and E. Campostrini. 2003. Chlorophyll a fluorescence analysis in response to excitation irradiance in bean plants (*Phaseolus vulgaris* L. and *Vigna unguiculata* L. Walp) submitted to high temperature stress. Photosynthetica 41:77-82.

Costa, J.W., M.P. Hasenfratz-Sauder, A.T. Pham-Thi, M.D.S. Lima, P. Dizengremel, Y. Jolivet, and D.F. de Melo. 2004. Identification in *Vigna unguiculata* (L.) Walp. of two cDNAs encoding mitochondrial alternative oxidase orthologous to soybean alternative oxidase genes 2a and 2b. Plant Sci. 167:233-239.

Cutforth, H.W., S.M. McGinn, K.E. McPhee, and P.R. Miller. 2007. Adaptation of pulse crops to the changing climate of the Northern Great Plains. Agron. J. 99:1684-1699.

Dai, Q., S. Peng, A.Q. Chavez, and B.S. Vergara. 1994. Intraspecific response of 188 rice cultivars to enhanced UV-B radiation. Environ. Exp. Bot. 34:433-442.

Davies, W.J., and J. Zhang. 1991. Root signals and the regulation of growth and development of plants in drying soil. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:55-76.

Davis, D.W., D.B. Marsh, and M.N. Alvarez. 1986. MN 13 and MN150 cowpea breeding lines. HortScience 21:1080-1081.

Davis, D.W., E.A. Oelke, E.S. Oplinger, J.D. Doll, C.V. Hanson, and P. D.H. 1991. Cowpea Alternative Field Crop Manual (online access date March 20, 2008, http://www.hort.purdue.edu/newcrop /afcm/cowpea.html).

Dow el-Madina, I.M., and A.E. Hall. 1986. Flowering of contrasting cowpea (*Vigna unguiculata* [L.] Walp.) genotypes under different temperatures and photoperiods. Field Crops Res. 14:87-104.

Drennen, P.M., M. Smith, D. Goldsworthy, and J. van Staten. 1993. The occurrence of trahaolose in the leaves of the desiccation-tolerant angiosperm *Myronthamnus flabellifoliius* Welw. J. Plant Physiol. 142:493-496.

Duke, J.A. 1981 Handbook of Legumes of World Economic Importance. Plenum Press, London, UK. p.p. 345.

Easterling, W.E., P.K. Aggarwal, P. Batima, K.M. Brander, L. Erda, S.M. Howden, A. Kirilenko, J. Morton, J.F. Soussana, J. Schmidhuber, and F.N. Tubiello. 2007. Food, fibre and forest products, p. 273-313, *In* M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden and C.E. Hanson, Eds. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

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

Edwards, G.E., and N.R. Baker. 1993. Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? Photosynthesis Res. 37:89-102.

Ehlers, J.D., and A.E. Hall. 1996. Genotypic classification of cowpea based on responses to heat and photoperiod. Crop Sci. 36:673-679.

Ehlers, J.D., and A.E. Hall. 1997. Cowpea (*Vigna unguiculata* L. Walp.). Field Crops Res. 53:187-204.

Ehlers, J.D., and A.E. Hall. 1998. Heat tolerance of contrasting cowpea lines in short and long days. Field Crops Res. 55:11-21.

Ellis, R.H., P.Q. Craufurd, R.J. Summerfield, and E.H. Roberts. 1995. Linear relations between carbon dioxide concentration and rate of development towards flowering in sorghum, cowpea and soyabean. Ann. Bot. 75:193-198.

Evan, L.T. 1998. Feeding the Ten Billion: Plants and Population Growth. Cambridge University Press, pp. 264.

Fang, J., C.C. Chao, P. Roberts, and J. Ehlers. 2007. Genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] in four West African and USA breeding programs as determined by AFLP analysis. Genet. Resour. Crop Evol. 54:1197-1209.

FAO. 1993. Food and Agriculture Organization of United Nations (FAO), Plant genetic Resources"Development Education Exchange Paper (DEEP), September 1993, pp.3.

FAO. 2004. The state of food and agriculture 2003-04. Agricultural Biotechnology: Meeting the Needs of the Poor, Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 208.

FAO. 2007. Food and Agriculture Organization of United Nations (FAO), Rome, Italy. (Online access date March 10, 2007, http://faostat.fao.org).

Faris, D.G. 1965. The origin and evolution of cultivated form of *Vigna sinensis*. Can. J. Genet. Cytol. 6:255-258.

Farquhar, G.D., and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. Ann. Rev. Plant Physiol. 33:317-145.

Fery, R.L. 1990. The cowpea: Production, utilization and research in the United States. Hort. Rev. 12:197-222.

Fery, R.L. 2002. New opportunities in *Vigna*, p. 424-428, *In* J. Janick and A. Whipkey, Eds. Trend in New Crop and New Uses. ASHS Press, Alexandria, VA.

Flexas, J., J.M. Escalona, and H. Medrano. 1998. Down-regulation of photosynthesis by drought under field conditions in grapevine leaves. Aust. J. Plant Physiol. 25:893-900.

Flexas, J., J. Bota, J.M. Escalona, B. Sampol, and H. Medrano. 2002. Effects of drought on photosynthesis in grapevines under field conditions: An evaluation of stomatal and mesophyll limitations. Funct. Plant Biol. 29:461-471.

Flint, S.D., and M.M. Caldwell. 1983. Influence of floral optical properties on the ultraviolet radiation environment of pollen. Am. J. Bot. 70:1416-1419.

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

Genty, B., J.M. Briantais, and N.R. Baker. 1990. Relative quantum efficiencies of the two photosystems of leaves in photorespiratory and not photorespiratory conditions. Plant Physiol. Biochem. 28:1-10.

Giardi, M.T., A. Cona, B. Geiken, T. Kučera, J. Masojídek, and A.K. Mattoo. 1996. Long-term drought stress induces structural and functional reorganization of photosystem II. Planta 199:118-125.

Gifford, R.M. 2004. The CO_2 fertilising effect – does it occur in the real world? New Phytol. 163:221-225.

Giorgi, R., G.A. Meehl, A. kattneberg, H. Grassl, J.F.B. Mitchell, R.J. Stouffer, T. Tokioka, A.J. Weaver, and T.M.L. Wigley. 1998. Simulation of regional climate change with global coupled climate models and regional modeling techniques, p. 427-437, *In* R.T. Watson, M.C. Zinyowera, R.H. Moss, Eds. The Regional Impacts of Climate Change: An Assessment of Vulnerability. Cambridge University Press, New York, NY.

Gobron, N., B. Pinty, F. Melin, M. Taberner, M.M. Verstraete, A. Belward, T. Lavergne, and J.L. Widlowski. 2005. The state of vegetation in Europe following the 2003 drought. Int. J. Remote Sens. 26:2013-2020.

Goto, E. 2003. Effects of light quality on growth of crop plants under artificial lighting. Environ. Control Biol. 41:121-132.

Govindjee. 1999. On the requirement of minimum number of four versus eight quanta of light for the evolution of one molecule of oxygen in photosynthesis: A historical note. Photosynthesis Res. 59:249-254.

Grantz, D.A., and A.E. Hall. 1982. Earliness of an indeterminate crop, *Vigna unguiculata* (L.) Walp., as affected by drought, temperature, and plant density. Aust. J. Agric. Res. 33:531-540.

Green, A., T. Sawada, and E. Shettle. 1974. The middle ultraviolet radiation reaching the ground. Photochem. Photobiol. 19:251-259.

Gross, Y., and J. Kigel. 1994. Differential sensitivity to high temperature of stages in the reproductive development of common bean (*Phaseolus vulgaris* L.). Field Crops Res. 36:201-212.

Gwathmey, C.O., and A.E. Hall. 1992. Adaptation to mid season drought of cowpea genotypes with contrasting senescence traits. Crop Sci. 32:773-778.

Gwathmey, C.O., A.E. Hall, and M.A. Madore. 1992. Adaptive attribute of cowpea genotypes with delayed monocarpic leaf senescence. Crop Sci. 32:765-772.

Haldimann, P., and U. Feller. 2005. Growth at moderately elevated temperature alters the physiological response of the photosynthetic apparatus to heat stress in pea (*Pisum sativum* L.) leaves. Plant Cell Environ. 28:302-317.

Hall, A.E. 2001. Crop Response to Environment. CRC Press, New York, NY, pp. 248.

Hall, A.E. 2004a. Breeding for adaptation to drought and heat in cowpea. Eur. J. Agron. 21:447-454.

Hall, A.E. 2004b. Comparative ecophysiology of cowpea, common bean, and peanut, p. 271-385, *In* T.N. Henry and B. Abraham, Eds. Physiology and Biotechnology Integration for Plant Breeding. Marcel Dekker, New York.

Hall, A.E., and L.H. Allen. 1993. Designing cultivars for climatic conditions of the next century, p. 291-297, *In* D.R. Buxton, R. Shibles, R.A. Forsberg, B.L. Blad, K.H. Asay, G.M. Paulsen and R.F. Wilson, Eds. International Crop Science I. Madison: Crop Sciences Society of America, WI.

Hall, A.E., and L.H. Ziska. 2000. Crop breeding strategies for the 21st century, p. 407–423, *In* K.R. Reddy and H.F. Hodges, Eds. Climate Change and Global Crop Productivity. CAB International, Oxon, UK.

Hall, A.E., A.E. Ismail, and J.D. Ehlers. 1997. Cowpea breeding. Plant Breed. Rev. 15:215-273.

Hall, A.E., R.G. Mutters, K.T. Hubick, and G.D. Farquhar. 1990. Genotypic differences in carbon isotope discrimination by cowpea under wet and dry field conditions. Crop Sci. 30:300-305.

Hall, A.E., N. Cisse, S. Thiaw, H.O.A. Elawad, J.D. Ehlers, A.M. Ismail, R.L. Fery, P.A. Roberts, L.W. Kitch, L.L. Murdock, O. Boukar, R.D. Phillips, and K.H. McWatters. 2003. Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. Field Crops Res. 82:103-134.

Hare, W.W. 1991. 'Mississippi Pinkeye' cowpea. HortScience 26:76-77.

Heber, U. 2002. Irrungen, Wirrungen? The Mehler reaction in relation to cyclic electron transport in C_3 plants. Photosynthesis Res. 73:223-231.

Hewitt, E.J. 1952. Sand and Water Culture Methods Used in the Study of Plant Nutrition. Tech. Comm. 22. Farnham Royal Commonwealth Agricultural Bureau, Bucks, UK.

Hofmann, R., B. Campbell, D. Fountain, B.R. Jordan, D.H. Greer, D. Hunt, and C. Hunt. 2001. Multivariate analysis of intraspecific responses to UV-B radiation in white clover (*Trifolium repense* L.). Plant Cell Environ. 24:917-927.

Houghton, J.T., Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, K. Maskell, C.A. Johnson, 2001. Climate Change 2001: The Scientific Basis. Contribution of Working Group to Third Assessment Report to the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, Cambridge, UK, pp. 892.

Hubbard, K.G., and H. Wu, 2005. Modification of a crop-specific drought index for simulating corn yield in wet years. Agron. J. 97:1478-1484.

IPCC. 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, pp. 996.

Ismail, A.M., and A.E. Hall. 1997. Chilling tolerance during emergence of cowpea associated with a dehydrin and slow electrolyte leakage. Crop Sci. 37:1270.

Ismail, A.M., and A.E. Hall. 1998. Positive and potential negative effects of heat-tolerance genes in cowpea. Crop Sci. 38:381-390.

Ismail, A.M., and A.E. Hall. 1999. Reproductive-stage heat tolerance, leaf membrane thermostability and plant morphology in cowpea. Crop Sci. 39:1762-1768.

Ismail, A.M., and A.E. Hall. 2000. Semidwarf and standard-height cowpea responses to row spacing in different environments. Crop Sci. 40:1618-1623.

Jablonski, L.M., X. Wang, and P.S. Curtis. 2002. Plant reproduction under elevated CO_2 conditions: A meta-analysis of reports on 79 crop and wild species. New Phytol. 156:9-26.

Johnson, D.E. 1998. Applied Multivariate Methods for Data Analysts. Duxbury Press, New York, NY, pp. 592.

Jones, H. 1998. Stomatal control of photosynthesis and transpiration. J. Exp. Bot. 49:387-398.

Jones, H.G. 2007. Monitoring plant and soil water status: Established and novel methods revisited and their relevance to studies of drought tolerance. J. Exp. Bot. 58:119-130.

Kakani, V.G., P.V.V. Prasad, P.Q. Craufurd, and T.R. Wheeler. 2002. Response of *in vitro* pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. Plant Cell Environ. 25:1651-1661.

Kakani, V.G., K.R. Reddy, D. Zhao, and S. Koti. 2003. Field crop responses to ultraviolet-B radiation: A review. Agric. For. Meteorol. 120:191-218.

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

Kakani, V.G., K.R. Reddy, S. Koti, T.P. Wallace, P.V.V. Prasad, V.R. Reddy, and D. Zhao. 2005. Differences in *in vitro* pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Ann. Bot. 96:59-67.

Kant, P., M. Gordon, S. Kant, S. Zolla, O. Davydov, Y.M. Heimer, V. Chalifa Caspi, R. Shaked, and S. Barak. 2008. Functional-genomics-based identification of genes that regulate *Arabidopsis* responses to multiple abiotic stresses. Plant Cell Environ. 31:697-714.

Kaspar, T.C., D.J. Pulido, T.E. Fenton, T.S. Colvin, D.L. Karlen, D.B. Jaynes, and D.W. Meek. 2004. Relationship of corn and soybean yield to soil and terrain properties. Agron. J. 96:700-709.

Kimball, B.A., K. Kobayashi, and M. Bindi. 2002. Responses of agricultural crops to free-air CO_2 enrichment. Adv. Agron. 77:293-368.

Kishor, P.B.K., Z. Hong, G. Miao, C. Hu, and D. Verma. 1995. Overexpression of Δ1-pyrroline-5-carboxylase synthase increases proline production and confers osmotolerance in transgenic plants. J. Plant Physiol. 108:1387-1394.

Koti, S., K.R. Reddy, V.G. Kakani, D. Zhao, and V.R. Reddy. 2004. Soybean (*Glycine max*) pollen germination characteristics, flower and pollen morphology in response to enhanced ultraviolet-B radiation. Ann. Bot. 94:855-864.

Koti, S., K.R. Reddy, V.R. Reddy, V.G. Kakani, and D.L. Zhao. 2005. 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. J. Exp. Bot. 56:725-736.

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

Krause, G.H. 1988. Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiol. Plant. 74:566-574.

Krupa, S.V. 1998. Elevated UV-B radiation and crop, p. 105-131, *In* S.V. Krupa, R.N. Kickert, and H.J. Jäger, Eds. Elevated Ultraviolet (UV)-B Radiation and Agriculture. Springer-Verlag Heidelberg and Landes Bioscience Georgtown, TX.

Kubien, D.S., and R.F. Sage. 2008. The temperature response of photosynthesis in tobacco with reduced amounts of Rubisco. Plant Cell Environ. 31:407-418.

Labanauskas, C.K., P. Shouse, and L.H. Stolzy. 1981. Effects of water stress at various growth stages on seed yield and nutrient concentrations of field-grown cowpeas. Soil Sci. 131:249-256.

Lawlor, D.W. 2002. Limitation to photosynthesis in water-stressed leaves: Stomata vs. metabolism and the role of ATP. Ann. Bot. 89:871-885.

Lefi, E., H. Medrano, and J. Cifre. 2004. Water uptake dynamics, photosynthesis and water use efficiency in field-grown *Medicago arborea* and *Medicago citrina* under prolonged Mediterranean drought conditions. Ann. Appl. Biol. 144:299-307.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthesis. Methods Enzymol. 148:350-352.

Lingakumar, K., P. Amudha, and G. Kulandaivelu. 1999. Exclusion of solar UV-B (280-315 nm) radiation on vegetative growth and photosynthetic activities in *Vigna unguiculata* L. Plant Sci. 148:97-103.

Lizana, C., M. Wentworth, J.P. Martinez, D. Villegas, R. Meneses, E.H. Murchie, C. Pastenes, B. Lercari, P. Vernieri, P. Horton, and M. Pinto. 2006. Differential adaptation of two varieties of common bean to abiotic stress: I. Effects of drought on yield and photosynthesis. J. Exp. Bot. 57:685-697.

Lobell, D.B., and G.P. Asner. 2003. Climate and management contributions to recent trends in U.S. agricultural yields. Science 299:1032.

Lobell, D.B., M.B. Burke, C. Tebaldi, M.D. Mastrandrea, W.P. Falcon, and R.L. Naylor. 2008. Prioritizing climate change adaptation needs for food security in 2030. Science 319:607-610.

Lois, R., and B.B. Buchanan. 1994. Severe sensitivity to ultraviolet radiation in an *Arabidopsis* mutant deficient in flavonoid accumulation II. Mechanisms if UV-resistance in *Arabidopsis*. Planta 194:504-509.

Long, S.P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? Plant Cell Environ. 14:729-739.

Long, S.P., and C.J. Bernacchi. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. J. Exp. Bot. 54:2393-2401.

Long, S.P., E.A. Ainsworth, A. Rogers, and D.R. Ort. 2004. Rising atmospheric carbon dioxide: Plants FACE the future. Annu. Rev. Plant Biol. 55:591-628.

Long, S.P., E.A. Ainsworth, A.D.B. Leakey, and P.B. Morgan. 2005. Global food insecurity. Treatment of major food crops with elevated carbon dioxide or ozone under large-scale fully open-air conditions suggests recent models may have overestimated future yields. Philos. Trans. R. Soc. London, Ser. B. 360:2011-2020.

Long, S.P., E.A. Ainsworth, A.D.B. Leakey, J. Nosberger, and D.R. Ort. 2006. Food for thought: Lower-than-expected crop yield stimulation with rising CO_2 concentrations. Science 312:1918-1921.

Longenberger, P.S., C.W. Smith, P.S. Thaxton, and B.L. McMichael. 2006. Development of a screening method for drought tolerance in cotton seedlings. Crop Sci. 46:2104-2110.

Lopez, F.B., T.L. Setter, and C.R. McDavid. 1987. Carbon dioxide and light responses of photosynthesis in cowpea and pigeonpea during water deficit and recovery. Plant Physiol. 85:990-995.

Lu, Z., R.G. Percy, C.O. Qualset, and E. Zeiger. 1998. Stomatal conductance predicts yields in irrigated pima cotton and bread wheat grown at high temperatures. J. Exp. Bot. 49:453-460.

Mackerness, S.A.H., B.R. Jordan, and B. Thomas. 1999. Reactive oxygen species in the regulation of photosynthetic genes by ultraviolet-B radiation (UV-B: 280-320 nm) in green and etiolated buds of pea (*Pisum sativum* L.). J. Photochem. Photobiol. B: Biol. 48:180-188.

Maréchal, R., M. Mascherpa and F. Stainer 1978. Étude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (*Papilionaceae*) sur la base données morphologiques et polliniques, traitées par l'analyse informatique. Boissiera 28:1-273.

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

Martin, B., and N.A. Ruiz-Torres. 1992. Effects of water-deficit stress on photosynthesis, its components and component limitations, and on water use efficiency in wheat (*Triticum aestivum* L.). Plant Physiol. 100:733-739.

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

Maxwell, K., and G.N. Johnson. 2000. Chlorophyll fluorescence: A practical guide. J. Exp. Bot. 51:659-668.

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

Medrano, H., J.M. Escalona, J. Bota, J. Gulias, and J. Flexas. 2002. Regulation of photosynthesis of C_3 plants in response to progressive drought: Stomatal conductance as a reference parameter. Ann. Bot. 89:895-905.

Meehl, T.G.A., and C. Tebaldi. 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science 305:994-997.

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

Morison, J.I.L., and R.M. Gifford. 1984. Plant growth and water use with limited water supply in high CO_2 concentrations. I. Leaf area, water use and transpiration. Aust. J. Plant Physiol. 11:375-384.

Murray, F.W. 1967. On the computation of saturation vapor pressure. J. Appl. Meteorol. 6:203-204.

Musil, C.F., S.B.M. Chimphango, and F.D. Dakora. 2002a. Effects of elevated ultraviolet-B radiation on native and cultivated plants of Southern Africa. Ann. Bot. 90:127-137.

Musil, C.F., L.O. Björn, M.W.J. Scourfield, and G.E. Bodeker. 2002b. How substantial are ultraviolet-B supplementation inaccuracies in experimental square-wave delivery systems? Environ. Exp. Bot. 47:25-38.

Nedunchezhian, N., and G. Kulandaivelu. 1997. Changes induced by ultraviolet-B (280-320 nm) radiation to vegetative growth and photosynthetic characteristics in field grown *Vigna unguiculata* L. Plant Sci. 123:85-92.

National Climate Data Center, (Online access date August 6, 2008, http://www.ncdc.noaa.gov/oa/ncdc.html)

Ng, N.Q. 1995. Cowpea *Vigna unguiculata* (Legumiosae-Papillionodeae), p. 326-323, *In* J. Smartt and N.W. Simmonds, Eds. Evolution of Crops. Longman, Harlow, UK.

Ng, N.Q., and R. Marechal. 1985. Cowpea taxonomy, origin and germplasm, p. 11-21, *In* S.R. Singh and K.O. Rachie, Eds. Cowpea Research, Production and Utilization. John Wiley & Sons Lt., Chichester, UK.

Nielsen, C.L., and A.E. Hall. 1985. Responses of cowpea (*Vigna unguiculata* [L.] Walp.) in the field to high night air temperature during flowering. I. Thermal regimes of production regions and field experimental system. Field Crops Res. 10:167-179.

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

Ohler, T.A., and C.A. Mitchell. 1995. Effects of carbon dioxide level and plant density on cowpea canopy productivity for a bioregenerative life support system. Life Support Biosph. Sci. 2:3-9.

Overdieck, D., C. Reid, and B.R. Strain. 1988. The effects of preindustrial and future CO_2 concentrations on growth, dry matter production and the C/N relationship in plants at low nutrient supply: *Vigna unguiculata* (cowpea), *Abelmoschus esculentus* (okra) and *Raphanus sativus* (radish). Angew. Bot. 62:119-134.

Padulosi, S., and N.Q. Ng. Origin, taxonomy, and morphology of *Vigna unguiculata* (L.) Walp., p. 1-12, *In* B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L. Jackai, Eds. Advances in Cowpea Research. IITA-JIRCAS, Ibadan, Nigeria.

Pal, M., A. Sharma, Y.P. Abrol, and U.K. Sengupta. 1997. Exclusion of UV-B radiation from normal solar spectrum on the growth of mung bean and maize. Agric. Ecosyst. Environ. 61:29-34.

Parry, M.A.J., J. Flexas, and H. Medrano. 2005. Prospects for crop production under drought: Research priorities and future directions. Ann. Appl. Biol. 147:211-226.

Patel, N.R., A.N. Mehta, and A.M. Shekh. 2001. Canopy temperature and water stress quantification in rainfed pigeonpea (*Cajanus cajan* [L.] Millsp.). Agric. For. Meteorol. 109:223-232.

Patel, P.N., and A.E. Hall. 1990. Genotypic variation and classification of cowpea for reproductive responses to high temperature under long photoperiods. Crop Sci. 30:614-621.

Prasad, P.V.V., K.J. Boote, and J.L.H. Allen. 2006. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [*Sorghum bicolor* (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. Agric. For. Meteorol. 139:237-251.

Prasad, P.V.V., K.J. Boote, L.H. Allen, and J.M.G. Thomas. 2002. Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (*Phaseolus vulgaris* L.). Global Change Biol. 8:710-721.

Prasad, P.V.V., K.J. Boote, L.H. Allen, Jr., and J.M.G. Thomas. 2003. Super-optimal temperatures are detrimental to peanut (*Arachis hypogaea* L.) reproductive processes and yield at both ambient and elevated carbon dioxide. Global Change Biol. 9:1775.

Premkumar, A., and G. Kulandaivelu. 1996. Influence of ultraviolet-B enhanced solar radiation on growth and photosynthesis of potassium deficient cowpea seedlings. Photosynthetica 32:521-528.

Premkumar, A., and G. Kulandaivelu. 1999. The influence of increased solar UV-B radiation on magnesium-deficient cowpea seedlings: Changes in the photosynthetic characteristics. Photosynthetica 36:21-29.

Premkumar, A., and G. Kulandaivelu. 2001. Influence of increased solar UV-B radiation on magnesium deficient cowpea seedlings: Changes in growth and foliar constituents. Plant Sci. 161:1-8.

Qaderi, M.M., and D.M. Reid. 2005. Growth and physiological responses of canola (*Brassica napus*) to UV-B and CO_2 under controlled environment conditions. Physiol. Plant. 125:247-259.

Qaderi, M.M., L.V. Kurepin, and D.M. Reid. 2006. Growth and physiological responses of canola (*Brassica napus*) to three components of global climate change: Temperature, carbon dioxide and drought. Physiol. Plant. 128:710-721.

Qaderi, M.M., D.M. Reid, and E.C. Yeung. 2007. Morphological and physiological responses of canola (*Brassica napus*) siliquas and seeds to UVB and CO_2 under controlled environment conditions. Environ. Exp. Bot. 60:428-437.

Rachie, K.O. 1985. Introduction, p. 21-28, *In* S.R. Singh and K.O. Rachie, Eds. Cowpea Research Production and Utilization. Wiley, Chisester, England.

Rajendiran, K., and M.P. Ramanujam. 2004. Improvement of biomass partitioning, flowering and yield by triadimefon in UV-B stressed *Vigna radiata* (L.) Wilczek. Biol. Plant. 48:145-148.

Rawal, K.M. 1975. Natural hybridization among wild, weedy and cultivated *Vigna unguiculata* (L.) Walp. Euphytica 24:699-707.

Reddy, K.R., and H.F. Hodges. 2000. Climate Change and Global Crop Productivity. CAB International, Oxon, UK, pp. 448.

Reddy, K.R., H.F. Hodges, and J.M. McKinion. 1997. A comparison of scenarios for the effect of global climate change on cotton growth and yield. Aust. J. Plant Physiol. 24:707-713.

Reddy, K.R., H.F. Hodges, J.J. Read, J.M. McKinion, J.T. Baker, L. Tarpley, and V.R. Reddy. 2001. Soil-Plant-Atmosphere-Research (SPAR) facility: A tool for plant research and modeling. Biotronics 30:27-50.

Rizhsky, L., H. Liang, and R. Mittler. 2002. The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol. 130:1143-1151.

Rizhsky, L., H. Liang, J. Shuman, V. Shulaev, S. Davletova, and R. Mittler. 2004. When defense pathways collide. The response of arabidopsis to a combination of drought and heat stress. Plant Physiol. 134:1683-1696.

Ro, H.M., P.G. Kim, I.B. Lee, M.S. Yiem, and S.Y. Woo. 2001. Photosynthetic characteristics and growth responses of dwarf apple (*Malus domestica* Borkh. cv. Fuji) saplings after 3 years of exposure to elevated atmospheric carbon dioxide concentration and temperature. Trees 15:195-203.

Robertson, B.M., A.E. Hall, and K.W. Foster. 1985. A field technique for screening for genotypic differences in root growth. Crop Sci. 25:1084-1090.

Rouhi, V., R. Samson, R. Lemeur, and P.V. Damme. 2007. Photosynthetic gas exchange characteristics in three different almond species during drought stress and subsequent recovery. Environ. Exp. Bot. 59:117-129.

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

Runeckles, V.C., and S.V. Krupa. 1994. The impact of UV-B radiation and ozone on terrestrial vegetation. Environ. Pollut. 83:191-213.

Saile-Mark, M., and M. Tevini. 1997. Effects of solar UV-B radiation on growth, flowering and yield of central and southern European bush bean cultivars (*Phaseolus vulgaris* L.). Plant Ecol. 128:115-125.

Samdur, M.Y., P. Manivel, V.K. Jain, B.M. Chikani, H.K. Gor, S. Desai, and J.B. Misra. 2003. Genotypic differences and water-deficit induced enhancement in epicuticular wax load in peanut. Crop Sci. 43:1294-1299.

SAS Institute Inc. 2004. SAS/STAT User's Guide, Version 9.1.3, Cary, NC.

Searles, P.S., S.D. Flint, and M.M. Caldwell. 2001. A meta-analysis of plant field studies simulating stratospheric ozone depletion. Oecologia 127:1-10.

Seckmeyer, G., B. Mayer, G. Bernhard, R.L. McKenzie, P.V. Johnston, M. Kotkamp, C.R. Booth, T. Lucas, T. Mestechkina, C.R. Roy, H.P. Gies, and D. Tomlinson. 1995. Geographical differences in the UV measured by intercompared spectroradiometers. Geophys. Res. Lett. 22:1889-1892.

Sekiya, N., and K. Yano. 2008. Stomatal density of cowpea correlates with carbon isotope discrimination in different phosphorus, water and CO_2 environments. New Phytol. In press, doi:10.1111/j.1469-8137.2008.02518.x.

Shackel, K.A., and A.E. Hall. 1983. Comparison of water relations and osmotic adjustment in sorghum and cowpea under field conditions. Funct. Plant Biol. 10:423-435.

Shand, H. 1997. Human Nature: Agricultural Biodiversity and Farm-Based Food Security. RAFI, Ottawa, Ontario, pp. 94.

Shouse, P., S. Dasberg, W.A. Jury, and L.H. Stolzy. 1981. Water deficit effect on water potential, yield and water use of cowpea. Agron. J. 73:333-336.

Sinclair, T.R., and L.C. Purcell. 2005. Is a physiological perspective relevant in a 'genocentric' age? J. Exp. Bot. 56:2777-2782.

Singh, A. 1995. Influence of enhanced UV-B radiation on tropical legumes. Trop. Ecol. 36:249-252.

Singh, A. 1996. Growth, physiological, and biochemical responses of three tropical legumes to enhanced UV-B radiation. Can. J. Bot. 74:135-139.

Singh, A. 1997a. Increased UV-B radiation reduces N_2-fixation in tropical leguminous crops. Environ. Pollut. 95:289-291.

Singh, B.B. 1997b. Recent advances in cowpea breeding, p. 30-49, *In* B.B. Singh, D.R. Mohan Raj, K.E. Dashiell and E.N. Jackai, Eds. Advances in Cowpea Research. IITA and JIRCAS, Ibadan, Nigeria.

Singh, B.B. 2004. Cowpea (*Vigna unguiculata* [L.] Walp.), p. 117-161, *In* R.J. Singh and P.P. Jauhar, Eds. Genetic Resources, Chromosome Engineering, and Crop Improvement. CRC Press, Boca Raton, FL.

Singh, B.B., and B. Sharma. 1996. Restructuring cowpea for higher yield. Indian J. Genet. Plant Breed. 56:389-405.

Singh, B.B., D.R. Mohan Raj, K.E. Dashiell, and L. Jackai. 1997. Advances in Cowpea Research. IITA-JIRCAS, Ibadan, Nigeria, pp. 375.

Singh, B.B., P. Hartmann, C. Fatokun, M. Tamo, S. Tarawali, and R. Ortiz. 2003. Recent progress on cowpea improvement. Chronica Hort. 43:8-12.

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

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

Souza, R.P., E.C. Machado, J.A.B. Silva, A.M.M.A. Lagôa, and J.A.G. Silveira. 2004. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. Environ. Exp. Bot. 51:45-56.

Steele, W.M., and K.L. Mehra. 1980. Structure, evolution and adaptation of farming system and environment in *Vigna*, p.p. 393-404, *In* R.J. Summerfield and A.H. Bunting, Eds. Advances in Legume Science. HMSO, London, UK.

Sullivan, J.H. 1997. Effects of increasing UV-B radiation and atmospheric CO_2 on photosynthesis and growth: Implications for terrestrial ecosystems. Plant Ecol. 128:195-206.

Summerfield, R.J., P.A. Huxley, P.J. Dart, and A.P. Hughes. 1976. Some effects of environmental stress on seed yield of cowpea (*Vigna unguiculata* [L.] Walp.) cv. Prima. Plant Soil 44:527-546.

Tardieu, F., and T. Simonneau. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: Modelling isohydric and anisohydric behaviours. J. Exp. Bot. 49:419-432.

Tegelberg, R., R. Julkunen-Tiitto, M. Vartiainen, R. Paunonen, M. Rousi, and S. Kellomäki. 2008. Exposures to elevated CO_2, elevated temperature and enhanced UV-B radiation modify activities of polyphenol oxidase and guaiacol peroxidase and concentrations of chlorophylls, polyamines and soluble proteins in the leaves of *Betula pendula* seedlings. Environ. Exp. Bot. 62:308-315.

ter Braak, C.J.F. 1983. Principal components biplots and alpha and beta diversity. Ecology 64:454-462.

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

Teramura, A.H., and J.H. Sullivan. 1994. Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. Photosynthesis Res. 39:463-437.

Teramura, A.H., J.H. Sullivan, and L.H. Ziska. 1990. Interaction of elevated ultraviolet-B radiation and CO_2 on productivity and photosynthetic characteristics in wheat, rice, and soybean. Plant Physiol. 94:470-475.

Thiaw, S., and A.E. Hall. 2004. Comparison of selection for either leaf-electrolyte-leakage or pod set in enhancing heat tolerance and grain yield of cowpea. Field Crops Res. 86:239-253.

Thiaw, S., A.E. Hall, and D.R. Parker. 1993. Varietal intercropping and the yields and stability of cowpea production in semiarid Senegal. Field Crops Res. 33:217-233.

Tubiello, F.N., J.S. Amthor, K.J. Boote, M. Donatelli, W. Easterling, G. Fischer, R.M. Gifford, M. Howden, J. Reilly, and C. Rosenzweig. 2007. Crop response to elevated CO_2 and world food supply: A comment on "Food for Thought..." by Long et al., Science 312:1918-1921, 2006. Eur. J. Agron. 26:215-223.

Turk, K.J., A.E. Hall, and C.W. Asbell. 1980. Drought adaptation of cowpea. I. Influence of drought on seed yield. Agron. J. 72:413-420.

U.N. Population Division, 2008. (Online access date August 6, 2008, http://esa.un.org/unpp).

U.S. Census Bureau, Population Division. 2008. U.S. and World Population Clocks – POPClocks (Online access date August 6, 2008, http://www.census.gov/main/www/popclock.html).

USDA. 2005. UV-B Monitoring and Research Program. Colorado State University, CO. (Online access date December 10, 2006, http://uvb.nrel.colostate.edu/UVB)

Vavilov, N.I. 1935. The Origin, Variation, Immunity and Breeding of Cultivated Plants (Translated by KS Chester) Chronica Botanica Co., Waltham, MA, pp.364.

Vinocur, B., and A. Altman. 2005. Recent advances in engineering plant tolerance to abiotic stress: Achievements and limitations. Curr. Opin. Biotechnol. 16:123-132.

Walton, G.H., P. Si, and B. Bowde. 1999. Environmental impact on canola yield and oil, p. 136-143, *In* N. Wratten and P.A. Salisbury, Eds. Proceedings of the 10th International Rapeseed Congres. The Regional Institute Ltd, Canberra, Australia.

Warrag, M.O.A. 1994. Autotoxicity of mesquite (*Prosopis juliflora*) pericarps on seed germination and seedling growth. J. Arid Environ. 27:79-84.

Warrag, M.O.A., and A.E. Hall. 1983. Reproductive responses of cowpea to heat stress: Genotypic differences in tolerance to heat at flowering. Crop Sci. 23:1088-1092.

Warrag, M.O.A., and A.E. Hall. 1984a. Reproductive responses of cowpea (*Vigna unguiculata* (L.) Walp.) to heat stress. II. Responses to night air temperature. Field Crops Res. 8:17-33.

Warrag, M.O.A., and A.E. Hall. 1984b. Reproductive responses of cowpea (*Vigna unguiculata* (L.) Walp.) to heat stress. I. Responses to soil and day air temperatures. Field Crops Res. 8:3-16.

Wentworth, M., E.H. Murchie, J.E. Gray, D. Villegas, C. Pastenes, M. Pinto, and P. Horton. 2006. Differential adaptation of two varieties of common bean to abiotic stress: II. Acclimation of photosynthesis. J. Exp. Bot. 57:699-709.

Williams, J.H., and K.J. Boote. 1995. Physiology and modelling predicting the unpredictable legume, p. 301-335, *In* H.E. Patte and H.T. Stalker, Eds. Advances in Peanut Science. APRES, Stillwater, OK.

Wingler, A., W.P. Quick, R.A. Bungard, K.J. Bailey, P.J. Lea, and R.C. Leegood. 1999. The role of photorespiration during drought stress: An analysis utilizing barley

mutants with reduced activities of photorespiratory enzymes. Plant Cell Environ. 22:361-373.

WMO. 2007. Scientific Assessment of Ozone Depletion: 2006, Global Ozone Research and Monitoring Project 50. Geneva, Switzerland, pp. 572.

Wu, J., S. Neimanis, and U. Heber. 1991. Photorespiration is more effective than the Mehler reaction in protecting the photosynthetic apparatus against photoinhibition. Botanica Acta 104:283-291.

Xiao, G., W. Liu, Q. Xu, Z. Sun, and J. Wang. 2005. Effects of temperature increase and elevated CO_2 concentration, with supplemental irrigation, on the yield of rain-fed spring wheat in a semiarid region of China. Agric. Water Manage. 74:243-255.

Yuan, L., Z. Yanqun, C. Haiyan, C. Jianjun, Y. Jilong, and H. Zhide. 2000. Intraspecific responses in crop growth and yield of 20 wheat cultivars to enhanced ultraviolet-B radiation under field conditions. Field Crops Res. 67:25-33.

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

Ziska, L.H. Evaluation of yield loss in field sorghum from a C_3 and C_4 weed with increasing CO_2. Weed Sci. 51:914-918.

Zoltán, T. 2005. Is the long-term elevated air CO_2 environment beneficial for plants, crops and vegetation?, p. 1-6, *In* Z. Tuba, Ed. Ecological Responses and Adaptations of Crops to Rising Atmospheric Carbon Dioxide. Haworth Press, Inc., New York, NY.