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Impact of root architecture and transpiration rate on drought tolerance in stay-green sorghum

Maduraimuthu Djanaguiraman[1] | Srinivasan Gowsiga[1] | Mahalingam Govindaraj[2,4] | Ephrem Habyarimana[2] | Alagarswamy Senthil[1] | Nallasamy Thavaprakaash[3] | Prabhakaran Jeyakumar[1] | Jayavel Kokilavani[1] | Chellapandiyan Chellammal[1]

[1]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

[2]International Crops Research Institute for the Semiarid Tropics (ICRISAT), Patancheru, Telangana, India

[3]Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

[4]Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT), Cali, Colombia

Correspondence
Maduraimuthu Djanaguiraman, Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641 003, India.
Email: jani@tnau.ac.in

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Abstract

Sorghum [*Sorghum bicolor* (L.) Moench] yield loss due to terminal drought stress is common in semiarid regions. Stay-green is a drought adaptation trait, and a deeper understanding of stay-green-associated traits is necessary for sorghum breeding. We hypothesize that the stay-green trait in sorghum may be associated with the root architecture and transpiration rate under drought stress. The objectives were to (i) identify the relationship among stay-green-associated traits, (ii) compare the root system architecture and transpiration rate of stay-green (B35 and 296B) and senescent (BTx623 and R16) genotypes under drought stress, and (iii) quantify the impacts of reproductive stage drought stress on gas exchange and grain yield of stay-green and senescent genotypes. A series of drought experiments were conducted with these genotypes. Under drought stress, the stay-green genotypes had an increased total root length in the top 30–60 cm (18%) and 60–90 cm of soil (45%) than the senescent genotypes. In contrast, under progressive soil drying, stay-green genotypes had a decreased transpiration rate (9%) than senescent genotypes by an early (~1 h) partial closure of stomata under high vapor pressure deficit conditions. The increased seed yield (43%) in stay-green genotypes is due to an increased photosynthetic rate (30%) and individual seed size (35%) than senescent genotypes. Overall, it is concluded that stay-green phenotypes had two distinct drought adaptive mechanisms: (i) increased root length for increased soil exploration for water and (ii) an early decrease in the transpiration rate to conserve soil moisture. Identifying genomic markers for these traits would accelerate drought-tolerant sorghum breeding.

1 | INTRODUCTION

Across the globe, drought is one of the most important challenges in achieving food and nutritional security in arid and semiarid regions (Campbell et al., 2016; Lobell et al., 2011). Rainfed agriculture is generally vulnerable to drought because the yield of the rainfed crop depends on rainfall intensity

Abbreviations: DAE, days after emergence; DAS, days after sowing; FC, field capacity; FTSW, fraction of transpirable soil water; PI_{ABS}, performance index; PS, photosystem; PVC, polyvinyl chloride; QTLs, quantitative trait loci; RBD, randomized block design; SPAD, soil plant analytical division; TTSW, total transpirable soil water; VPD, vapor pressure deficit.

and duration (Batisani & Yarnal, 2010; Romero et al., 1998). Sorghum [*Sorghum bicolor* (L.) Moench.] is one of the major food and fodder crops in rainfed areas, adapted to hot and dry conditions, and is grown in more than 30 million ha worldwide. In sorghum, reproductive (anthesis and grain-filing) stages are the most sensitive to drought stress than other growth stages (Assefa et al., 2010; De-Camargo & Hubbard, 1999; P. V. V. Prasad et al., 2019; V. B. R. Prasad et al., 2021). Drought tolerance possesses complex traits, dependent on the crop development stage and severity of stress. Therefore, there is a need to mine novel traits for increased drought stress adaptation to sustain and increase grain yield in sorghum (Abdelrahman et al., 2017).

Stay-green is one of the important drought adaptation traits in sorghum (Borrell & Hammer, 2000). During post-flowering drought stress, the stay-green phenotype is characterized by a higher photosynthetic rate over a long time than senescent phenotypes because of delayed leaf senescence, resulting in an increased grain yield (∼31%) than senescent genotypes (Borrell et al., 2000; Thomas & Ougham, 2014). In sorghum, the stay-green trait had an oligogenic inheritance, and four major quantitative trait loci (QTLs), namely, *Stg1*, *Stg2*, *Stg3*, and *Stg4*, have been associated with stay-green trait. The chromosome mapping showed that *Stg1* and *Stg2* were localized in chromosome 3 and *Stg3* and *Stg4* on chromosomes 3 and 5 (Borrell et al., 2014). Studies showed that these QTLs influence leaf nitrogen content (Harris et al., 2006), plant size (Borrell et al., 2014), and nodal root angle (Mace et al., 2012).

Results of the several stay-green experiments indicate that the stay-green trait is a complex phenotype since it includes component traits that directly influence leaf senescence or indirectly through water uptake and water loss (Borrell & Hammer, 2000; Borrell et al., 2014; Mace et al., 2012). In addition, it is observed that the stay-green trait is associated with reduced water use during the early vegetative stage by limited transpiration under high vapor pressure deficit (VPD) (Karthika et al., 2019) or enhanced sensitivity to soil drying (Sinclair et al., 2017). However, analysis of stay-green sorghum genotypes for the expression of restricted transpiration under high VPD showed no relationship between stay-green and restricted transpiration (Choudhary et al., 2013). Similarly, there was no relationship between stay-green and sensitivity to soil drying. Hence, we can hypothesize that stay-green trait may be associated with root traits because the response to stomata to the transpiration rate in stay-green genotypes varies. Tuinstra et al. (1998) showed a positive association between xylem pressure potential and grain yield. Likewise, a positive relationship between the xylem pressure potential and the expression of the stay-green trait was observed (Tuinstra et al., 1998). The DSSAT-CERES sorghum model indicated that the simulated optimal rooting depth for rainfed sorghum was between 110 and 140 cm (Lopez et al., 2017). Similarly, Hammer et al. (2009) proposed that deep root systems with increased water uptake in maize may be associated with grain yield.

Core Ideas

- Sorghum genotypes with contrasting leaf senescence traits were evaluated under drought stress for stay-green.
- Stay-green and nodal root angle has been positively associated with terminal drought tolerance in sorghum.
- Stay-green genotypes had a significantly increased root length than senescent genotypes under drought stress.
- Stay-green genotypes had an earlier decrease in the transpiration rate than senescent genotypes under drying soil.

Research to identify the "designer root" for drought tolerance in sorghum was not studied in detail. For instance, Lynch (2013) proposed that a steep, cheap, and deep root system is the preferred cereal phenotype for water-limited ecosystems. Mace et al. (2012) and Singh et al. (2012) showed a strong negative association between nodal root angle and sorghum grain yield; that is, the genotype with a narrow (smaller) nodal root angle had a high yield under drought. In addition, three QTLs associated with nodal root angle were co-located with identified QTLs for stay-green, explaining a 58.2% variability and a strong relationship between nodal root angle and stay-green (Borrell et al., 2014; Mace et al., 2012). Overall, it is clear from the above findings that under drought stress, stay-green sorghum genotypes may acquire more water than the senescent sorghum genotypes through an increased root length at different soil profiles, but this has not been documented.

In general, plants must counteract the drought stress effect through the modulation of photosynthesis, osmoregulation, hormonal content, and reactive oxygen species concentration (Blum, 1996; Hoekstra et al., 2001; Kwak et al., 2003). Severe drought stress reduces growth and yield through the decreased photosynthetic rate and partitioning of carbohydrates to the developing grain (Jung et al., 2019; V. B. R. Prasad et al., 2021; Sankarapandian et al., 2013). The decreased photosynthetic rate under drought stress is associated with stomatal and non-stomatal processes (Yang et al., 2021). The stomatal limitation was due to the partial closure of the stomata, thereby causing a limitation to the transpiration rate under high VPD conditions (Kholova et al., 2014). The non-stomatal limitation is caused by photodamage and biochemical limitations (Keshavarz-Afshar et al., 2015). A deeper understanding of drought adaptive traits such as photosynthesis, transpiration, and photosystem (PS) II quantum yield under drought stress in stay-green and senescent genotypes was not thoroughly researched.

Therefore, this study was conducted to (i) identify the relationship among stay-green-associated traits, (ii) compare the root system architecture and transpiration rate of stay-green (B35 and 296B) and senescent (BTx623 and R16) genotypes under drought stress, and (iii) quantify the impacts of reproductive stage drought stress on gas exchange and grain yield of stay-green and senescent genotypes.

2 | MATERIALS AND METHODS

2.1 | Materials

In experiment 1, 38 sorghum genotypes (296B, ICSB 38, ICSB 52, ICSB 101, ICSB 403, ICSB 541, ICSB 544, ICSB 627, ICSB 24001, ICSB 24002, ICSB 24003, ICSB 24004, ICSB 24005, B 35, ICSR 101, ICSR 196, ICSR 89016, ICSR 89058, ICSR 24009, ICSR 25001, ICSR 14002, ICSR 14005, ICSV 700, ICSV 89039, ICSV 89106, ICSV 15013, ICSV 17022, ICSV 17028, ICSV 17037, CSV 13, Isiap dorado, Macia, PVK 801, ICSV 745, S 35, ICSV 93046, NTJ 2, and R 16) were used, and in experiments 2–5, four genotypes (B35, 296B, BTx623, and R16) were used.

2.2 | Classification of sorghum genotypes to drought stress and identification of traits associated with drought

A field experiment was conducted in a split-plot design with two replications using the above genotypes during the *Kharif* (summer) season at Tamil Nadu Agricultural University, Coimbatore (11˚N; 77˚E; 426.7 m MSL), India. The main plots and subplots were irrigation regimes and genotypes, respectively. The main plot had two levels: (1) irrigated control, where plants are irrigated at 5-day intervals and (2) drought stress, which involved withholding water from the booting to the physiological maturity stage. The subplot had 38 levels (mentioned earlier). Each genotype was sown at 45 × 7.5 cm in two ridges, measuring 3 m long. The fertilizer dose of 90:45:45 kg N:P_2O_5:K_2O ha^{-1} was followed. The nitrogenous fertilizer was applied at two doses, one at sowing and another at 30 days after sowing (DAS). In addition, phosphorus and potassium fertilizers were added at sowing. During the drought stress period, there was no rainfall.

At booting, five plants were tagged in each replication for measuring green leaf area plant^{-1}, chlorophyll index (soil plant analytical division [SPAD] units), stay-green score, stomatal frequency, and seed-set percent at the grain-filling stage. However, plant height, number of senesced leaves, individual seed size, and total dry matter production were recorded at maturity. The maximum green leaf length and leaf width of the second leaf from the top were measured using a ruler, and the value was multiplied by a factor of 0.7 to arrive at the green leaf area (Mahalakshmi & Bidinger, 2002). This value was multiplied by the number of the green leaves at the grain-filling stage to arrive the green leaf area plant^{-1}. The chlorophyll index was measured in the fourth leaf from the top using a chlorophyll meter (SPAD; Model 502, Spectrum Technologies) and expressed as SPAD units. The stay-green score was recorded on a scale from 0 to 5, where 0–1 represents 80% green-leaf area, 1–2 represents 60% green-leaf area, 2–3 represents 40% green-leaf area, 3–4 represents 20% green-leaf area, and 4–5 represents <10% green-leaf area. The second leaf from the top was cleaned gently with a brush, and in the middle of the leaf, a thin layer of nail polish was applied on the upper side and allowed to dry. After 30 min, the nail polish peel was removed using a needle and examined in a Nikon upright microscope under a 40× objective. The stomatal frequency (the number of stomata per unit area) was determined by manual counting (Neil et al., 1990). The seed-set percent was determined as described by Djanaguiraman et al. (2018). Plant height was measured as the distance from the soil surface to the tip of the fully expanded leaf and expressed as centimeters. The number of senesced leaves at maturity was counted and expressed as numbers per plant. Individual seed size was estimated as described by P. V. V. Prasad et al. (2015). At maturity, five plants were harvested and dried. Total dry matter production was determined by weighing the whole dried plant and expressed as g plant^{-1}.

2.3 | Response of stay-green and senescent sorghum genotypes to drought stress at the vegetative stage: Root system and leaf physiological traits

2.3.1 | Measurement of root angle

Nodal root angle

A pot culture experiment was conducted in a completely randomized design with five replications. The seeds of B35, 296B, BTx623, and R16 were surface sterilized using sodium hypochlorite (10% v/v) for 5 min, followed by washing in deionized water five times and sown in a plastic pot (25.7 × 25.5 × 22.1 cm; L × B × H) containing 9 kg of sandy clay loam soil. There were holes in each pot at the bottom for drainage. In each pot, three seeds were sown at a depth of 3 cm. After seedling emergence, the seedlings were thinned to one per pot. After thinning, 2 g urea, 1 g diammonium phosphate, and 1 g potash were added to each pot. The plants were irrigated on alternate days from emergence to 21 days after emergence (DAE). On the 22nd day, the soil was washed from the pot by flushing water, and the plants were carefully removed from the pots. The roots were washed with deionized water with a hand to remove the debris and stone. The roots of the upper node were tough and thicker than the lower nodes, and the root angle remained in the original shape after removing the soil with water. The root angle of the first nodal root from the top of the plant was determined by measuring the root angle between the horizontal line moving along the soil surface and the line along the slope of the nodal root at 2 cm from the root base using a protractor (Ali et al., 2015).

Seminal root angle

A laboratory experiment was conducted in a completely randomized design with five replications. The seeds of B35, 296B, BTx623, and R16 were surface sterilized as described earlier, then germinated in a Petri plate (100 mm × 15 mm; diameter × thickness) using Whatman number 42 filter paper, and moistened with 5 mL of deionized water for 2 days. In each Petri plate, 10 seeds were placed. A 2% (w/v) agar (Type A; Sigma Chemicals) solution was prepared and autoclaved at standard temperature (121°C) and pressure (15 lbs sq in.$^{-1}$) for 20 min. Under the aseptic condition, the sterilized agar was poured into the Petri plate (12 × 12 × 1.7 cm; L × B × H) up to the rim and solidified. Following this, all the sides of the Petri plates were sealed using clear cellophane tape (Amazon Basics Tape 1.9 cm × 1605 cm). On the second day, a uniformly sized seedling with a radicle length of 0.2–0.3 cm was selected, and one seed per plate was placed through the cuts of the Petri plates containing agar in a vertical position with the radicle facing downward. The Petri plates were positioned vertically in an incubation room maintained at 24.5 ± 1.5°C for 14 days. On the 15th day, the angle of the first pair of seminal roots was measured at a 3-cm distance from the seed relative to a vertical line passing through the stem base (Manschadi et al., 2006).

2.3.2 | Effects of vegetative stage drought stress: Root column experiment

Plant materials and crop husbandry

An outdoor experiment in a randomized block design (RBD) with a split-plot treatment structure with three replications was conducted at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore (11°N; 77°E; 426.7 m MSL), India. Before starting the experiment, the clay-loamy soil was sieved through a 2-mm sieve, and the soil fraction that did not pass through the sieve was discarded. Next, the soil fraction (<2 mm) was sieved using a 1-mm sieve, and the fraction which passed through the sieve was collected, and the material that did not pass through was discarded. Finally, 5 kg of vermicompost was added to 200 kg of sieved soil and thoroughly mixed and filled in the column.

The above soil was sampled to quantify the available soil moisture using a pressure plate apparatus (L. Richards, 1941). Analysis indicated that the available soil moisture was 18 ± 0.5%. The soil pH and electrical conductivity were 7.7 and 0.32 dS m^{-1}, respectively. The plant was grown in a polyvinyl chloride (PVC) column (100 × 7.5 cm; length × inner diameter), and the bottom of the column had a plastic cap with a central hole of 1-cm diameter for drainage. Before sowing, each PVC column was filled with 6.5 kg of soil; 2 g of urea, 2 g of diammonium phosphate, and 1 g of muriate of potash were added on top of the soil and mixed well. Then, the column was irrigated to 100% field capacity (FC). Three seeds of respective sorghum genotypes (B35, 296B, BTx623, and R16) were sown in a PVC column at a depth of 3 cm. After seedling emergence, the seedling was thinned to one and maintained till the completion of the experiment. The plants were grown under natural sunlit conditions and irrigated daily in the evening from sowing to the five-leaf stage (40 DAE). Afterward, the plants were divided into two groups; one served as irrigated control, and the other was drought stressed.

Imposition of drought stress

The total transpirable soil water (TTSW) content was determined as the difference between the soil water content at FC and wilting point (WP). The FC and WP were determined through a pressure plate apparatus, and using the FC and WP values, the fraction of transpirable soil water (FTSW) was calculated (FTSW = [actual water content − water content at the wilting point]/TTSW) (Sinclair & Ludlow, 1986). Drought stress was imposed by maintaining the plants at 0.4 FTSW, and the control plants were held at 0.9 FTSW.

At the five-leaf stage, all the plants were irrigated to FC; then, the top of the column was completely covered with a PVC sheet by making a small slit. The slit was covered with white packing adhesive tape around plant collars such that there was no space between the plant and the slit to minimize evaporative losses from the soil surface (Fracasso et al., 2016). Then, the column weight was recorded in the morning (06:00–07:00 hours) to arrive at the weight corresponding to FC. The irrigated plants were weighed daily at 07:00 hours, and the quantity of water required to bring 0.9 FTSW was worked out and added. In the drought-stressed plants, the irrigation was withheld until the soil moisture dropped to 0.4 FTSW. From that point, the required quantity of water to maintain 0.4 FTSW was added. The plants were held at 0.9 or 0.4 FTSW for 5 days. In our previous study, no variation between the genotypes was observed for an FTSW value of 0.3 or further below (Gowsiga et al., 2021); hence, 0.4 FTSW was maintained for imposing the drought stress.

In the present study, the plants at 0.4 FTSW on the second day showed leaf-rolling symptoms, and the drought stress was imposed for 5 days. Based on the difference in fresh and dry weight of the sample, the leaf water content of irrigated plants was 75%–80%, and the drought-stressed plants had a leaf water content of 34%–38%. During the drought, the daytime maximum temperature ranged from 37 and 38°C, and the nighttime minimum temperature ranged from 22 to 23°C. Similarly, the daytime relative humidity ranged from 45% and 50%, and the nighttime humidity from 90% to 95%. The plants were harvested after 5 days of drought stress.

Traits recorded

Growth traits. Plant height was measured as the distance from the soil surface to the tip of the fully expanded leaf and expressed as centimeters. At harvest, the PVC column was gently tilted at about 80°, and water was flushed slowly to the bottom of the column. The wet loose soil was removed by hand, so the soil particle alone slipped out of the column. The entire root system was washed thrice in water by hand gently to remove the attached soil particles from the root system. Then, it was laid on a flat surface and straightened to measure the maximum root length (rooting Depth; measured from the base of the stem to the tip of the root) and expressed as centimeters. Washed roots were stored in a plastic container containing 30% ethanol until further analysis (Fenta et al., 2014).

The whole root system was cut into 30-cm long portions, and each part was carefully spread in a transparent tray (20 × 15 × 2 cm; L × W × H) containing water to minimize root overlap and scanned using an Epson photo scanner (Epson Perfection V800 with 100 dpi resolution; Epson). Images of scanned roots were analyzed using the WinRHIZO Pro image system (Regent Instruments, Inc.) to estimate the total root length from top 0 to 30 cm, 30 to 60 cm, 60 to 90 cm, and 0 to 90 cm as explained by McPhee (2005) and Singh et al. (2011) and expressed in m plant^{-1}.

The maximum quantum yield of PS II, leaf area, photosynthetic rate, stomatal conductance, transpiration rate, and total dry matter production. The maximum quantum yield of PS II (F_v/F_m ratio) was measured using a modulated fluorometer (OS30p+; Optisciences) in 30 min dark adapted attached fully expanded top leaf from each replication between 10:00 and 14:00 hours on the fifth day of stress. The leaf-level gas exchange measurements (photosynthesis, stomatal conductance, and transpiration rate) were done on the tagged fully expanded top leaf using a LI-COR 6400XT portable photosynthesis system (LI-COR). The gas exchange measurements were recorded at the daytime growth temperature and ambient CO_2 level (410 ppm). The internal light-emitting diode light source was set at 1600 µmol m^{-2} s^{-1} to ensure a constant, uniform light across all measurements (Djanaguiraman et al., 2014). At harvest, the leaves were removed, and the leaf area was measured using a benchtop leaf area meter (LI-COR 3000; Lincoln) and expressed as cm^2 plant^{-1}. Total dry matter production was obtained by drying the samples at 60°C for 10 days, weighed, and expressed as g plant^{-1}.

2.4 | Stay-green and senescent sorghum genotypes response to drought stress at the booting stage: Leaf transpiration rate and associated physiological traits

An outdoor progressive soil drying experiment was conducted in an RBD with five replications. The soil previously described was used for this experiment. The available soil moisture was measured as described above. The pot (30 cm in diameter and 35.6 cm in height) with three holes in the bottom was filled with 22 kg of soil, and to each pot, 3 g of urea, 2 g of diammonium phosphate, and 2 g of potash were added and mixed well. Three seeds of respective sorghum genotypes (B35, 296B, BTx623, and R16) were sown in each pot and irrigated to 100% FC, after seedling emergence thinned to one per pot. On alternate days, the plants were watered from emergence to 50 DAE.

On 51 DAE, the pots were irrigated to FC at 18:00 hours; the top surface of the pot with the plant was covered with a PVC sheet by making a small slit, and there was no space between the plant and the sheet/slit (Fracasso et al., 2016). On the following morning, at 7:00 hours, the pot weight was recorded, and the water required to achieve 100% FC was added. Afterward, the pots were weighed hourly from 8:00 to 15:00 hours for 4 days to measure the hourly transpiration rate. The plant transpiration rate was calculated as the change in pot weight between two consecutive weight measurements (Xin et al., 2008). The traits such as chlorophyll index (SPAD units), PS II quantum yield (F_v/F_m ratio; unitless), performance index (PI_{ABS}), photosynthetic rate (µmol m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}), and transpiration rate (mmol m^{-2} s^{-1}) were recorded from day 1 to day 4 of drying soil as explained earlier.

2.5 | Stay-green and senescent sorghum genotypes response to drought stress at the seed development stage to the milky stage: Leaf physiological and yield traits

An outdoor pot culture experiment was conducted in a factorial RBD with five replications. Factors 1 and 2 are irrigation regimes and genotypes, respectively. The crop husbandry was the same as in the above experiment. On alternate days, the plants were watered from emergence to 7 days after the flowering stage (seed development stage). Drought stress was imposed by withholding water for 12 days. At the end of the stress, the traits such as chlorophyll index (SPAD units), PS II quantum yield (F_v/F_m ratio; unitless), photosynthetic rate (μmol m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}), transpiration rate (mmol m^{-2} s^{-1}), and green leaf area (cm^2 $plant^{-1}$) were recorded. After the drought stress period, all the plants were irrigated and maintained until harvest, and at harvest, the traits such as individual seed size (mg $seed^{-1}$), seed yield (g $plant^{-1}$), and harvest index (%) were recorded. Seed-set percent was arrived as explained by Djanaguiraman et al. (2018).

2.6 | Stay-green and senescent sorghum genotypes response to drought stress from the seed-set to dough stage: Leaf physiological and yield traits

A field experiment was conducted in a split-plot design with three replications. The main plot and subplots were irrigation regimes and genotypes, respectively. The main plot had two levels: (1) irrigated control, where plants are irrigated at 7-day intervals and (2) drought stress, which involved withholding water for 25 days from 5 days after the flowering stage. The subplot had four levels (genotypes: 1. B35, 2. 296B, 3. R16, and 4. BTx623).

Sorghum genotypes were sown in the ridge at a spacing of 60 × 30 cm at a depth of 3 cm. The fertilizer dose of 90:45:45 kg $N:P_2O_5:K_2O$ ha^{-1} was followed. The nitrogenous fertilizer was applied in two doses, one at sowing and another at 30 DAS. In addition, phosphorus and potassium fertilizers were added during sowing. The crop was irrigated once in seven days until the five days after the flowering stage. Afterward, the drought-stressed plants were not irrigated until harvest. At the flowering stage, the primary stalk and the top fully expanded leaf were tagged to record the gas exchange traits and plant height (cm $plant^{-1}$). In addition, attributes such as green leaf area (cm^2 $plant^{-1}$), total biomass (g $plant^{-1}$), individual seed size, and seed yield were recorded at harvest as described earlier.

2.7 | Statistical analyses

The classification of sorghum genotypes for drought tolerance was performed using the principal component analysis using the XLSTAT program. The data from drought stress were used for the classification of genotypes as described by Djanaguiraman et al. (2018). The seminal and nodal root angles data were analyzed using the PROC TTEST procedure of the SAS 9.4 version (SAS Institute Inc.). A p-value ≤ 0.05 was considered statistically significant. The root column experiment was designed in an RBD with a split-plot treatment structure. The irrigation regime was the main plot factor, and the genotype was the subplot factor. The PROC MIXED procedure of SAS was used for split-plot data analysis. In the progressive soil drying, the hourly transpiration data were analyzed by considering irrigation regime, genotype, days of observation, and time of observation (hourly transpiration rates) as fixed factors and block as a random factor. The transpiration rate was analyzed using linear and nonlinear regression techniques to quantify the transpiration response to the time of day. The broken stick or bilinear equation provided the highest R^2 value and smallest root mean squared deviation for the transpiration rate and was used to estimate the breakpoint in the transpiration rate. The data on chlorophyll index, photosynthetic rate, F_v/F_m ratio, stomatal conductance, transpiration rate, and PI_{ABS} were analyzed by considering irrigation regime and genotypes as fixed factors and block as a random factor. The Tukey–Kramer adjustment was used to separate the treatment means. Drought stress from seed development to the milky stage was conducted in a factorial RBD with five replications, and the data were analyzed through the PROC MIXED procedure. Likewise, drought stress from seed set to the dough stage was conducted in a split-plot design with three replications. The main plot was the irrigation regime, and the subplot was the genotype. The PROC MIXED procedure of SAS was used for data analysis.

3 | RESULTS

3.1 | Association among the traits and classification of sorghum genotypes to drought stress

The first two principal component vectors (PC1 and PC2) explained 55.6% of the total variation (Figure 1a,b). Among the traits in PC1, the highest variation was described by the stay-green score (19.4%). In PC2, the maximum variability was observed for the seed-set percent (20%). As anticipated, a negative relationship was observed between the stay-green score and seed yield (g $plant^{-1}$). Vectors of total dry matter production (g $plant^{-1}$), seed size (mg $seed^{-1}$), and green leaf area (cm^2 $plant^{-1}$) were generally in the same direction to seed yield but not as close as chlorophyll index (SPAD units) and seed-set percent, indicating that chlorophyll index and seed-set percent were closely related to grain yield. The genotypes 296 B, B35, and ICSB 24009 (+PC1 scores) were classified as stay-green genotypes. The genotypes R16, Macia, and ICSB 544 (−PC1 scores) were grouped as senescent genotypes. BTx623 was a known senescent genotype; therefore, it was not used in this experiment.

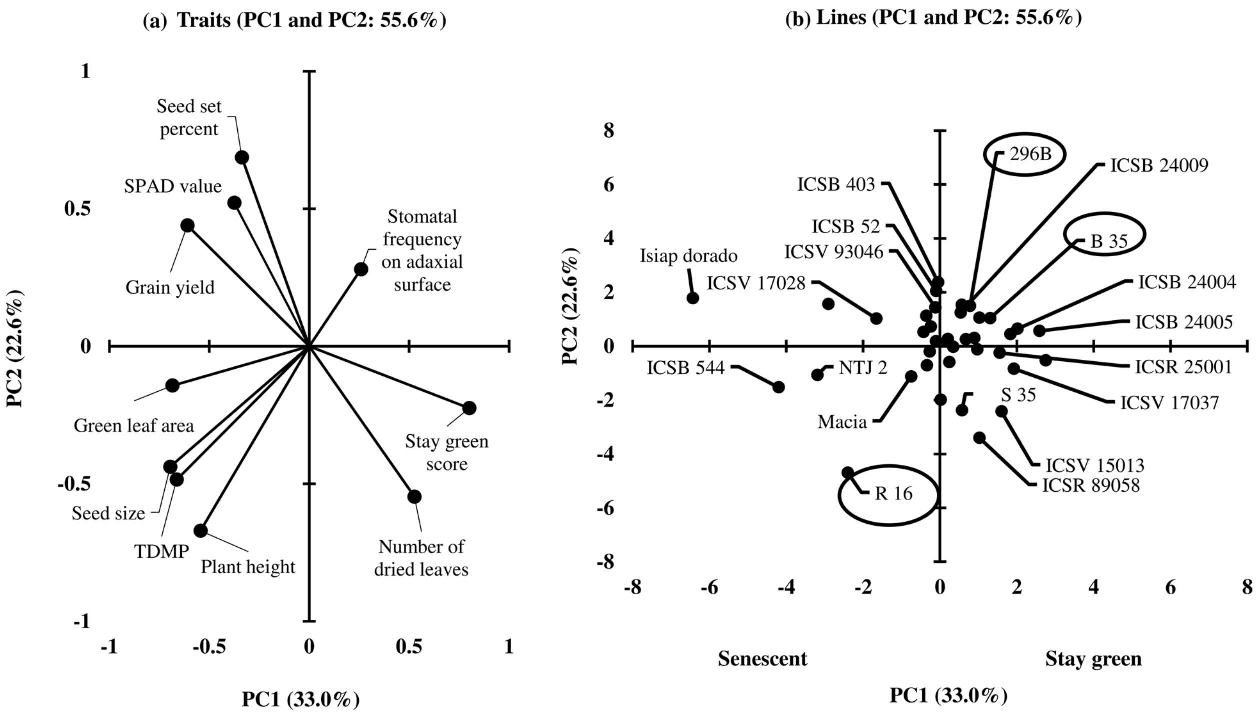

FIGURE 1 First and second principal component scores (PC1 and PC2) for identifying traits conferring drought stress tolerance and stay-green genotypes: (a) the factor loading value for traits is indicated by thick lines radiating from the center showing the direction (angle) and magnitude (length) of the trait's contribution to the principal component and (b) classification of 38 sorghum genotype based on the factor scores of PC1 and PC2. The principal components are shown in the axis, and the variance contributed by each principal component is indicated inside the parentheses. SPAD, soil plant analytical division ; TDMP, total dry matter production.

3.2 | Response of stay-green and senescent sorghum genotypes to drought stress at the vegetative stage: Root system and leaf physiological traits

The effect of genotype on the nodal (Figure 2a,c,d) and seminal (Figure 2b,e,f) root angle (°) was significant. The effect of genotypes, moisture regime, and interaction of genotype and moisture regime was significant ($p < 0.05$) for total root length from 0 to 30 cm (m plant^{-1}; Figure 3a), 30 to 60 cm (m plant^{-1}; Figure 3b), 60 to 90 cm (m plant^{-1}; Figure 3c), 0 to 90 cm (m plant^{-1}; Figure 3d), maximum root length (cm plant^{-1}; Figure 4b), PS II quantum yield (F_v/F_m ratio [relative units; Figure 4e), photosynthetic rate (μmol m^{-2} s^{-1}; Figure 4f), stomatal conductance (mol m^{-2} s^{-1}; Figure 4g), and transpiration rate (mmol m^{-2} s^{-1}; Figure 4h). In contrast, plant height (cm plant^{-1}; Figure 4a), leaf area (cm^2 plant^{-1}; Figure 4c), and total biomass (g plant^{-1}; Figure 4d) varied significantly ($p < 0.05$) between the genotypes.

Between the moisture regime, drought stress during the vegetative stage significantly ($p < 0.05$) reduced the total root length and root lengths at all soil layers. Similarly, drought stress significantly ($p < 0.05$) decreased maximum rooting depth, PS II quantum yield, photosynthetic rate, stomatal conductance, and transpiration rate compared with irrigated control. Compared to senescent, the stay-green genotypes had a significant ($p < 0.05$) wider nodal root angle (~11°) and a narrow seminal root angle (~5°) at the seedling stage (Figure 2a,b). The stay-green genotypes had a significant ($p < 0.05$) higher root length at all soil layers and total root length (27%) under drought stress conditions than the senescent genotypes. In stay-green genotypes, the most substantial increase in total root length was observed from the top 30–60 cm (45%), followed by 60–90 cm (29%). The stay-green genotypes had an increased maximum root length (8%), PS II quantum yield (4%), photosynthetic rate (32%), and transpiration rate (42%) than senescent genotypes under drought stress.

3.3 | Response of stay-green and senescent sorghum genotypes to drought stress at the booting stage (progressive soil drying): Leaf transpiration rate and associated physiological traits

The hourly transpiration rate (g plant^{-1}) differed significantly ($p < 0.05$) for genotypes, time of the day, and interaction

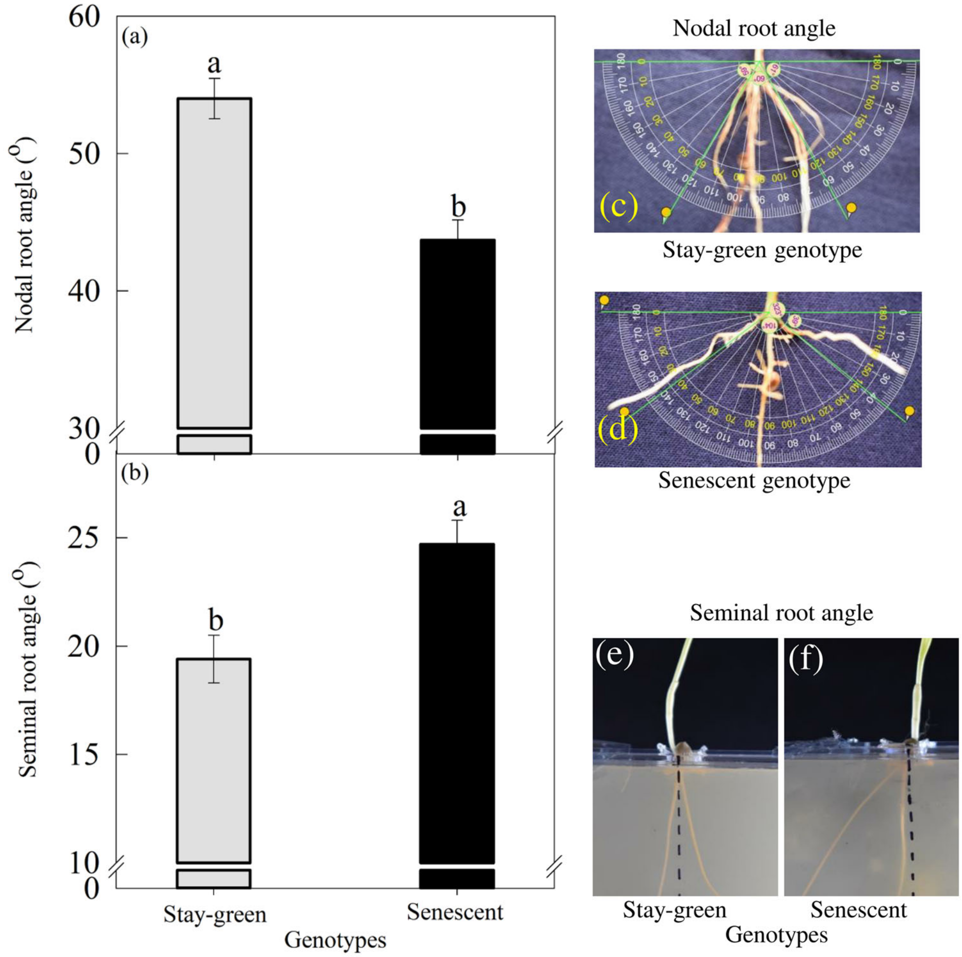

FIGURE 2 Variation in (a) nodal and (b) seminal root angle (°) of stay-green (B35 and 296B) and senescent (R16 and BTx623) sorghum genotypes. (c) and (d) are representative images of nodal root angle, and (e) and (f) are representative images of seminal root angle of stay-green and senescent genotypes. Each datum is the mean ± SEM of 10 observations (two genotypes and five replications). Within a column, values not sharing a common lowercase letter are significantly different at $p < 0.05$.

of time of day and genotypes (Figure 5a,b). The chlorophyll index (SPAD units; Figure 6a), PS II quantum yield (F_v/F_m ratio [relative units]; Figure 6b), performance index (PI_{ABS}; Figure 6c), photosynthetic rate (μmol m^{-2} s^{-1}; Figure 6d), stomatal conductance (mol m^{-2} s^{-1}; Figure 6e), and transpiration rate (mmol m^{-2} s^{-1}; Figure 6f) varied significantly ($p < 0.05$) for genotypes, days of drought stress, and their interactions.

The chlorophyll index, PS II quantum yield, performance index, and gas exchange parameters were significantly ($p < 0.05$) decreased by increasing the duration of soil drying. Across the days of observation, stay-green genotypes had a reduced transpiration rate than the senescent genotype (Figure 5a,b).

Under progressive soil drying, in stay-green genotypes, on days 1 and 2, the transpiration rate decreased from 12:20 hours. However, in the senescent genotype, it dropped between 12:45 hours. Similar early partial closure of stomata (1 h) was observed in the stay-green genotypes on days 3 and 4 compared to senescent genotypes (10:45 hours vs. 11:45 hours). Considering the response of the transpiration rate to naturally occurring VPD, there was no apparent difference between stay-green and senescent genotypes because both genotypes had a breakpoint in the transpiration rate under high VPD conditions (Figure 5a,b).

On average, under drought stress, between the genotypes, the stay-green genotypes had a significantly ($p < 0.05$) higher chlorophyll index (8%), PS II quantum yield (3%), performance index (23%), photosynthetic rate (26%), and stomatal conductance (20%) than senescent genotypes. However, the transpiration rate was decreased by 18% in stay-green genotypes than in senescent genotypes (Figure 6).

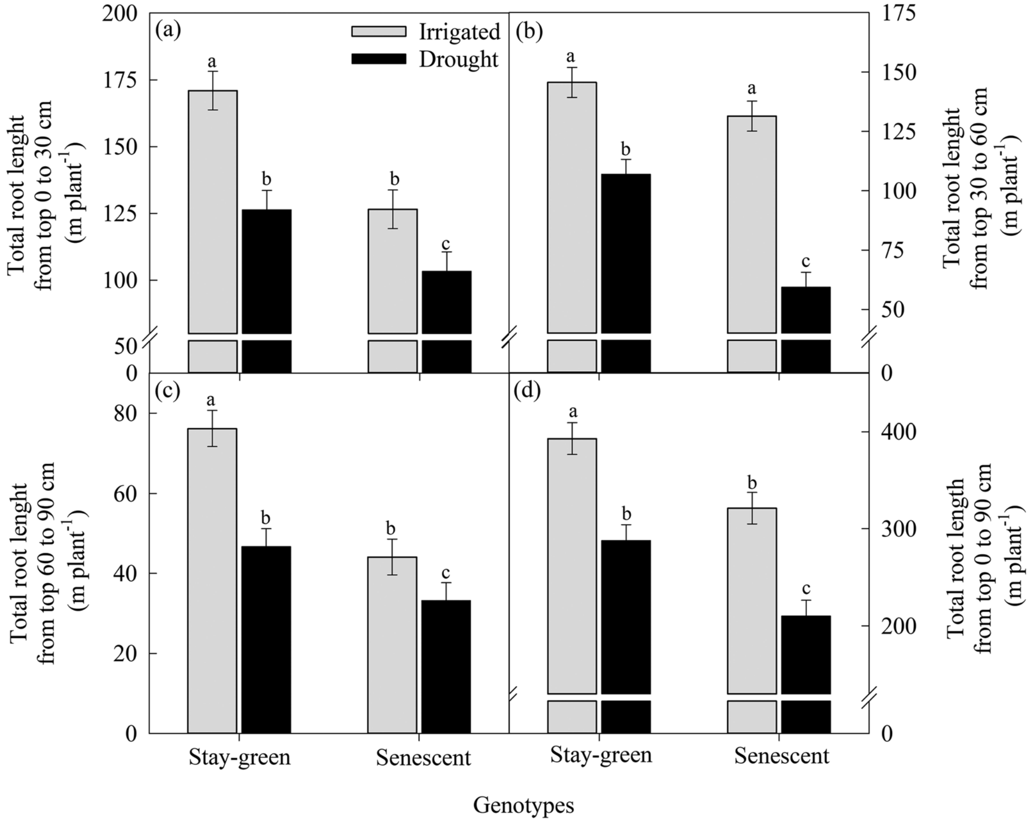

FIGURE 3 Interaction effects of irrigation regime (irrigated: 0.9 fraction of transpirable soil water [FTSW] and drought: 0.4 FTSW) and genotypes (stay-green [B35 and 296B] and senescent [R16 and BTx623]) on (a) total root length from top 0 to 30 cm (m plant^{-1}), (b) total root length from top 30 to 60 cm (m plant^{-1}), (c) total root length from top 60 to 90 cm (m plant^{-1}), and (d) total root length from top 0 to 90 cm (m plant^{-1}). The drought stress was imposed at the peak vegetative stage (35–40 days after emergence [DAE]; five leaf stages) for 5 days. Each datum is the mean ± SEM of six observations (two genotypes and three replications). Within a column, values not sharing a common lowercase letter are significantly different at $p < 0.05$.

3.4 | Response of stay-green and senescent sorghum genotypes to drought stress from the start of the seed development stage to the milky stage: Leaf physiological and yield traits

The effect of genotypes, moisture regime, and interaction of genotype, and moisture regime was significant ($p < 0.05$) for PS II quantum yield (F_v/F_m ratio [relative units]; Figure 7c), stomatal conductance (mol m^{-2} s^{-1}; Figure 7e), photosynthetic rate (μmol m^{-2} s^{-1}; Figure 7d), transpiration rate (mmol m^{-2} s^{-1}; Figure 7f), individual seed size (mg seed^{-1}; Figure 7h), seed yield (g plant^{-1}; Figure 7i), and harvest index (%; Figure 7j). In contrast, the chlorophyll index (SPAD units; Figure 7a), green leaf area (cm^2 plant^{-1}; Figure 7b), and seed-set percent (Figure 7g) were significant ($p < 0.05$) for genotypes.

Similar to progressive soil drying, drought stress at the seed development stage significantly ($p < 0.05$) decreased the PS II quantum yield (18%), photosynthetic rate (22%), stomatal conductance (30%), transpiration rate (26%), individual seed size (28%), seed yield (27%), and harvest index (22%) compared to irrigated conditions (Figure 7). In contrast, under drought stress, the stay-green genotypes had an increased PS II quantum yield (19%), photosynthetic rate (25%), stomatal conductance (47%), transpiration rate (42%), individual seed size (56%), seed yield (43%), and harvest index (32%) than the senescent genotypes.

3.5 | Response of stay-green and senescent sorghum genotypes to drought stress from the seed-set to dough stage: Leaf physiological and yield traits

The effect of genotypes, moisture regime, and interaction of genotype and moisture regime was significant ($p < 0.05$) for green leaf area (cm^2 plant^{-1}; Figure 8b), total biomass (g plant^{-1}; Figure 8c), photosynthetic rate (μmol m^{-2} s^{-1};

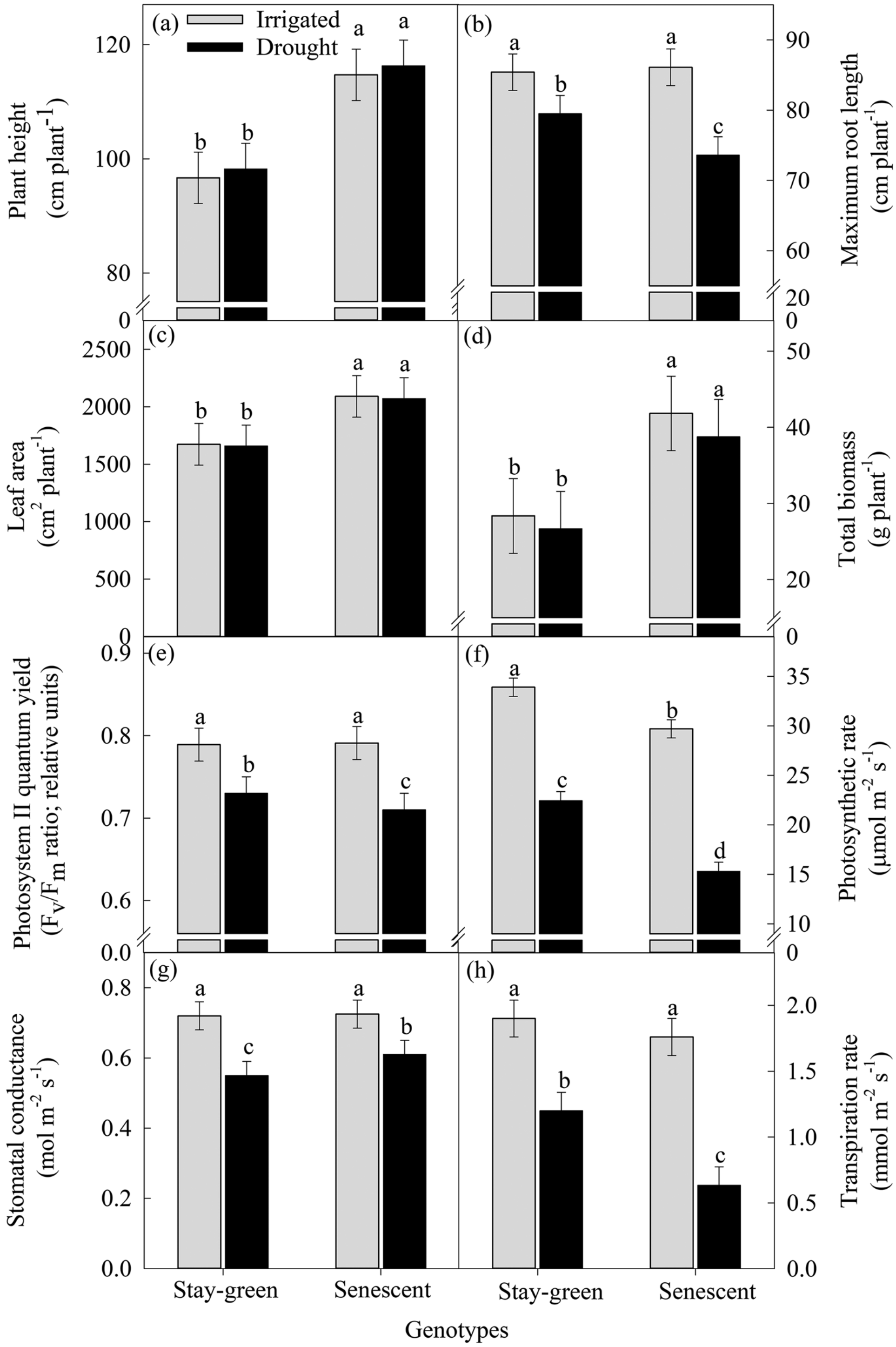

FIGURE 4 Interaction effects of irrigation regime (irrigated: 0.9 fraction of transpirable soil water [FTSW] and drought: 0.4 FTSW) and genotypes (stay-green [B35 and 296B] and senescent [R16 and BTx623]) on (a) plant height (cm plant^{-1}), (b) maximum root length (cm plant^{-1}), (c) leaf area (cm^2 plant^{-1}), (d) total biomass (g plant^{-1}), (e) photosystem II quantum yield (F_v/F_m ratio; relative units), (f) photosynthetic rate (µmol m^{-2} s^{-1}), (g) stomatal conductance (mol m^{-2} s^{-1}), and (h) transpiration rate (mmol m^{-2} s^{-1}). The drought stress was imposed at peak the vegetative stage (35–40 days after emergence [DAE]; five leaf stages) for 5 days. Each datum is the mean ± SEM of six observations (two genotypes and three replications). Within a column, values not sharing a common lowercase letter are significantly different at $p < 0.05$.

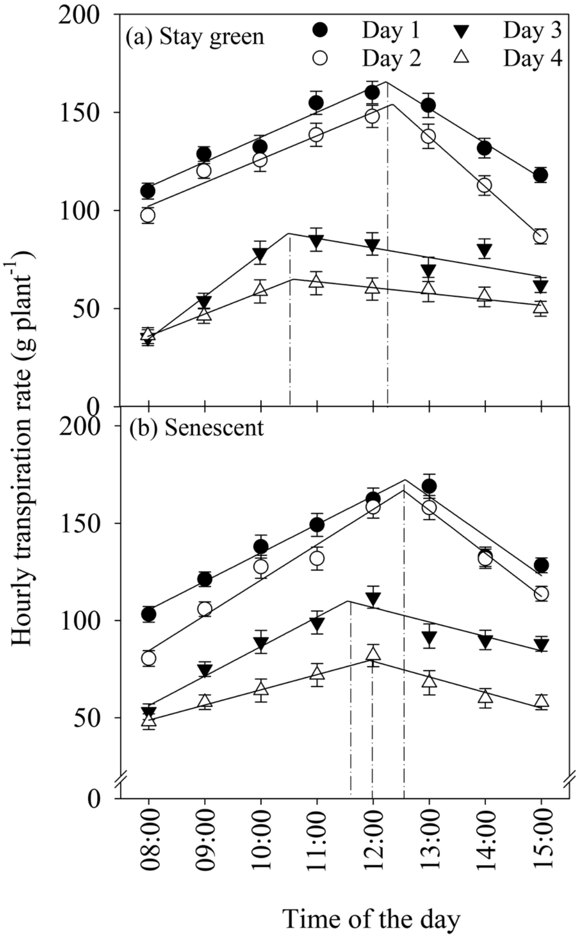

FIGURE 5 Effects of progressive soil drying on hourly transpiration rate (g $plant^{-1}$) in (a) stay-green (B35 and 296B) and (b) senescent (R16 and BTx623) genotypes. The drought stress was imposed at the booting stage for 4 days. Each datum is the mean ± SEM of 10 observations (two genotypes and five replications). Vertical dashed lines represent the time at which the breakpoint occurs.

Figure 8d), stomatal conductance (mol m^{-2} s^{-1}; Figure 8e), transpiration rate (mmol m^{-2} s^{-1}; Figure 8f), individual seed size (mg $seed^{-1}$; Figure 8g), and seed yield (g $plant^{-1}$; Figure 8h). In contrast, the plant height (Figure 8a) was significant ($p < 0.05$) for genotypes.

Similar to progressive soil drying and seed development to milky stage results, drought stress from the seed-set to dough stage significantly ($p < 0.05$) reduced the traits associated with leaf photosynthesis, namely, photosynthetic rate (36%), and stomatal conductance (29%), transpiration rate, and yield traits, namely, total biomass (42%), individual seed size (42%), and seed yield (59%) in comparison with irrigated conditions. In contrast, under drought stress, the stay-green genotypes had an increased photosynthetic rate (31%), individual seed size (33%), and seed yield (31%) in comparison with senescent genotypes.

3.6 | Analysis of the relationship between root length and physiological traits

The result indicated that the photosynthetic rate ($r^2 = 0.43$***), chlorophyll index ($r^2 = 0.52$***), and seed yield ($r^2 = 0.50$***) were positively and significantly correlated with the total root length at the top 0–30 cm of soil (Figure 9i,e,a). A similar positive and significant relationship was observed for the above physiological traits with total root length at the top 30–60 cm of soil and total root length at 0–90 cm (Figure 9b,f,j,d,h,l). A positive linear relationship was observed between the photosynthetic rate and total root length at the top 60–90 cm of soil (Figure 9k).

4 | DISCUSSION

The main findings under drought stress are as follows: (i) stay-green genotypes had deep and prolific root systems and reduced transpiration rate; in contrast, the senescent genotypes produced a moderate and less prolific root system and increased transpiration rate; (ii) conservation of soil water through reduced stomatal conductance coupled with increased root length increased the photosynthetic rate and grain yield of sorghum; (iii) the decrease in photosynthetic rate was linked with both stomatal and non-stomatal limitations; and (iv) drought stress at all growth stages had an increased grain yield in stay-green genotypes than senescent genotypes.

This study showed significant variations among 38 sorghum genotypes assessed for their stay-green score, seed-set percent, seed size, and seed yield under drought stress, thereby supporting our hypothesis that there is an amble genetic variability for the seed-set, seed size, and seed yield in sorghum germplasm for breeding drought stress tolerance varieties. This study also showed that the correlation between the seed-set percent and seed yield ($r = 0.43$) was higher than the chlorophyll index and seed yield ($r = 0.41$) and seed size and grain yield ($r = 0.27$), indicating that grain yield under reproductive stages (from booting stage to maturity) and drought stress can be improved through targeted breeding for the seed-set percent in stay-green genotypes, and carbohydrate translocation efficacy from source to sink (Borrell & Hammer, 2000; Fu et al., 2009). Among the genotypes, B35 and 296B were identified as stay-green genotypes and R16 as senescent genotypes.

The root traits determine the crop plant's drought tolerance ability (Sharma et al., 2010). The stay-green genotypes had a narrow seminal and wider nodal root angle compared to senescent genotypes (Figure 2). As explained in the previous studies, the genotype with a narrow seminal root angle had a deeper root system and was drought tolerant (Djanaguiraman et al., 2019; Manschadi et al., 2006; Uga

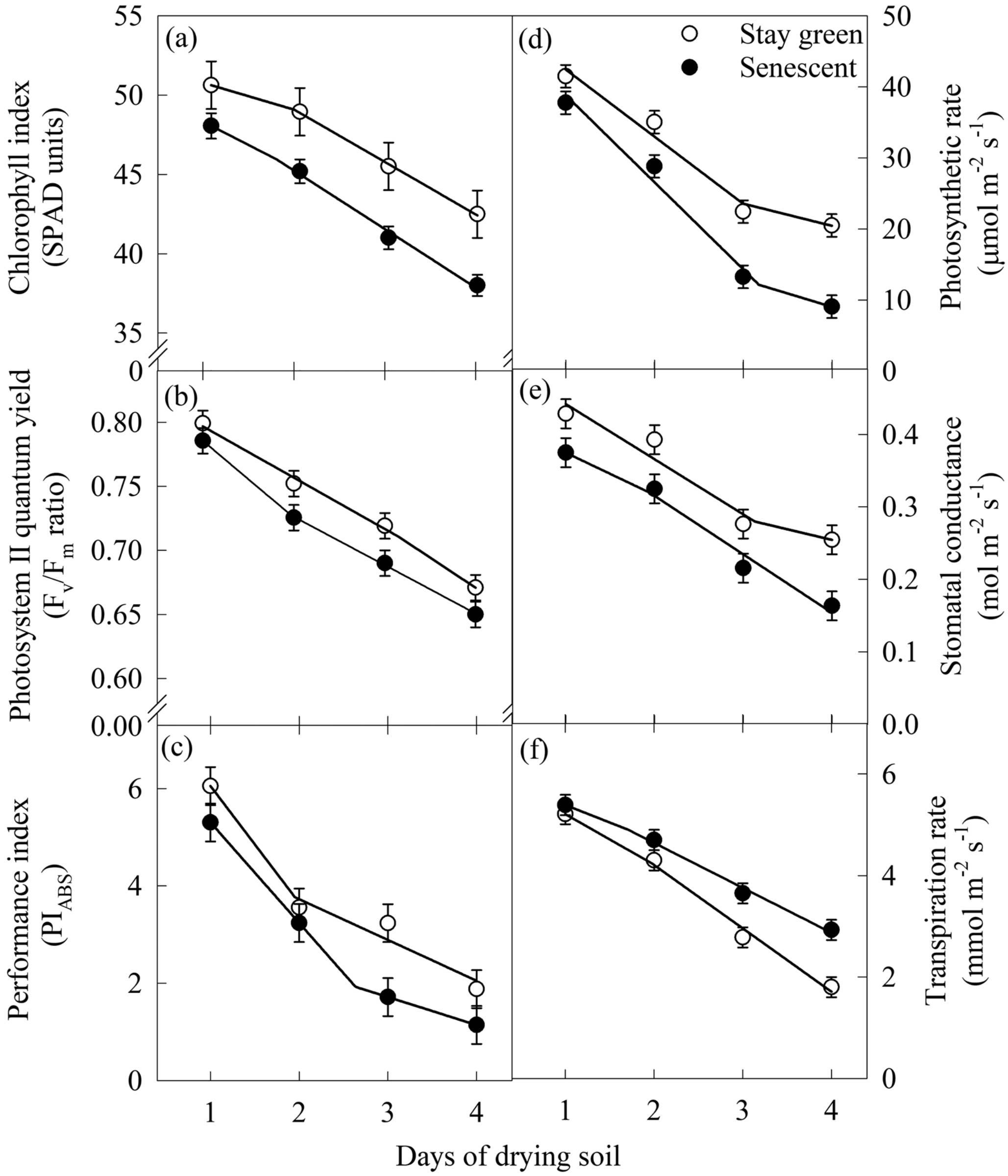

FIGURE 6 Effects of progressive soil drying on (a) chlorophyll index (soil plant analytical division [SPAD] units), (b) photosystem II quantum yield (F_v/F_m ratio; unitless), (c) performance index (PI_{ABS}), (d) photosynthetic rate (µmol m^{-2} s^{-1}), (e) stomatal conductance (mol m^{-2} s^{-1}), and (f) transpiration rate (mmol m^{-2} s^{-1}) in stay-green (B35 and 296B) and senescent (R16 and BTx623) genotypes. The drought stress was imposed at the booting stage for 4 days. Each datum is the mean ± SEM of 10 observations (two genotypes and five replications).

et al., 2013; Williams et al., 2022). Similarly, in the stay-green barley (*Hordeum vulgare* L.) genotypes, the seed yield was positively associated with a deep root system, not total root length under drought stress (Williams et al., 2022). In contrast, in wheat (*Triticum aestivum* L.), the seed yield is positively associated with the total root length (Djanaguiraman et al., 2019; Manschadi et al., 2006; Williams et al., 2022). In line with this, Uga et al. (2013) also showed that *DRO1*, an allele associated with a deep and prolific root system in rice (*Oryza sativa* L.), had increased the seed yield under drought stress. The cereal root lacks vascular cambium, so the plant needs increased root length for water absorption and transport (Rich & Watt, 2013; Vadez et al., 2013). This study also showed a positive linear significant relationship between total root length and seed yield (r^2 = 0.52, Figure 9d). The results of the study should be interpreted with caution because deep and prolific root systems will extract more water (Metselaar et al., 2019; Passioura, 1983), which may also lead to the exhaustion of soil water before or at critical growth stages (Palta et al., 2011). However, in this study, the stay-green genotypes had an increased grain yield under drought stress (Figures 7 and 8) compared to the senescent genotype, indicating that water uptake under drought through deep and prolific root systems was at the

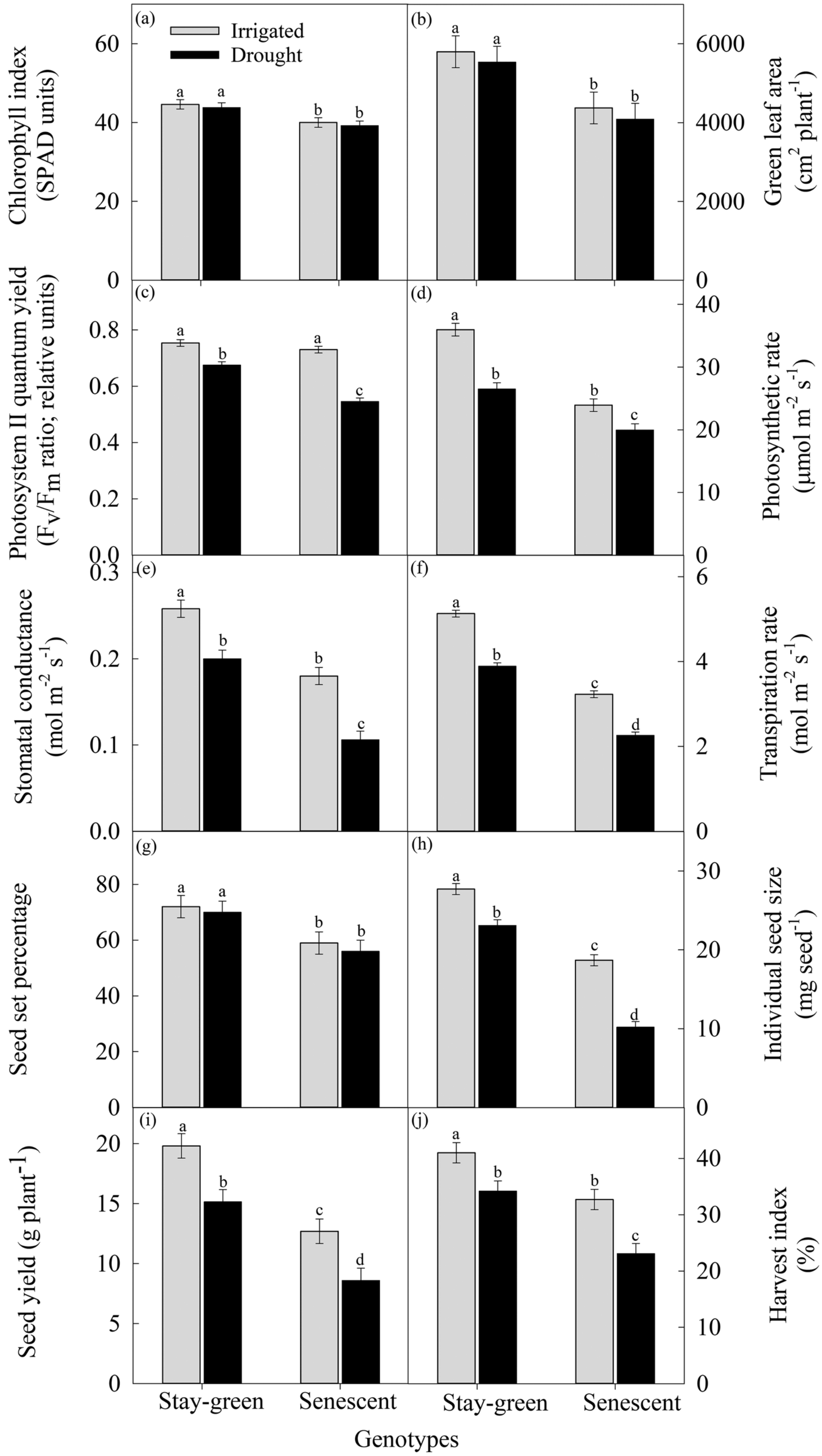

FIGURE 7 Interaction effects of irrigation regime (irrigated: irrigated once in 2 days and drought: water withheld for 12 days from start of seed development stage [7 days after flowering stage]) and genotypes (stay-green [B35 and 296B] and senescent [R16 and BTx623]) on (a) chlorophyll index (soil plant analytical division [SPAD] units), (b) green leaf area (cm^2 $plant^{-1}$), (c) photosystem II quantum yield (F_v/F_m ratio; unitless), (d) photosynthetic rate (µmol m^{-2} s^{-1}), (e) stomatal conductance (mol m^{-2} s^{-1}), (f) transpiration rate (mmol m^{-2} s^{-1}), (g) seed-set percent (h) individual seed size (mg $seed^{-1}$), (i) seed yield (g $plant^{-1}$), and (j) harvest index (%). Each datum is the mean ± SEM of 10 observations (two genotypes and five replications). Within a column, values not sharing a common lowercase letter are significantly different at $p < 0.05$.

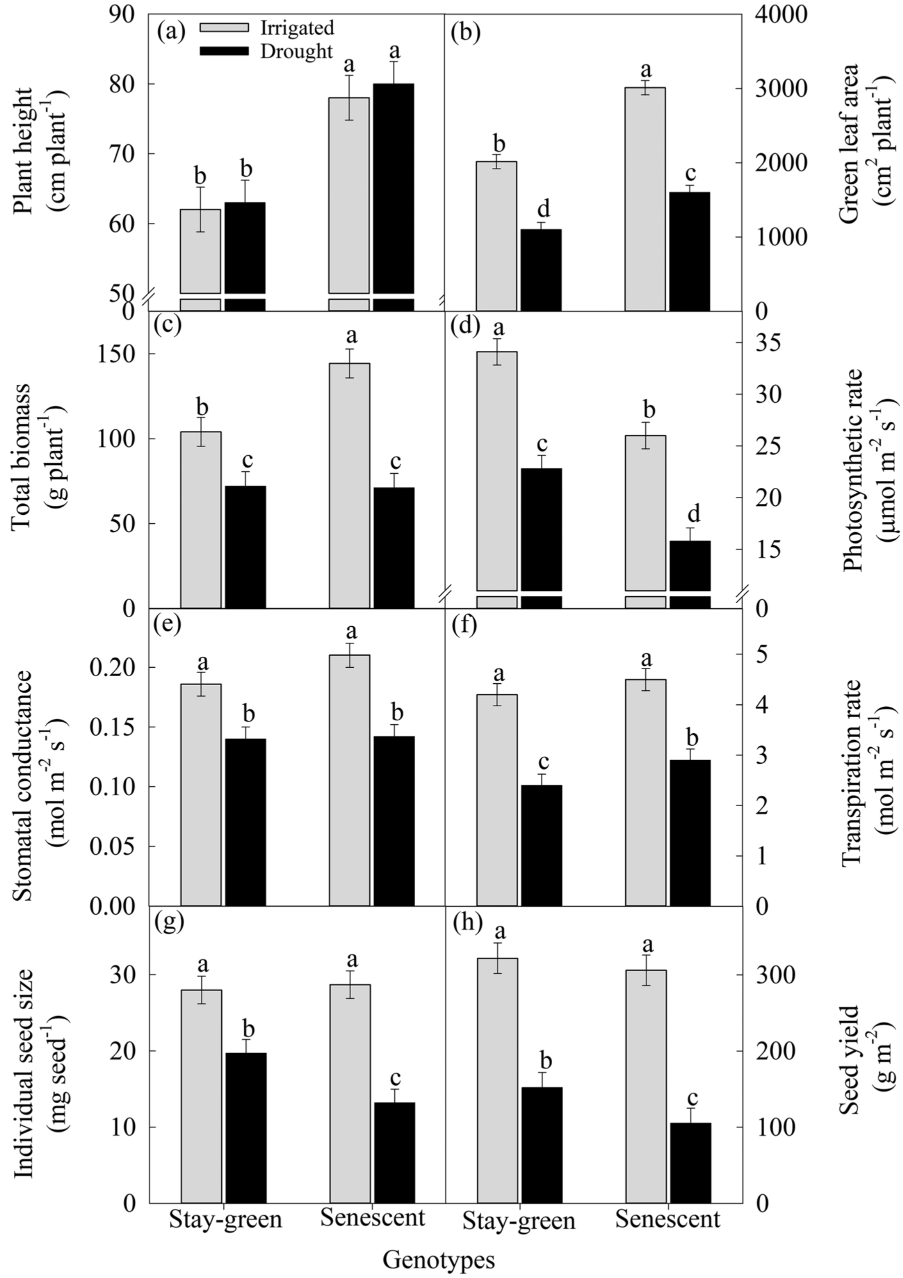

FIGURE 8 Interaction effects of irrigation regime (irrigated: irrigated once in 7 days and drought: water withheld for 25 days from 5 days after flowering stage) and genotypes (stay-green [B35 and 296B] and senescent [R16 and BTx623]) on (a) plant height (cm plant^{-1}), (b) green leaf area (cm^2 plant^{-1}), (c) total biomass (g plant^{-1}), (d) photosynthetic rate (µmol m^{-2} s^{-1}), (e) stomatal conductance (mol m^{-2} s^{-1}), (f) transpiration rate (mmol m^{-2} s^{-1}), (g) individual seed size (mg seed^{-1}), and (h) seed yield (g m^{-2}). Each datum is the mean ± SEM of six observations (two genotypes and three replications). Within a column, values not sharing a common lowercase letter are significantly different at $p < 0.05$.

critical growth stage, namely, seed development (Rathod et al., 2022).

The driving force for transpiration is the VPD differences between the leaf and the surrounding atmosphere (Kramer, 1983). Our previous study indicated that the FTSW reached a value of 0.3 on the fourth day of progressive soil drying. At 0.3 FTSW, the photosynthetic rate and stomatal conductance were decreased by 70% and 81%, respectively, from the initial value (Gowsiga et al., 2021). Apart from this, Gano et al. (2021) reported that in sorghum, at 0.3 FTSW, the plants experienced drought stress, as revealed by leaf rolling. Under progressive soil drying, across the day of stress, the stay-green genotypes had a decreased transpiration rate (9%) than the senescent genotypes (Figure 5a,b), and both the genotypes had a breakpoint in the transpiration rate in response to naturally occurring VPD (Figure 5a,b), indicating that the response of VPD on the transpiration rate in both genotypes are similar. However, the stay-green genotypes exhibited an

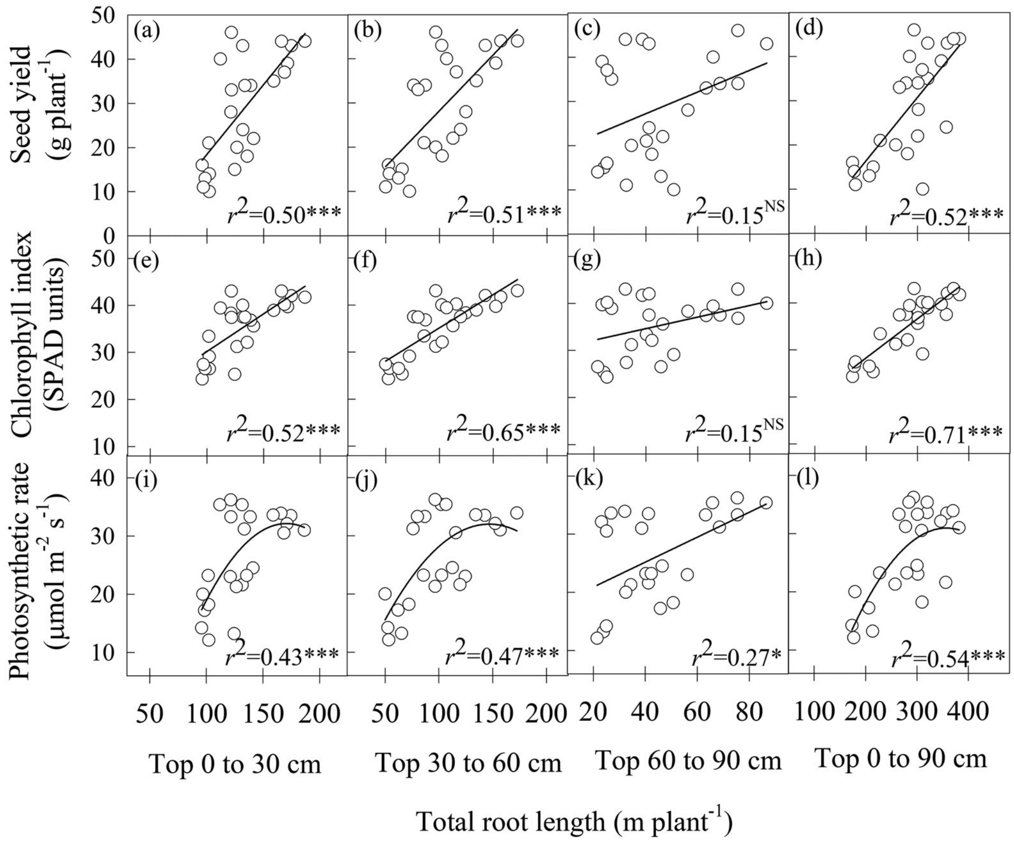

FIGURE 9 Relationship between total root length from top 0 to 30 cm (m plant^{-1}) and seed yield (g plant^{-1}), chlorophyll index (soil plant analytical division [SPAD] units), and photosynthetic rate (μmol m^{-2} s^{-1}) (a, e, and i), from top 30 to 60 cm and seed yield , chlorophyll index , and photosynthetic rate (b, f, and j) from top 60 to 90 cm and seed yield , chlorophyll index , and photosynthetic rate (c, g, and k), and from 0 to 90 cm and seed yield , chlorophyll index and photosynthetic rate (d, h, and l) in sorghum under irrigated and drought stress conditions. *$p < 0.05$; ***$p < 0.001$; NS, nonsignificant.

early breakpoint in the transpiration rate compared to senescent genotypes indicating an early partial (~1 h) closure of stomata during the high VPD conditions, thereby saving soil moisture for later growth stages (R. A. Richards & Passioura, 1989; Sinclair et al., 2005, 2017). In maize, the significance of the limited transpiration trait under drought stress was proved by AQUAmax hybrids (Gaffney et al., 2015); in this hybrid, the limited transpiration trait alone has increased the grain yield from 22 to 53 g m^{-2}.

The decrease in chlorophyll content and F_v/F_m ratio under drought may be related to ultrastructural damage to the chloroplast (Zhang et al., 2020) and photoinhibition (Huang et al., 2013), respectively. Across the experiments, stay-green genotypes had a higher value of chlorophyll content (15%), F_v/F_m ratio (18%), and PI (23%) than senescent genotypes under drought stress, explaining their higher drought tolerance ability than senescent genotypes. The decreased photosynthetic rate under drought stress could be also linked to stomatal and/or non-stomatal factors (Yang et al., 2021). This study showed that short-term drought stress decreased the photosynthetic rate primarily by stomatal inhibition, as evidenced by a higher decrease in stomatal conductance (30%) than F_v/F_m ratio (18%) and PI (23%) (Figure 6). The relationship between the photosynthetic rate and total root length at different soil depths was significant and positive (Figure 9; Shimshi & Ephrat, 1975), indicating that increased root length and prolific roots could support the photosynthetic rate under drought stress by enhanced water uptake from the deeper zone of soil.

5 | CONCLUSIONS

In general, the study discovered that drought stress decreased the total root length at all soil profiles, gas exchange traits, and grain yield in both stay-green and senescent sorghum genotypes. The decrease in the photosynthetic rate under mild drought stress is associated with stomatal limitations. A deep and prolific root system and decreased transpiration rate under drought stress in stay-green genotypes have increased its drought tolerance ability. In contrast, the senescent sorghum genotypes had a moderate root system and increased transpiration rate under drought stress, which resulted in the exhaustion of soil water, and thus became drought sensitive. Compared to senescent genotypes at all the reproductive growth stages, stay-green genotypes had an increased grain yield by 43% under drought stress, through an increased individual seed size (35%). This study warrants further investigation on stay-green germplasm, parents, and varieties for systematic genetic and genomic aspects to identify genic markers for accelerated breeding and release of drought-tolerant sorghum varieties and hybrids in semiarid regions of Asia and Sub-Saharan Africa.

AUTHOR CONTRIBUTIONS

Maduraimuthu Djanaguiraman: Conceptualization; data curation; formal analysis; funding acquisition; investigation; project administration; writing—original draft. **Srinivasan Gowsiga**: Formal analysis; investigation. **Mahalingam Govindaraj**: Conceptualization; formal analysis; funding acquisition; writing—review and editing. **Ephrem Habyarimana**: Writing—review and editing. **Alagarswamy Senthil**: Writing—review and editing. **Nallasamy Thavaprakaash**: Writing—review and editing. **Prabhakaran Jeyakumar**: Writing—review and editing. **Jayavel Kokilavani**: Writing—review and editing. **Chellapandiyan Chellammal**: Writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Maduraimuthu Djanaguiraman https://orcid.org/0000-0003-2231-7562
Mahalingam Govindaraj https://orcid.org/0000-0003-0559-4015

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