1. Introduction

Drought stress reduces global soybean yield more than 50%, annually (Wang et al. 2003). The effect of drought on soybean yield depends on the severity, duration, and timing of the stress about the growth stage (Brar et al. 1990). Soybean is most susceptible to drought stress during the reproductive stage (Westgate and Peterson 1993; Wijewardana et al. 2018, 2019); however, when the plants are subjected to long-term severe water stress during the vegetative growth stage, the stress may be great enough to cause substantial yield losses.

The effects of water stress on soybean physiological and biochemical changes are not clearly understood (Manalavan et al. 2009). Soil moisture stress induces various morpho-physiological and biochemical adaptations that, subsequently, inhibit growth, lower photosynthesis, reduce stomatal conductance and transpiration, decrease chlorophyll content, and cause changes in proteomics (Lu and Zhang 1999; Cornick 2000; Reddy et al. 2004; Wijewardana et al. 2017). Photosynthetic and gas exchange processes are very dynamic, involving complex regulation by several factors, and thus, these should be taken as integrative parameters to assess soybean photosynthetic responses under soil moisture stress during its vegetative stage. The effects of drought stress on photosynthetic parameters is hotly debated, with most questioning if soil moisture stress limited photosynthesis through stomatal closure or metabolic impairment (Lauer and Boyer 1992; Cornick 2000; Lawson et al. 2003). Many noted that stomatal limitation was the main factor reducing photosynthesis under drought stress (Cornick 2000). However, others reported limitations such as photophosphorylation (Meyer and Genty 1999), diminished ribulose-1,5-bisphosphate (RUBP) caused by low ATP synthesis (Tezara et al. 1999), and increased oxygenase activity of Rubisco (Medrano et al. 2002) are responsible for reduced photosynthesis under drought stress. Soil moisture stress decreases leaf water potential (LWP), which reduces the swelling pressure and subsequently, stomatal closure (Schulze 1993). Plants experience soil moisture stress when the rate of transpiration becomes very high or when the water supply to roots become difficult (Reddy et al. 2004). Wang et al. (2006) observed a strong correlation of stomatal conductance with transpiration compared to net photosynthesis. This could be due to the soil moisture stress-induced abscisic acid (ABA) which is stimulated by soil drying through the transpiration stream (Reddy et al. 2004), resulting in stomatal closure. Moreover, a significant increase in ABA was reported by Mutava et al. (2015) in tolerant soybean genotypes under water stress condition compared to susceptible ones.

Chlorophyll content has also known to be affected by soil moisture stress. Makbul et al. (2011), reported a 28% decrease in chlorophyll content when the leaf water potential decreased from −0.88 to −1.18 MPa in soybean. Decreased chlorophyll content and increased carotenoids contents...
during drought stress have been reported in many species (Wang et al. 2003; Gajanjake and Reddy 2016; Wijewardana et al. 2016), depending on the severity and duration of the stress. Loss of chlorophyll content is considered as the main cause of reduced photosynthesis under drought stress.

Soybean water status can be determined remotely through hyperspectral readings. Remote sensing is quick and can provide one with an array of spectral indices that estimate plant water status, economically (Pénelas et al. 1993; Prasad et al. 2007; Gutiérrez et al. 2010; Pask et al. 2012). Canopy reflectance has been used to assess the water status in various crops including cotton, corn, and wheat. These reflectance indices are often correlated with midday LWP (Pénelas et al. 1993; Gutiérrez et al. 2010; Elsayed and Darwish 2017). Reflectance fluctuations in the Near-Infrared region (NIR; 700–1300 nm) can be used to evaluate relationships between canopy water status and spectral indices. NIR wavelengths penetrate deeper into the canopy than middle infrared (1300–2500 nm), thereby allowing one to estimate water content in the entire canopy rather than only in the uppermost layers (Pénelas et al. 1993). Some studies suggested a better correlation of crop water status and canopy reflectance using normalized difference vegetation index (NDVI; (R900–R680)/(R900 + R680)), water index (R900/R970), normalized water index (NWI; (R970–R880)/(R970 + R880)), and normalized difference water indices (Pénelas et al. 1993; Yi et al. 2007; Wu et al. 2009; Elsayed et al. 2011; Winterhalter et al. 2011). Because the NDVI includes both near infrared and red light, it can be used to predict photosynthetic activity, final yield, crop nutrient deficiency, and long-term water stress.

Many of the studies on hyperspectral remote sensing measurements in soybean mainly focused on predicting yield, estimating crop nutrient status, monitoring chlorophyll content, or to understand the relationship with leaf area index (Ma et al. 2001; Patil et al. 2007; Gray et al. 2010). To the best of our knowledge, there is very little information available on capturing the effects of soil moisture variations on soybean canopy reflectance indices at its vegetative growth phase. A combination of measurements, including soil moisture content, midday LWP, canopy photosynthesis and gas exchange traits, and pigment content would significantly improve our limited understanding of soybean physiological responses to varying levels soil moisture conditions. Therefore, the objectives of this study were to quantify the responses of leaf gas exchange, chlorophyll fluorescence, midday leaf water potential, and canopy reflectance to series of soil moisture stress levels in two soybean cultivars with different growth habits and to discuss possible differences among the cultivars in relation to the physiological mechanisms triggered by soil moisture stress.

2. Materials and methods

2.1. Plant culture and experimental details

The research was conducted in Soil-Plant-Atmosphere-Research (SPAR) chambers located at the Environmental Plant Physiology Laboratory at Mississippi State University, MS, USA in 2015. Four seeds from two soybean cultivars from maturity group V with differing growth habits (indeterminate type-Asgrow AG5332 and determinate type-Progeny P5333RY), were sown into individual PVC (polyvinyl chloride) pots (15.2-cm diameter by 30.5-cm high). Each pot was filled with a 3:1 sand: top soil classified as a sandy loam (87% sand, 2% clay, and 11% silt) with a 500 g of gravel and a small hole at the bottom for excess water drainage. Nine days after seeding (DAS) the plants were thinned to one per pot. Throughout the experimental period, plants were fertigated with full-strength Hoagland’s nutrient solution delivered through a computer-controlled and automated drip irrigation system to ensure favorable water and nutrient conditions for plant growth.

The SPAR units consist of 1.27-cm thick Plexiglas chambers (2.5-m tall by 2-m long by 1.5-m wide) to accommodate aerial plant parts and a steel soil bin (1-m deep by 2-m long by 0.5-m wide) to house the root system. The SPAR units allow for precise control of CO2 and air temperature at near ambient levels of photosynthetic active radiation (PAR). The chamber CO2 concentration was set to 400 μmol mol⁻¹. The relative humidity of each chamber was monitored with a humidity and temperature sensor (HMV 70Y, Vaisala, Inc., St. Louis, MO) installed in the returning path of air ducts. The air temperature was set at 29/21°C (day/night) and monitored and adjusted every 10 s and maintained within ±0.5°C of the treatment set points measured with aspirated thermocouples. The daytime temperature was initiated at sunrise and returned to the nighttime temperature 1 h after sunset. From the air temperature and relative humidity measurements, vapor pressure deficit (VPD) was estimated according to the procedure described by Murray (1967). Around the edges of plant canopy, variable density shade cloths (Hummert Seed CO., St. Louis, MO) were placed and adjusted regularly to match canopy height and to eliminate the need for border plants. More details of the operation of the SPAR units have been described by Reddy et al. (2001). The mean values of day/night temperature, chamber CO2 concentration, and VPD are given in Table S1.

2.2. Treatments

The soil moisture treatments included 100%, 80%, 60%, 40%, and 20% of the evapotranspiration (ET) values. Initially, all the plants were irrigated with the same water of volume as in 100% ET treatment. Thirty-one DAS, ET-based irrigation treatments were imposed and continued until harvest, 65 DAS. The ET of each treatment was measured on a ground area basis (L d⁻¹) as the rate at which condensate was removed by cooling coils at 900 s intervals by measuring the mass of water in collecting devices connected to a pressure transducer (McKinion and Hodges 1985; Reddy et al. 2001). By accounting the ET values recorded on the previous day, the amount of water provided for each treatment was adjusted by changing the duration of irrigation.

2.3. Measurements

2.3.1. Soil moisture content and midday leaf water potential

Soil moisture was monitored using Decagon soil moisture sensors (STM Soil Moisture and Temperature Sensor, Decagon Devices, Inc., Pullman, WA) inserted at a depth of 15 cm in five random pots of each treatment. Midday leaf water potential (LWP) was measured using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). LWP was measured 42, 46, 52, and 57 DAS by selecting
three random plants from each cultivar in each soil moisture treatment and taking four readings from the youngest fully expanded leaves between 1000 and 1300 h after imposing the treatments.

2.3.2. Phenology and growth
Plant height and number of nodes on the main stem was recorded weekly. Plants were harvested 65 DAS, and roots, stems, and leaves were separated and dry weight determined by oven drying to constant weight.

2.3.3. Gas exchange and fluorescence measurements
Leaf net photosynthesis (Pn), stomatal conductance (gs), transpiration rate (E), chlorophyll fluorescence (Fv/Fm) were measured three times from the youngest fully expanded leaves between 1000 and 1300 h, from three individual plants per cultivar from each treatment using a LI-6400 portable photosynthesis meter (Li-COR Inc., Lincoln, NE). The temperature in the leaf cuvette was set to the 29°C daytime air temperature, and CO₂ was controlled by the CO₂ injection system to match the 400 µmol mol⁻¹ chamber CO₂ concentration. The relative humidity inside the cuvette was maintained at 50%, and the PAR provided by a 6400–02 LED light source was set to 1500 µmol m⁻² s⁻¹. The instrument itself computes the data for Pn, gs, E, leaf internal CO₂ concentration, and Fv/Fm. The internal (Ci) to external CO₂ (Ca) ratio was calculated as Ci/Ca considering Ca as 400 µmol mol⁻¹ (ambient CO₂ concentration) and photosynthetic water-use efficiency (WUE) was calculated and expressed as the ratio of Pn/gs.

2.3.4. Measurements of photosynthetic pigments
Photosynthetic pigment content, chlorophyll a, chlorophyll b, and carotenoids were measured by taking leaf samples collected from three youngest fully expanded leaves from each soil moisture stress treatment at 41–56 days after seeding. Five leaf discs, each with 2 cm² area, were collected randomly and placed in vials containing 5 mL of dimethyl sulfoxide (DMSO) for chlorophyll and carotenoids extraction. After 24 h incubation, the absorbance of the supernatant was measured with a Bio-Rad ultraviolet/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA) at 470, 648, and 663 nm. The total chlorophyll and carotenoids were estimated by the equations of Lichtenthaler (1987) according to the procedure described by Chappelle et al. (1992) and expressed on a leaf area basis (µg cm⁻²).

2.3.5. Canopy reflectance measurements
Starting from 46 DAS, canopy reflectance measurements were made three times (46, 52, and 57 DAS) on sunny days between 1100 and 1200 h above the canopy in all the treatments using a portable ASD FieldSpec FR spectroradiometer (Analytical Spectral Devices Inc., Boulder, CO, USA). Reflectance was measured at wavelengths ranging from 350 to 2500 nm in each plant, setting the distance to half a meter between the optical head of the spectroradiometer and the plant terminal. At the beginning of each treatment before taking canopy reflectance measurements, the ASD instrument was optimized, and a reference signal was obtained using a Spectralon (Labsphere, Inc., Sutton, NH, USA) white panel. Therefore, for each soil moisture stress treatment, the canopy reflectance was computed as the ratio of canopy radiance to the radiance from the white reference panel. The nine spectral reflectance measurements for each cultivar in each treatment were averaged, and the mean values were used for graphical and statistical analysis. Reflectance values in the range of 400–1400 nm were selected for the data analysis while omitting the other values due to noise and location of these bands within regions of atmospheric water absorption (Zhao et al. 2007). The six different reflectance indices (Table S2) were calculated based on the equations provided in the previous studies (Prasad et al. 2007; Elsayed et al. 2011; Winterhalter et al. 2011; Pask et al. 2012). The reflectance values for each treatment were averaged over the sampling dates, to determine the relationships between reflectance indices and midday leaf water potential, and a single regression line was fitted across all the measurements.

2.4. Data analysis
The data were analyzed using SAS 9.2 (SAS Institute, Cary, NC) as a completely randomized design with nine replications. Analysis of variance was used to determine soybean responses to soil moisture stress and midday leaf water potential. Means were separated and compared using the least significant difference at P < 0.05 probability. The regression analyses were performed using SigmaPlot version 13 (Systat Software Inc., San Jose, CA). The relationships among the midday leaf water potential and different physiological, gas exchange, and canopy reflectance traits were tested for linear and quadratic functions, and the best fit regressions were selected.

3. Results and discussion
This research was conducted to evaluate the effect of drought stress that occurs during vegetative stages on gas exchange, canopy reflectance, and midday leaf water potential in soybean cultivars with divergent growth habits. The effect of drought stress on soybean must be understood to increase agricultural productivity in a changing environment. Data from this study increases our understanding of how drought stress affects soybean during vegetative stages so that we can improve soybeans tolerance and yield potential in environments with low soil moisture.

3.1. Soil moisture, evapotranspiration, and midday leaf water potential
Soil moisture content measured using decagon soil moisture sensors showed a significant difference (P < 0.05) among the five ET-based soil moisture stress treatments (Table S1) during the experimental period from 31 to 65 DAS, however, the treatments were not significantly different (P > 0.05) among the two soybean cultivars (Figure 1(A)). Soil moisture content was inversely correlated with the ET replacement. Similar to soil moisture variations, measured ET values also differed among the soil moisture treatments (Table S1). Evapotranspiration was reduced by 60% when the treatment changed from control (100% ET) to severe water deficit (20% ET). The other environmental variables, such as day and night average temperature, CO₂, and vapor pressure deficit (VPD) were not different (P > 0.05) among the treatments and cultivars (Table S1).
Midday LWP is a reliable measurement to assess the water status of plants, due to its interrelationships with leaf gas exchange and other growth and developmental parameters (Williams and Araujo 2002). In the present study, midday leaf water content was correlated with soil moisture content, and it was not different between cultivars (Figure 1B). Exposure to severe soil moisture stress resulted in decreasing midday LWP by 45% in stressed plants as compared to the control (Figure 1B). Wang et al. (2006) reported a drop of LWP from −1.4 (control) to −2.2 MPa (drought stress) for five days of water stress in soybean at the pre-flowering stage. A 34% decline in LWP was reported by Makbul et al. (2011) and they have suggested that the decrease was due to the 28% decrease in chlorophyll content in drought-stressed soybean.

### 3.2. Photosynthetic physiology

Pooled over cultivars, all gas exchange parameters were positively correlated with midday LWP (Figure 2). Pn (Figure 2A) and gs (Figure 2B) were highly correlated with midday LWP, while Ci/Ca was weakly correlated with midday LWP (Figure 2C). Our data indicate, therefore, that the decrease in Pn is mainly due to stomatal closure. Moreover, under severe soil moisture stress, Pn was 62% smaller as compared to the control, while gs was 71% smaller than the control (Figure 2A and B). The ratio of Ci/Ca was slightly decreased, and the reduction was 3% in stressed plants compared to the control (Figure 2C). Thus, the stomatal limitation is likely the primary cause for reduced net photosynthesis in soybean exposed to drought stress. The stomatal behavior of the leaf is typically regulated either to minimize water loss or to maximize carbon gain, depending on the plant water status and photosynthetic rate. Many studies have reported decreased Pn and gs, in response to soil moisture stress (Makbul et al. 2011; Ku et al. 2013). Tezara et al. (1999) suggested that the decrease of Pn could be due to low ATP content, caused by decrease of photosynthesis (Fv/Fm), transpiration (E), water use efficiency (WUE), the ratio of internal to external CO2 concentration (Ci/Ca), fluorescence (Fv/Fm'), and electron transport rate (ETR), Normalized difference vegetation index (NDVI), Red normalized difference vegetation index (RNDVI), Normalized water index (NWI).

#### Table 1. Analysis of variance across the irrigation treatments (Trt), cultivars (Cul), and their interaction (Cul × Trt) with soybean vegetative growth, development, physiological, and canopy reflectance traits measured at 65 days after sowing (DAS).

| Source              | Soil moisture (Trt) | Cultivar (Cul) | Trt * Cultivar (Cul) |
|---------------------|---------------------|----------------|----------------------|
| Plant height, cm    |                   |                |                      |
| Node no. plant⁻¹    |                   |                |                      |
| Leaf area, cm²      |                   |                |                      |
| Pod no. plant⁻¹     |                   |                |                      |
| Leaf weight, g plant⁻¹ |             |                |                      |
| Stem weight, g plant⁻¹ |              |                |                      |
| Root weight, g plant⁻¹  |            |                |                      |
| Pod weight, g plant⁻¹  |             |                |                      |
| Total dry matter, g |                   |                |                      |
| Chlorophyll a, µg cm⁻² |             |                |                      |
| Chlorophyll b, µg cm⁻² |             |                |                      |
| Total chlorophyll, µg cm⁻² |            |                |                      |
| Carotenoids, µg cm⁻² |                   |                |                      |
| Photosynthesis, µmol CO₂ m⁻² s⁻¹ | |                |                      |
| gs, mol H₂O m⁻² s⁻¹ |                   |                |                      |
| E, mmol H₂O m⁻² s⁻¹ |                   |                |                      |
| Ci/Ca               |                   |                |                      |
| WUE, mmol CO₂ mol⁻¹ H₂O |             |                |                      |
| Fv/Fm'              |                   |                |                      |
| ETR, µmol m⁻² s⁻¹   |                   |                |                      |
| Reflectance         |                   |                |                      |
| NDVI                |                   |                |                      |
| RNDVI               |                   |                |                      |
| NWI                 |                   |                |                      |
| NW4                 |                   |                |                      |
| NW5                 |                   |                |                      |
| NW6                 |                   |                |                      |

†, ‡, §, *** represent Significance levels at P ≤ 0.05, P ≤ 0.01, and P ≤ 0.001. NS represents P > 0.05. Stomatal conductance (gs), transpiration (E), water use efficiency (WUE), the ratio of internal to external CO₂ concentration (Ci/Ca), fluorescence (Fv/Fm'), and electron transport rate (ETR), Normalized difference vegetation index (NDVI), Red normalized difference vegetation index (RNDVI), Normalized water index (NWI).
drought adaptive mechanism which might have resulted from lower transpiration rate under moisture stressed conditions.

Chlorophyll fluorescence (Fv/Fm) is the most reliable indicator and widely used parameter to assess the photosystem II (PS-II) efficiency. In our study, both ETR (Figure 2(F)) and Fv/Fm (Figure 2(G)) had a declining trend concerning midday LWP. The ETR decreased by 21% under severe soil moisture stress condition, whereas the decrease in Fv/Fm was 14% compared to the control treatment. A marked decrease in Fv/Fm under soil moisture stress indicated more photoinhibition and decreased PS-II efficiency compared to the well-irrigated soybean plants. Moreover, this may be attributed to a lesser number of open PS-II reaction centers which may inhibit light harvesting and energy transduction leading to lower CO2 assimilation. The lower Fv/Fm further suggests that the absorbed light energy in soil moisture stressed plants was not utilized in photochemical quenching but could be dissipated in the form of heat energy resulting in lower sink capacity.

3.3. Chlorophylls and carotenoids

Chlorophyll is the major chloroplast component for photosynthesis and is important for harvesting light and generating reducing power. The limitation of photosynthesis through metabolic impairment is a result of reduced photosynthetic pigments. In the present study, total chlorophyll content declined linearly, and in contrast, carotenoids increased linearly with declining soil moisture content (Figure 2(H)). However, the two soybean cultivars exhibited similar responses and did not differ (P > 0.05) for both pigments (Table 1). Chlorophyll decreased 24% under severe soil moisture stress condition, whereas the increase in carotenoids was 38% compared to the control (Figure 2(H)). Soil moisture stress-induced reduction in chlorophylls is considered as an indicator of oxidative stress which is attributed to chlorophyll degradation, pigment photo-oxidation, and insufficient synthesis of chlorophylls. By doing such, light absorption by the chloroplasts would be minimized to prevent...
photochemical damage to PS II under water-limited conditions. Decreased chlorophyll and increased carotenoid contents have been reported for many crop species under soil moisture stress (Massacci et al. 2008; Guha et al. 2010; Gajanayake and Reddy 2016). Carotenoids form a key part of the plant antioxidant defense system; however, they are susceptible to oxidative damage. The major role of carotenoids is to prevent the generation of singlet oxygen and protect from oxidative damage through direct quenching of triplet chlorophyll (Farooq et al. 2009). Therefore, increased carotenoid content is an antioxidant defense mechanism in soybean to limit oxidative damage under soil moisture stress.

3.4. Growth and developmental attributes

Soil moisture stress is a major limiting factor at the initial vegetative phase of soybean growth and establishment. Drought stress affects cell elongation, expansion, and leaf development during vegetative growth stages. Among many growth and developmental traits, node number, plant height, length of internode, and leaf area expansion have been proposed as indicators of soil moisture stress tolerance in soybean (Desclaux et al. 2000; Ku et al. 2013; Khan et al. 2014). In the present study, soil moisture stress decreased the plant height in both the cultivars (Figure 3). The reduction of plant height could associate with the decline in the cell enlargement (Ku et al. 2013). Plant height of two cultivars showed significant differences ($P < 0.001$) under both stressed and control conditions (Table 1). The plant height of indeterminate type, Asgrow AG5332 (Figure 3(A)), exhibited 33% reduction at 20% ET treatments; whereas, Progeny P5333RY showed 20% reduction compared to the control (Figure 3(B)). Asgrow AG5332 plants were taller regardless of the treatment effects compared to the Progeny P5333RY. This difference could be due to their growth habits in which Asgrow AG5332, the indeterminate type, grows continuously while Progeny P5333RY ceases its vegetative activity at or soon after photoperiod-induced floral induction (Wijewardana et al. 2018).

The mild or severe soil moisture stress reduced both node and pod numbers (Figure 4(A and B)). Similar to plant height, two cultivars showed a significant difference ($P < 0.001$) for node and pod no. (Table 1). The determinate type did not show much variation for the node no. among the different soil moisture stress levels; however, node numbers decreased by 10% for Asgrow AG5332 as soil moisture changed from 0.22 to 0.09 m$^3$ m$^{-3}$. Pod numbers exhibited a linear decline with decreasing soil moisture (Figure 4(B)).

![Figure 3](image_url)  
**Figure 3.** Time series analysis of plant height in two soybean cultivars (A) Asgrow AG5332 and (B) Progeny P5333RY across five soil moisture stress regimes. Each data point is a mean of nine individual plants, and standard errors of the mean are shown when larger than the symbols.

![Figure 4](image_url)  
**Figure 4.** Relationships between soil moisture and (A) node no., (B) pod no., and (C) leaf area of soybean measured at the harvest. Bars represent standard errors of the mean ($n = 9$) and are shown when larger than the symbols.
At the time we harvested soybean plants (65 DAS); they were at the beginning of the pod development stage. Therefore, the total pod no. that was given in the present study does not provide the true potential of the cultivars for pod number. Averaged over the treatments, pod no. of Asgrow AG5332 and Progeny P5333RY grown under optimum soil moisture level (0.22 m³ m⁻³) was 11 and 14% higher than the pod no. under severe moisture stress (0.09 m³ m⁻³). Soybean pod set and seed development are more susceptible to soil moisture stress, especially when initiated at R2 or R4 (Brown et al. 1985), causing a substantial yield reduction. This could be the result of reduced mainstem nodes and branches at the early vegetative stage (Brevedan and Egli 2003) and later stages, from accelerated leaf senescence and shortened seed filling period. Some studies have reported a 93% reduction in a number of pods per plant at the beginning of pod development in soybean (Atti et al. 2004). The reduction was 82% during pod lengthening due to the cumulative effects of reduced no. of flowers, pod abortion, and reduced pod production under soil moisture stress conditions (Atti et al. 2004; He et al. 2017; Wijewardana et al. 2018). Total leaf area per plant also declined with increasing soil moisture stress (Figure 4(C)). Progeny P5333RY exhibited larger leaf area under all the soil moisture stress treatments compared to Asgrow AG5332. However, under severe water deficit, the percent of leaf area reduction was higher than the Asgrow AG5332 cultivar (Figure 4(C)). Development of optimum leaf area is important for increased photosynthesis, more biomass, and greater yield. Lower leaf area is a drought avoidance mechanism to reduce transpiration by restricting leaf area expansion as the soil moisture content declines to conserve more water within the plant. Moreover, by reducing the leaf area, the plant manages water loss by maintaining fewer stomata which in turn reduces stomatal conductance. Overall, reduced stem elongation, node no., and leaf size is a likely result of the inhibition of cell elongation from the lower photosynthetic efficiency (Farooq et al. 2009) or caused by dehydration of protoplasm and reduced turgidity resulting in reduced cell division and expansion (Khan et al. 2014).

3.5. Biomass production

All the biomass components (leaf, stem, root, and pod) decreased linearly and exhibited positive correlations with decreasing soil moisture. Like for most of the growth and developmental traits, the two soybean cultivars were significantly different (P < 0.001) for dry weights components (Table 1). The leaf (Figure 5(A)), stem and root (Figure 5(B)), and pod (Figure 5(C)) dry weights declined by 13, 52, 1, and 56% for Asgrow AG5332 and 30, 45, 12, and 3% for Progeny P5333RY correspondingly, at 20% ET level (0.09 m³ m⁻³) compared with the control treatment (100% ET). Shoot dry weight loss was greater than root dry weight loss in both the cultivars. Progeny P5333RY showed higher leaf, stem, and root weight under all the soil moisture stress treatments except for pod dry weight. Lower pod dry weight in Progeny P5333RY is possibly due to the production of a lower number of pods at the time of harvest due to its determinate growth habits. The higher shoot weight was closely related to the increased leaf area in Progeny P5333RY across all the treatments compared to the Asgrow AG5332. The higher root dry weight is desirable under drought for efficient uptake of water and nutrients that could increase drought

![Figure 5. Relationships between soil moisture and (A) leaf weight, (B) stem and root weight, (C) pod weight, and (D) total dry weight of soybean measured at the harvest. Bars represent standard errors of the mean (n = 9) and are shown when larger than the symbols.](image-url)
resistance. Therefore, at the vegetative growth stage, Progeny P5333RY exhibited better sustainability with having increased shoot and root weights under sub-optimal water conditions. Greater plant fresh and dry weights under soil moisture stress are desirable characters (Farooq et al. 2009) that contribute to optimum canopy development.

Plant productivity is strongly related to the temporal biomass distribution and biomass partitioning (Specht et al. 2001). Evapotranspiration based irrigation treatments reduced total dry weights in both the cultivars (Figure 5(D)). By our findings, reduced biomass was reported by Specht et al. (2001) in soybean under limited water conditions. The total dry weight under 0.09 m$^3$ m$^{-2}$ compared to their control treatments was 29% in Asgrow AG5332 and 43% in Progeny P5333RY. The ET-based irrigation treatments imposed at the reproductive stage on the same two cultivars (Wijewardana et al. 2018) showed 43% (Asgrow AG5332) and 54% (Progeny P5333RY) reduction in the total dry matter, respectively, at 20% ET level compared to the control. This implies that although shoot and root dry weights were higher for Progeny P5333RY, it was more sensitive to soil moisture stress compared to Asgrow AG5332.

### 3.6. Canopy reflectance and the relationship between LWP and reflectance indices

The spectral reflectance is the radiance that is reflected from the leaf expresses as a percentage of incident radiance (Carter 1993). Typically, crop canopy reflectances differ in the visible red region (550–700 nm) and near infrared region (700–1300 nm) of the electromagnetic spectrum (Kumar and Silva 1973). In our study, different response curves were observed for five soil moisture stress treatments (Figure 6(A)); however, the difference among the cultivars and sampling dates was not significant ($P > 0.05$) (Table 1). Therefore, the crop reflectances were averaged among the cultivars and sampling dates. The resulting five different spectra imply that soil moisture stress influenced the spectral features of the soybean canopy. Soil moisture stress increased soybean canopy reflectance in the visible range of the spectrum (400–700 nm), especially for 60, 40, and 20% ET compared to the control and 80% ET (Figure 6(B)). This is probably due to a decrease in the absorption of visible radiation by chlorophylls and other leaf pigments (Bowman 1989; Carter 1991, 1993; Wang et al. 2001). Previous studies reported that the absorptivity of chlorophyll is relatively low in the visible spectral range; therefore, the reflectance is higher at those wavelengths. Typically, visible light, or the short wavelength photons absorbed by leaf pigments, is used for photochemical reactions and overall photosynthesis processes (Wang et al. 2001). Therefore, soil moisture stress-induced photosynthetic reduction was better explained by the decreased radiation absorbed by the soybean canopy or the increased reflectance in the visible region. In the range of near-infrared (NIR; 700–1300 nm), absorption by pigments and water is relatively low, thus, the reflectance is relatively high (Carter 1991, 1993). In accordance with the previous findings, a greater increase in canopy reflectance was observed in the NIR region for the control treatment and low reflectances for soil moisture stress treatments, suggesting that the red region of the spectrum is a good indicator of soil moisture stress (Carter 1993). According to Wang and Shannon (1999), higher absorption of infrared radiation resulted in leaf heating and transpiration. Therefore, the lower reflectance in the NIR region further confirmed the soil moisture stress-induced reduction in transpiration and stomatal conductance in soybean canopy at its vegetative stage. Beyond the NIR region (1300–2500 nm) which is a function of leaf water content and thickness, has also been reported to increase spectral reflectance in soil moisture stressed plants (Carter 1991, 1993; Carter et al. 1992) by stimulating leaf dehydration and reducing absorption of radiation by leaf internal water. In contrast to the previous findings, the soybean canopy reflectance continued to exhibit decreased values for the mid-infrared region (MIR) in our study. This infers that soil moisture stress had little effect on increasing leaf dehydration or the water stress might not be severe enough to cause the leaves to dehydrate completely.

Soybean midday leaf water potential was strongly correlated (Figure 7) with all the calculated spectral indices (Table S2). The NDVI (Figure 7(A)), RNDVI (Figure 7(B)), NW1 (Figure 7(C)), NW14 (Figure 7(D)), and NW15 (Figure 7(E)) increased linearly with the increase in LWP, however NW16 (Figure 7(F)) exhibited a negative correlation with increasing LWP. NDVI was most highly correlated ($R^2 = 0.82$) with midday LWP (Figure 7(A)). In general, NDVI is an indication of ground coverage of vegetation canopy and greenness (Zhao et al. 2007). Soil moisture stress reduces chlorophyll and other photosynthetic pigments and canopies size, probably resulting in a low NDVI index. RNDVI (Figure
(B)), which is an indication of photosynthetic capacity and leaf N status, also showed a higher positive correlation than NWI4 and NWI5 with midday LWP \((Y = 0.30 - 0.07X, r^2 = 0.72)\) similar to NDVI. The four water stress indices tested in the present study demonstrated strong associations with the midday LWP, with NWI showing the strongest association \((r^2 = 0.75)\). These indices have widely been associated with diverse water relation parameters to sense canopy water status in different plant species such as corn, wheat, barley, sorghum, and pepper. Some studies suggested that wavelengths in the area of the NIR region are more suitable to describe the canopy water mass under water stress conditions (Elsayed et al. 2015). In accordance with our findings, Lobos et al. (2014) also observed higher coefficient of determinations \((r^2 > 0.60)\) for NWI and NWI4 with the grain yield under mild water stress. Moreover, Winterhalter et al. (2013) reported a strong correlation of NWI6 with water content and biomass in maize corroborating with our present findings on soybean. In summary, our results indicated that soybean midday leaf water potential was associated with NDVI, RNDVI, NWI, NWI4, NWI5, and NWI6 regardless of sampling date across all the treatments, with higher \(r^2\) values \((r^2 > 0.50)\). Therefore, the linear algorithms and the developed reflectance indices may be useful to predict leaf water potential and crop water status under sub-optimal water conditions.

4. Conclusions

The current study is an effort to investigate plant water status using quantitative measurements of soil moisture, midday leaf water potential, leaf gas exchange, and canopy reflectance. The results of the study indicated that soybean water status could be quantitatively monitored using measures of midday leaf water potential, soil moisture, and gas exchange. The leaf gas exchange traits had relatively strong correlations with midday leaf water potential. The two soybean cultivars at the vegetative growth phase showed the marked difference for growth and phenological traits under soil moisture stress; however, physiological and gas exchange traits were not different. The cultivar Asgrow AG5332 was more soil moisture stress tolerant than Progeny P5333RY concerning...
physiological adaptation associated with growth and developmental attributes. The decrease in stomatal conductance led to the reduction in net photosynthesis; however, the non-stomatal limitation may occur under severe soil moisture stress which also leads to impairment of photosynthetic activity. The soil moisture stress-induced reduction in chlorophyll exhibited oxidative stress; whereas, increased carotenoids could be a protective mechanism to overcome oxidative damage to photosystem II. The five spectral indices showed strong correlations with midday leaf water potential suggesting that those indices are good indicators to detect water status in soybean under suboptimal soil moisture conditions. The quantified physiological responses could be used by genetic engineering or/and breeding programs concerning soil moisture stress adaptation of soybeans for both current and future climates. Moreover, the tested canopy reflectance indices may be used to understand plant water relations and canopy structure to help soybean growers to make field management decisions during the growing season.

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