Uncovering Different Physiological Mechanisms of Peanut Drought Tolerance Under Mid-season Drought in Automated Rain-out Shelters

By

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 11th, 2021

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Abstract

Peanut is an economic cash crop mainly planted in arid and semi-arid regions where the drought causes around 20% loss of peanut production every year. Research suggested that crops have various physiological mechanisms against drought stress, such as reduction of transpiration while maintaining photosynthesis which leads to water use efficiency (WUE), maintain water extraction through a deeper and more complex root system and differences in the partitioning of dry matter to pods. There are few field studies about which physiological characteristics are responsible for peanut drought tolerance in the Southeast United States. To find which physiological characteristics are responsible of mid-season drought tolerance in peanut, a 2-year experiment testing cultivars known by its drought tolerance and sensitiveness was carried out in rain-out shelters. Plants were grown under irrigated conditions and at the beginning of pod filling (70 DAP) the drought treatment started and lasted until 100 DAP, at which time plants were rewatered until harvest. Photosynthetic rate, and specific leaf area were measured 4 times at different development stages in 2019 and measured 7 times in 2020. After harvest, pod yield and HI were collected. ^{13}C and ^{15}N isotope discrimination and N content were measured for pods and shoot biomass. HI, seeds $\Delta^{13}C$, total N in the whole plant, photosynthesis and stomatal conductance were strong and positively correlated with yield under drought. $\delta^{15}N$ was negatively correlated with yield under drought. Different varieties have significant differences in photosynthetic rate, pod yield and carbon isotope discrimination. Moreover, when photosynthesis and stomatal conductance were measured several times during the drought period these parameters had a stronger correlation with yield than $\Delta^{13}C$. In this research, we found two different mechanisms responsible of drought tolerance. The cultivars PI 502120 and AU-17 showed the highest yield under drought due to a higher photosynthesis and stomatal conductance probably due with a higher capacity to extract water, and therefore they were classified as water spender. On the other hand, two other high yield cultivars, AU 16-28 and Line-8, showed relatively high photosynthesis but low stomatal conductance, which means they might have high water use efficiency under drought, and therefore were classified as water savers. In order to improve our understanding of the mechanisms involved in tolerance, a combination of physiological and genomics approaches needs to be applied in the future research.

Key words: Peanut, drought tolerance, photosynthesis, nitrogen fixation, water use efficiency, effective use of water

Acknowledgements

I would like to thank Dr. Charles Y. Chen, Dr. Phat M. Dang, and Dr. William Batchelor for their support professional advice while conducting this experiment. I would like to thank my major professor, Dr. Alvaro Sanz-Saez, for his support, patience, and guidance throughout the completion of this thesis. I am thankful to my family for their support and love.

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CHAPTER I: Literature Review

Origin and importance of peanut

Peanut originated from South America and was brought to North America around 1700s after being introduced into Europe, Asia and Africa (Hammons et al., 2016). Peanut is an important food and oil crop mainly grown in tropic and semi-tropic regions of Asia, Africa and America (Hammons et al., 2016). Peanut is an annual species with indeterminate flowers (Putnam et al., 1991). In U**.**S**.** peanuts are planted around May and harvested after a 120-160 days growing period, usually in September-October.

Peanut (*Arachis Hypogaea L.*) includes two subspecies: *A. hypogaea subsp. Hypogaea* (Virginia, Runner, and hirsuta) and *A. hypogaea subsp. Fastigiate* (Valencia and Spanish). There are four peanut commercial types grown in US, including Runner, Virginia, Spanish, and Valencia. Runner type is the most popular in Georgia, Alabama, Florida, and Mississippi accounting for 80% of peanut production in US and covering 1.3 million acres of planted area in 2020 (USDA, 2020). Runner type peanuts are mainly used for peanut butter and snacks. As the name suggested, runner types occupy more bare ground due to the prostrate or runner growth habit, meaning the cotyledonary laterals grow longer than the main stem. Flowering in lateral branches in runner peanut contributes to higher yields than other types (Coffel, 1974). In this research, we will focus on runner type peanuts due to its importance for Alabama and the U.S. economy. Virginia peanuts are mainly planted in South Carolina, North Carolina, and Virginia, and are famous for their large size seed that is perfect for boiled peanut snacks in stadiums. Virginia peanuts also have a prostrate growth habit similar to runners. On the other hand, Spanish and Valencia peanuts are considered as "bunch" growth, presented on their curved upward branches from the base and relative taller main stem in comparison with runner types. The Spanish and Valencia types are only planted in

Texas and Oklahoma and are known for their red skins and nutty flavor and grown in those areas for its known drought tolerance.

Worldwide, there are around 29.6 million hectares (ha) of crop land planted with peanut and 48 million metric tons of peanut are produced every year. World peanut production is led by China, India, Nigeria, and followed by U.S. (FAOSTAT, 2020). The U.S. with an annual production of around 3 million tons of peanut, has become an indispensable exporting country in the worldwide peanut market (USDA FAS, 2020). In 2020, The U.S. harvested 3 million tons from 673,490 hectares, which averages a yield of 4.25 tons/ha (NASS, 2020). In the U.S., over half of the peanut production is used for peanut butter manufacturing and consumption in the US. Over 500,000 tons of peanut production is exported per year, valued at over $675 million (National Peanut Board, 2020). Alabama is the 3rd largest peanut producer state with 74,866 hectares of planting area and 0.3 million tons of production in 2020 (USDA/NASS, 2020).

Contrary to soybean and corn, in the US, peanut is important as it is dedicated for human consumption. In the US, 56% of the peanut production is used for peanut butter production and the rest is used for snacks and food supplements. In other countries such as China, India and Nigeria, peanut production is mainly used for the extraction of cooking oil (National Peanut Board, 2020). Peanut seed contains 38-56% oil, 28-33% protein, 20% carbohydrates and enriched vitamins and minerals (USDA, Food Composition Database, 2019). Peanut is high in healthy unsaturated fatty acids accounting for 80% content in oleic and linoleic acids (Prasad et al., 2010), effectively preventing heart disease and gallstones when included moderately in the diet (Tsai et al., 2004; Jones et al., 2014). Peanuts are also used as a source of dietary protein when separated from the oil (Singh and Singh, 1991), and it has gradually became an important raw material for food manufacturers to increase the content of protein and mineral elements to effectively alleviate the problem of malnutrition in growing children in developing countries (Asibuo et al., 2008). Peanuts are also used in livestock feed, soap, shampoo, and fuel (Micucci, 2000).

Peanut is an important legume in Southeast agricultural system

In the Southeastern coastal plain of U.S., peanut also plays an important role in agriculture cropping system as a rotation crop with cotton and corn. Experimental results suggests that a peanut-cotton rotation effectively reduce boll weevil (*Anthonomus grandis, Boheman*) and other pests in cotton, increasing yield in both crops (Johnson et al., 2001). The other benefit of rotation with peanuts is the available supplementary nitrogen source released from decomposition of dead legume roots and nodules that can be absorbed as a natural N fertilizer by the next crop (Ta & Faris, 1987; Dubach & Russelle, 1994). A result found in an experiment in China showed that rice yield increased 29-37% when rotated with peanut which improved the N status of the soils (Chu et al., 2004). This is because peanut is capable of fixing atmospheric nitrogen (N_2) by associating with symbiotic soil microorganisms bacteria called rhizobia (Fabra et al., 2010), which allows them to grow in N poor soils as other legume crops (Hartzog & Adams, 1988; Chu et al., 2004; Li et al., 2018).

To establish the symbiosis in peanut plants, peanut roots exudate flavonoids and phenolic acids in to the rhizosphere (Taurian et al., 2008; Fabra et al., 2010; Wang et al., 2021) which attracts rhizobium to infect root hairs making hotspots of microbial activity in the root (Yakov and Evgenia, 2015). Plants and bacteria form specialized organs, called nodules, where rhizobium is housed to fix atmospheric N_2. In return for the N, the rhizobium obtains carbohydrates derived from photosynthesis, like malate that is used as energy source for the bacteria metabolism and to fuel N_2-fixation. Although biologic N_2-fixation (BNF) is a beneficial process for plant and bacteria, it is a very expensive process for plants. It requires high energy by hydrolyzing 20-30 ATP per fixed N_2 molecule (Burrisl and Roberti, 1993) and therefore only occurs with an adequate carbohydrate supply to feed the nitrogenase energy requirements (Carnahan et al., 1960; Mortenson, 1964; Gibson, 1966).

Plant BNF has been proved to be tightly related and regulated by photosynthesis (Lindstrom et al., 1952) due to the dependency of the symbiotic bacteria from the sugars derived from the photosynthesis (Lawn and Brun, 1974). Lawn and Brun (1974) demonstrated that BNF decreased remarkably at the start of pod-filling stage, moment at which the nodules start to compete for photoassimilates coming from the leaves with the filling seed (Lawn and Brun, 1974). However, it has been demonstrated that having a higher BNF rate usually results in higher yields. In some studies of peanut, cultivars KK 60-3, ICGV 98305 (Pimratch et al., 2007, 2012; Wunna et al., 2009), and NC 6 (Arrenddell et al., 1985) fixed more N_2 than other cultivars under well water and drought conditions showing higher yields (Arrenddell et al., 1985; Pimratch et al., 2007, 2012; Wunna et al., 2009).

Needs for increasing yield under abiotic stress conditions

Since the 1960s, the Chinese population has increased from 0.7 billion to 1.4 billion, and the Indian population almost tripled in the last 60 years (MPC, 2015). This increase in population has increase the demand for food supplies and therefore for agricultural production. For example, from 1990-2019**,** China’s net import of soybean products increased from 2 to 91 million tons respectively, and shelled peanuts net imports increased 8 times (FAOSTAT, 2020). Additionally, food and agricultural demand is expected to increase in the future due to an increasing population that could reach 10 billion. By 2050, crop production needs to double with respect of the 2006 records in order to meet the increase in food demand (World Population Prospects, 2019). Although expanding planting area may increase crop production, yield improvement is the more sustainable way to feed the world due to the limitation of available agricultural land (Masuda and Goldsmith, 2009).

Thanks to a better understanding of genetics, improved varieties produced by breeding programs and advanced agricultural management, crop yield has increased significantly in the last century. Nowadays, global yield of corn, rice, wheat, and soybean, have increased a 1.6%, 1%, 0.9%, and 1.3% per year respectively (Ray et al., 2013). For example, newer released soybean cultivars have shown higher yields due to improvements in some physiological and agronomic characteristics such as high light interception efficiency, partitioning efficiency and radiation use efficiency (Koester et al., 2014). In the case of corn, grain yield has experienced two significant changes, which resulted from the introgression of double-cross hybrid corn in 1940s-1960s; and genetic breeding accompanied by advanced agricultural management practice including application of N fertilizer after 1960s (Kucharik and Ramankutty, 2005). For peanut, before the 1930´s, production was increased due to an increase on peanut acreage and a stagnant yield (USDA, 2019; Holbrook, 2019). After that time, application of new fertilizers, herbicides (Warren, 1998), fungicides (Worthing and Hance, 1991), and insecticides, drove production and yield even though the planted area has been shrinking ever since. At the same time, peanut yield potential has been increasing due to new germplasm developed by breeding programs (Smil, 2001; Pimratch et al., 2007). For example in the last century, peanut mean yield has increased from 740 to 4473 kg ha-1 (Duncan et al., 1978, USDA, 2019). Yield potential is the yield produced in ideal growing condition that is not restricted by biotic and abiotic stress. However, record yields and yield potential can be achieved through crop improvement and management practices. The highest overall peanut yield, 8 tons ha^{-1}, had been reported in a small family farm in Georgia in 2017 (Thompson and Editor, 2017).

Peanut yield potential has increased due to improved varieties that have been selected for better agronomic and physiological characteristics (Richards, 1996). For example, in 1970s, breeding programs started to select varieties with prostrated growth habit such as in runner types because of the higher growing rates that resulted in higher yield in comparison with erect growth habit varieties (Warren, 1998; Haro et al., 2013). Another physiological modification that improves yield is the selection for longer growth cycle and flowering time which allow plants to potentially increase the amount of biomass accumulated by photosynthesis and derived it to reproductive organs during seed filling stage increasing pod weigh and yield (Duncan et al., 1978; Richards, 1996; Haro et al., 2013). In peanut, yield increases have been in part achieved by selecting for cultivars that are able to partition more C and thus biomass from vegetative organs such as leaves and stems to pods, therefore selecting for higher harvest index (HI). This has been demonstrated by growing peanut cultivars developed at different times during the 1900´s under the same field management conditions and comparing the yield, total aboveground biomass and harvest index (Duncan et al., 1978; Coffelt et al., 1989; Blum, 2009). In these experiments the authors found that newer cultivars showed higher yields due to a higher biomass accumulation that was accompanied by a higher HI (Duncan et al., 1978; Coffelt et al., 1989; Blum, 2009). Although the increases in peanut yield are promising with a 0.05% per year (USDA, 2019); and could meet theoretically meet the United Nations demand for 2050 (Ray et al., 2013), yield will be negatively affected by abiotic stresses such as drought that will be intensified in the next century due to effects of climate change (IPCC, 2007).

Effect of drought on crop yield and economic loss

Drought stress is the most universal abiotic stress and easily triggers other stresses such as high temperature, decreasing significantly crop productivity (Mittler, 2006; Hamidou et al., 2013).

Plants have strong responses to drought, such as stunting plant growth by suppressing cell elongation and cell division (Farooq et al., 2009; Yadav et al., 2020). Substantial reduction in stomatal conductance and photosynthesis which largely decrease the carbohydrate sources for plants (Lawlor and Cornic, 2002; Flexas et al., 2004), and also increase night respiration which aggravates the loss of C (Mittler, 2006; Mutava et al., 2015; Fahad et al., 2017). The drought of Summer 2012 was the most severe drought recorded in recent years and caused $18 billion on crop losses (Schnoor, 2012) including a production loss of 52% for corn, 51% for sorghum and 30% for cotton (Lal et al., 2012). When the yield of 2012 is compared with the USDA database from years before, yield of corn, sorghum, and soybean was reduced 26%, 24%, and 9%, respectively due to drought (Schnoor, 2012).

Drought is one of the top two abiotic stresses for peanut in the world. Due to erratic and insufficient rainfall during the growing season in semitropic regions, the yield of peanut fluctuates significantly with the duration, frequency, capacity of rainfall and stages of growth (Wright et al., 1991; Shin et al., 2010a). In India, Hamidou et al. (2013) studied the yield response to drought of 268 genotypes and found that drought reduced pod yield by 55%. In Southeastern US, Shin et al. (Shin et al., 2010a) demonstrated that drought imposed by lack of seasonal precipitation during the years 1911-2006 reduced peanut yield by 43% (Shin et al., 2010a).

Societal, environmental, and economic factors are increasingly causing restrictions on water resources available for irrigation. Considering that fresh water is a limited resource, and that 80% of global human water use is related to agricultural production (Chapagain and Hoekstra, 2004), crops that have high yield per unit of water supply, also called water use efficiency, are desirable. Peanut is such a crop, since tree nuts require between 15-25 times more water to produce the same yield as peanut (Mekonnen and Hoekstra, 2011). However, the U.S. peanut industry still suffers $50 million of annual losses associated with drought (USDA-ARS, 2019). Much of this loss is due to extended drought in non-irrigated (rainfed) fields. Indeed, the lack of irrigation systems further increases the critical need for drought tolerance breeding, since dryland cropping is common throughout the Southeast U.S, accounting for 65% of the planted area, with Alabama having a 90% of the peanut growing under rainfed conditions. In regions like the Southwestern U.S., where peanut crop irrigation is historically common, competition for water resources has led to increased adoption of dryland peanut production (Steward et al., 2013). Both the current cost to production and continuing use of dryland cropping systems demonstrates that peanut production will dramatically benefit by breeding varieties that yield well under dry conditions.

Physiological effects of drought on crop plants and peanut

Drought stress negatively influences plant growth, photosynthesis, metabolisms, and yield of peanut (Jeyaramraja and Thushara, 2013). Water deficiency restricts the leaf elongation rate due to reduced cell division rate due a drop in cell turgor (Avramova et al., 2015), subsequently decreasing growth and development of the plant.

Yield reduction caused by drought stress, is in part caused by the negative effects of drought over the photosynthesis of the plant which is the process that obtains the energy and C for growth. Photosynthesis occurs in the chloroplast of leaves, with the light reactions located in the thylakoid membrane and the Calvin cycle in the stroma of the chloroplast. In the light reactions, the photons of the light captured by chlorophyll give energy to the oxygen evolving complex in the photosystem II (PSII) that uses that energy to perform the photolysis of water. In this reaction the energy of light breaks the water to H^+ and O_2 and donates the electrons to the electron transport chain. Electrons flow through PSII and PSI until the protein ferredoxin reductase donates the electrons to form NADPH that is used later as reductor power to form carbohydrates in the Calvin

Cycle. A decrease in the amount of water in the plant implies a reduction of the photolysis of water and therefore the amount of electrons that are available to form NADPH and thus sugars (Ferreyra et al., 2003; Chaves et al., 2009; Zlatev and Lidon, 2012).

In the case of moderate drought, before the reduction in the photolysis of water happens, some plants that are not very water use efficient continue to uptake the limited water that is available in the soil and use it for transpiration and photosynthesis (Al-Khafaf et al., 1978; Bates and Hall, 1981). This happens until the soil water content is reduced to around 20% (Ritchie, 1973; Meyer and Green, 1980), the moment at which the stomata shut down completely limiting the amount of CO_2 that reach the chloroplast and reducing photosynthesis (Bates and Hall, 1981; Reggelbrugge, 1997; Devi et al., 2010). As CO_2 is less available in the plant, the NADPH that would be used in the CO_2 fixation is accumulated, reducing the amount of $NADP^+$, its precursor, that is the receptor of the electrons coming from the chlorophylls. Although the photolysis of water and the $NADP^+$ are reduced under drought conditions that does not mean that the photons from the light stop giving energy to the thylakoids. Under well water conditions the energy of these photons is used for the photolysis of water and to reduce $NADP^+$ to NADPH. However, under drought, the electrons that would be used for the photolysis of water or for the reduction of $NADP^+$ are derived to alternative electron acceptors such as oxygen and water generating superoxide anion ($O_2{\bullet}^{-}$) and water peroxide (H_2O_2) respectively, also called reactive oxygen species (ROS) (Asada, 1999; Apel and Hirt, 2004; Akcay et al., 2010; Laxa et al., 2019). Reactive oxygen species have a very oxidative capacity being able to disrupt the delicate balance of redox-regulating function (Avramova et al., 2015), and proline and abscisic acid (ABA) signaling which results on leaf premature senescence (Reddy et al., 2004; Zhao et al., 2016). Accumulation of ROS due to drought has been related with lipid peroxidation that results in membrane damage, instability and leakage as 50% of the thylakoid membrane is composed by lipids (Quinn and Williams, 1983; Lauriano et al., 2000; Quilambo, 2004). As the thylakoid membrane is where all the proteins and enzymes of the electron transport chain have their anchorage, the damage by ROS results on damage in the b_6 *f* complex (Wu et al., 2013; van Eerden et al., 2015), ATP synthase (Staehelin and van der Staay, 1996), and effectiveness of PSII and PSI (Fujii et al., 2014; Kobayashi, 2016) which reduces the overall efficiency of photosynthesis. Stomatal closure due to drought and the following reduction of CO_2 concentration near the active site of the enzyme rubisco, are going to cause an indirect reduction of photosynthesis, as under these circumstances Rubisco is going to fix oxygen instead of CO_2 increasing the photorespiration of the plant (Apel and Hirt, 2004). In photorespiration, oxygen is attached to RuBP forming a molecule of glycolate and H_2O_2 which has to be detoxified in the peroxisomes, process that is very expensive energetically and reduce the efficiency of the photosynthesis (Apel and Hirt, 2004).

In peanut, physiological impacts on drought also include disordered water potential in leaf, disturbed osmosis potential and relative water content (Clavel et al., 2005; Yadav et al., 2020), damage of integrity of cell membranes and therefore the electron transport chain (Percival and Sheriffs, 2002; Clavel et al., 2005) also reduces the amount of chlorophyll in the leaves (Earl and Davis, 2003). As drought affects photosynthesis it will also decrease directly biomass accumulation and peanut yield (Wright and Rao, 1994; Pimratch et al., 2007). However, drought can also reduce peanut pod yield indirectly. For example, droughts harden the topsoil that impedes the pegs of peanuts penetrating the soil and therefore decrease seed-set and subsequent seed number (Sarma and Sivakumar, 2012). Previous research have shown that drought stress during pod filling stage will reduce yield due to less pegs penetrating the soil and due to less pod filling properly due to reduced photosynthesis and C partitioning to the seed (Rao et al., 1985).

Drought not only decreases plant growth and yield through a decrease in photosynthesis (M Vanaja, 2015). Drought also decrease BNF (Pimratch et al., 2007). Nitrogen fixation is more vulnerable to drought than photosynthesis or dry matter accumulation, probably due to a change in the permeability of O_2 in the nodule, and to reduction in the export of the fixed N_2 in the nodule that is accumulated in form of aminoacids and inhibits BNF (Thomas et al., 2004; Peoples et al., 1986; Parsons and Sunley, 2001; Vessey et al., 2005). Soil dehydration has also been shown to reduce nitrogenase activity (Pimratch et al., 2008a) and nodule formation (Vessey et al., 2005) resulting in less N supply for plants which is a high demand element for growth. Selection of genotypes with better BNF is one target to improve yield under drought for breeding programs (Sinclair et al., 2007; Devi et al., 2013).

Peanuts shows different water needs at different developmental stages, being the reproductive stages, between beginning of flowering to pod filling, the stages with more water consumption due to a more dense canopy, and its higher water consumption to maintain an increasing photosynthesis to fill the pods (Stansell and Pallas, 1985; Rowland et al., 2012a). In the Southeastern U.S. it is very common to have drought periods during the months of July and August, also called "mid-season drought" that coincides with the flowering and pod filling period (Climate United States, 2021). Previous studies have showed that mid-season drought can result in yield decreases of at least 30% (Chapman et al., 1993; Carvalho et al., 2017; Rita Valentin et al., 2018). For this reason, in this literature review, our current research we will focus on sources of drought tolerance during mid-season drought events.

Physiological phenotypes for drought tolerance in peanut

Drought tolerance in crops, from the physiological and breeding standpoint, means to maintain acceptable yields under water-limited conditions (Tardieu and Tuberosa, 2010; Sinclair, 2011; Tardieu et al., 2018). High yields under drought conditions have been associated with different physiological mechanisms such as high water use efficiency, effective use of water, nitrogen fixation, photosynthetic activity, root density, biomass accumulation, harvest index, and water potential in other crops such as corn, wheat and soybean (Pimratch et al., 2007; Tardieu and Tuberosa, 2010; Sinclair, 2011; Tardieu et al., 2018; Ye et al., 2018). Although the general physiological mechanisms of drought tolerance for some of these traits have been identified, the introgression of those physiological traits in breeding lines has been scarce (Sinclair et al., 2005; Tuberosa and Salvi, 2006; Passioura, 2010), and is mainly represented by two success stories. Increased wheat yields under terminal drought were achieved by selecting for water use efficiency in Australia (Condon et al., 2002; 2006), and drought tolerance was introduced in soybean by selecting for high BNF under drought (Chen et al., 2007). However, in peanut, physiological traits responsible for drought tolerance have not been studied as deeply as in other crops and have never been purposely introgressed in elite lines.

Historically, drought-tolerant peanuts have been bred by selecting high-yielding varieties under drought stress but without selecting for a specific physiologic tolerance traits (Devi et al., 2010). In addition, the research investigating drought tolerance mechanisms in peanut has been more limited than in other more economically important crops such as corn (Ribaut et al., 2009; Adee et al., 2016), soybean (Sinclair, 2000; King et al., 2014; Iqbal et al., 2019), and wheat (Condon et al., 2002, 2006), reducing the investigated mechanisms to a handful of traits. In this literature review, we will focus our efforts on describing four mechanisms that have been related to drought tolerance in other crops and in peanut and that later will be tested in the field under drought simulator conditions.

1. Water Use Efficiency (WUE) is the amount of yield produced by a crop divided by the amount of water transpired during the whole growing season. Cultivars that detect early the drought and respond to it by reducing transpiration will conserve soil moisture maintaining a low but stable photosynthesis and allowing those cultivars to deliver acceptable yields (Devi et al., 2010; Sinclair, 2011; Vadez and Ratnakumar, 2016; Sinclair et al., 2018). Crop transpiration can be measured by weighing pots and/or installing mini lysimeters in order to calculate the amount of water in the soil that is transpired by the plant, which is very time consuming and very difficult to implement under field conditions (Vadez and Ratnakumar, 2016), and therefore is not easily applicable for breeding and genomic work. Carbon isotope discrimination ($\Delta^{13}C$) has been proven to predict WUE accurately in peanut (Wright et al., 1994), therefore, it could be used to screen for cultivars that are more water-use efficient than others by looking for low values of $\Delta^{13}C$, as has been done in wheat (Condon et al., 2002, 2006) and soybean (Kvien et al., 1986; Zhou et al., 2016; Dhanapal et al., 2015; Buezo et al., 2019). However, in peanut, this approach has only been tested to compare a limited number of commercial lines that did not present a lot of genotypic variation under rain-fed or irrigated conditions (Rowland et al., 2012b). Such a screening would allow us to find and select breeding lines that could be introgressed in breeding programs. In order to differentiate between cultivars that show low $\Delta^{13}C$ (high WUE) due to a complete stomatal closure and therefore low photosynthesis and yield and cultivars with high WUE that maintain acceptable photosynthesis and yield, $\Delta^{13}C$ sampling needs to be complemented with photosynthesis and stomatal conductance measurements. Photosynthesis measurements using gas exchange portable equipment such as the LI-6400 (LI-COR Biosciences, Lincoln, NE) has been demonstrated as a useful tool to measure crop response to abiotic stress (Sanz-Sáez et al., 2017; Pilon et al., 2018; Buezo et al., 2019; Soba et al., 2020).

2. Effective use of water (EUW) is the amount of water that a cultivar can extract from the soil during the entire growing season. In common bean, cultivars that have high effective use of water can maintain transpiration and photosynthesis for more time and result in higher yields under drought (Polania et al., 2016; Sanz-Saez et al., 2019). This mechanism has been detected and easily estimated by measuring the $\Delta^{13}C$ of the biomass and selecting for high $\Delta^{13}C$ (Polania et al., 2016, 2017; Sanz-Saez et al., 2019). The capacity of these EUW cultivars to use more water is directly related to deeper or more abundant roots. White et al. (White et al., 1990) showed that common bean cultivars with high $\Delta^{13}C$ had more root surface area in deeper parts of the soil, showing drought tolerance. This mechanism has not yet been demonstrated in peanut. However, it has been documented in rhizobox and pot experiments that peanut cultivars with deeper or more dense roots in deeper areas of the soil are able to tolerate drought (Kvien et al., 1986; Zhou et al., 2016; Thangthong et al., 2016, 2018, 2019). In addition, it has been observed that drought tolerant cultivars with this characteristic stimulate more root growth when the drought intensity increases (Songsri et al., 2008a).

3. Maintaining high biological N_2-fixation (BNF) under drought has been documented as a tolerant trait for legumes. In fact, using different physiological techniques, crop biologists and breeders have been able to introgress this trait in soybean elite lines that resulted in commercial lines with high BNF and yield under drought (Sinclair, 2000; Chen et al., 2007; King et al., 2014). In peanut, it has been also demonstrated that cultivars that maintain a high BNF are able to accumulate more biomass and also yield (Sinclair et al., 1995; Pimratch et al., 2008b; a, 2010; Devi et al., 2013). However, there has been no reports in the literature in which a sizeable number of peanut cultivars have been screened for BNF and then introgressed in elite lines, in part because determining BNF *in-situ* in the field is very difficult and costly. In soybean and common bean, tolerance of BNF to drought has been screened in diverse populations using the ^{15}N natural abundance method (Hardarson and Danso, 1993) with the objective of finding new breeding lines (Dhanapal et al., 2015; Steketee et al., 2019; Oladzad et al., 2020). In our research we will screen a set of 36 peanut cultivars grown under mid-season drought conditions using the ^{15}N natural abundance method to estimate BNF.

4. Maintaining high biomass accumulation and increased harvest index under drought conditions have been demonstrated to be two good selection traits for drought tolerance (Vadez and Ratnakumar, 2016). The drought tolerant mechanisms listed before may result in an increase in above ground biomass accumulation. However, if the biomass is not properly partitioned into pods due to a low harvest index, the final yield and therefore the consideration of drought tolerance may be tainted (Polania et al., 2016; Vadez and Ratnakumar, 2016). It has been found that more intense partitioning during pod filling results in higher harvest index (HI) and pod yield (Harris et al., 1988). For example, Spanish type peanuts that are known for their higher drought tolerance, accumulate more above ground biomass during the first part of the season where there is not drought stress that later partition to the pods during the drought periods that usually coincides with the pod filling stages (Wright et al., 1991). Previous studies demonstrated that culitvars ICGV 98324, ICGV 98348, Q18801 had better pod yield because they maintained a higher HI in drought (Chapman et al., 1993; Koolachart et al., 2013).

Objectives

During this literature review we have shown that peanut is an important legume crop for the U.S. and the World, and the need for this commodity is going to increase in the following years as a consequence of a growing population. Historical advances in breeding and management in peanut have increased peanut yield to the point that we can meet the demand of a growing population. However, negative effects on plant growth derived from different abiotic stress such as drought will decrease crop yield and pose a threat for food security. In order to keep increasing peanut yield under drought conditions, we need to understand the physiological effects of drought over peanut so we can later breed for drought tolerant cultivars. Drought tolerance can be achieved by many mechanisms. In peanut, four mechanisms have been introduced as the main sources of drought tolerance which are: 1) Increased water use efficiency; 2) Increased water uptake due to a more developed root system; 3) Higher BNF; and 4) Maintaining high biomass accumulation and an increased harvest index under drought conditions. However, there is not any report at which these mechanisms have been tested together in sizable number of cultivars. For these reasons the objectives of these Master Thesis are:

1. Selecting the best drought tolerant peanut cultivars based on their yields and drought indices in rainout shelters.
2. Describe physiological variation in photosynthesis, water use efficiency, water use, nitrogen fixation, and final biomass partitioning between cultivars to differentiate between the 4 above mentioned drought tolerance mechanisms.

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Chapter II: Physiological characteristics of peanut varieties to mid-season drought stress in rain-out shelters

Abstract

Peanut is an economic cash crop mainly planted in arid and semi-arid regions where the drought causes around 20% loss of peanut production every year. Research suggested that crops have various physiological mechanisms against drought stress, such as reduction of photosynthetic rate, closure of stomatal, amelioration of water use efficiency (WUE) and efficient use of water (EUW), and differences in the partitioning of dry matter to pods. There have been few field studies about which physiological characteristics are responsible to drought tolerant traits of peanut in the Southeast United States due to the fact that severe rain events can happen during the season. To study the physiological effects of drought stress on peanuts, a 2-year experiment was carried out in rain-out shelters planting different peanut varieties with different drought tolerance performances. Plants were grown under irrigated conditions until 70 days after plant (DAP), at which the drought treatment started and lasted until 100 DAP. Photosynthetic rate, and specific leaf area were measured 4 times at different development stages in 2019 and measured 7 times in 2020. After harvest, pod yield and HI were collected. ^{13}C and ^{15}N isotope discrimination and N content were measured for pods and shoot biomass. HI, seeds $\Delta^{13}C$, total N in the whole plant, photosynthesis and stomatal conductance were positively correlated with yield under drought. Different varieties have significant differences in photosynthetic rate, pod yield and carbon isotope discrimination. Moreover, when photosynthesis and stomatal conductance were measured several times during the drought period, these parameters were strongly correlated with yield and this correlation was stronger than between $\Delta^{13}C$ and yield. In the current research, there were two drought tolerant mechanisms that influenced peanut drought tolerance. PI 502120 and AU 17 showed highest yield under drought and had a high photosynthesis and high stomatal conductance which means they are cultivars with high water use efficient (EUW) and therefore they were classified as water spenders. On the other hand, the other two high yielding cultivars, AU 16-28 and Line-8, showed relatively high photosynthesis but with low stomatal conductance which means they may be high WUE cultivars, also called water savers. In order to increase our understanding of the mechanisms involved in tolerance, a combination of physiological and genomics approaches need to be applied in the future.

Abbreviations:

LWC Leaf water content (%), **SLA** specific leaf area (mm^2 mg^{-1}), $\mathbf{g_s}$ stomatal conductance, **DAD** (Days After Drought), **BNF** biological N_2-fixation, $\mathbf{\Delta^{13}C}$ carbon isotope discrimination (‰), $\mathbf{\delta^{15}N}$ nitrogen isotope discrimination, **HI** harvest index, **TN** total nitrogen, **EUW** efficient use of water

Key words: Peanut, drought tolerance, photosynthesis, nitrogen fixation, water use efficiency, effective use of water.

Introduction

Peanut (*Arachis Hypogaea* L), also called groundnut, is an important oilseed and food crop as peanut seed contains 38-56% oil, 28-33% protein, 20% carbohydrates and enriched vitamins and minerals (FoodData Central, 2019). In the U.S., more than half of the peanuts are used for peanut butter and snacks, also used for livestock feed, soap, and shampoo et al (Micucci, 2000). Additionally, peanut is a significant legume in Southeast agricultural system as a rotation crop for preventing boll weevil in cotton (Johnson et al., 2001) and supplying available biological fixed nitrogen for the crop in rotated, usually cotton or corn (Ta & Faris, 1987; Dubach & Russelle, 1994).

In 2020, U.S. peanut production was 3 million metric tons, of which 80% of the production was runner type, mostly planted in Georgia, Alabama, Florida, and Mississippi, covering 1.3 million acres of planted area in 2020 (USDA, 2020). For that reason, this research focused on yield performance under drought of runner type peanuts. Although peanut production has increased in the past year, the need of higher peanut production is increasing due to higher demand from increasing population in developing countries (IPCC, 2007; World Population Prospects, 2019; FAOSTAT, 2020). Peanut yield has significantly increased from 740 to 4,473kg ha^{-1} in the last century due to breeding programs and advanced agricultural managements (Duncan et al., 1978, USDA, 2019). Aabiotic stress derived from Global Warming such as more intense droughts, extensively and negatively reduced peanut yield in the past and this trend is expected to continue (Duncan et al., 1978; IPCC AR4 SYR, 2007; USDA, 2019). In the Southeastern US, Shin et al. (2010) demonstrated that drought imposed by lack of seasonal precipitation during the years 1911-2006 reduced peanut yield by 43% (Shin et al., 2010).

Drought is the most important abiotic stress in Alabama, as only 10% of peanut fields are irrigated, and peanuts are especially sensitive to drought periods. Furthermore, mid-season drought is very common in Alabama when irrigation is critical for producing yield during the pod-filling stage (Rao et al., 1985). Therefore, it is crucial to conduct research and breed for drought tolerant cultivars to improve peanut yield under dry conditions, which requires a better understanding of the physiological characteristics that makes plants tolerant to this stress.

Drought stress negatively influences plant growth, photosynthesis, and metabolism, reducing peanut yield (Jeyaramraja and Thushara, 2013). Water deficiency restricts the leaf elongation rate due to reduced cell turgor pressure, subsequently stunting shoot growth of the plant and reducing biomass accumulation (Avramova et al., 2015). Moreover, drought induces ABA accumulation and triggers stomatal closure which limits CO_2 absorption and therefore photosynthesis (Reddy et al., 2004; Zhao et al., 2016). Soil dehydration has also been shown to reduce nitrogenase activity and nodule formation resulting in less N supply from N_2-fixation, which is a high demand element for growth (Vessey et al., 2005; Pimratch et al., 2008a).

Understanding the physiological characteristics that make peanut drought tolerant and finding genotypic variation in these characteristics under drought is a precondition for selection and breeding (Pimratch et al., 2007; Tardieu and Tuberosa, 2010; Sinclair, 2011; Tardieu et al., 2018). Different physiological mechanisms associated with high yield under drought have been reported in other crops, for example high water use efficiency in wheat (Condon et al., 2004; Sinclair, 2011), effective use of water in common bean (Polania et al., 2016; Sanz-Saez et al., 2019), nitrogen fixation in soybean (Sinclair, 2000; Chen et al., 2007) , root depth and complexity (Ye et al., 2018), and high biomass accumulation combined with harvest index, (Pimratch et al., 2007; Tardieu and Tuberosa, 2010; Tardieu et al., 2018).

In peanut, four mechanisms have been introduced as the main sources of drought tolerance : 1) Increased water use efficiency; 2) Increased water uptake due to a more developed root system; 3) Higher BNF; and 4) Maintaining high biomass accumulation and an increased harvest index under drought conditions.

High water use efficiency (WUE) refers to the capacity of some cultivars to accumulate high biomass and yield while having a low transpiration. This is achieved by closing the stomata and decreasing transpiration and maintain a stable photosynthesis that will allow acceptable yields under drought (Devi et al., 2010; Sinclair, 2011; Vadez and Ratnakumar, 2016; Sinclair et al., 2018). The most accurate way to calculate WUE is the use of mini-lysimeters which can measure the amount of water that is transpired by the plant during the whole season (Vadez and Ratnakumar, 2016). However, this method is very labor intensive and therefore limits the number of cultivars that can be analyze. Water use efficiency can be also estimated by measuring the plant's gas exchange and dividing the photosynthesis value with the stomatal conductance, also known as instantaneous water-use efficiency or WUEi (Medrano et al., 2015). However, this measurement is highly variable as it is based on a punctual measurement and does not represent the WUE during the whole plant cycle (Medrano et al., 2015). For these reasons, many researchers have used the carbon isotope discrimination ($\Delta^{13}C$) technique to estimate WUE in many crops (Condon et al., 2002, 2006; Buezo et al., 2019; Sanz-Saez et al., 2019) and also in peanut (Wright et al., 1994). In these experiments, researchers showed that cultivars with lower $\Delta^{13}C$, showed limited transpiration and higher yield under terminal drought conditions (Condon et al., 2002, 2006) .

Effective use of water (EUW), the capacity of plants to absorb and use more water and maintain a high photosynthetic rate and therefore yield, has been demonstrated in common bean

(Polania et al., 2016; Sanz-Saez et al., 2019). In those experiments, cultivars with deeper roots had more access to water resulting in higher photosynthesis and drought tolerance (Polania et al., 2016; Sanz-Saez et al., 2019). The higher access to water can be demonstrated by higher stomatal conductance and $\Delta^{13}C$, that result in higher photosynthesis and biomass accumulation. This type of drought tolerance mechanism has been described only once in peanut for one cultivar (Wright et al., 1994) but not confirmed in a large number of cultivars with $\Delta^{13}C$ and gas exchange measurements.

Maintaining high biological N2-fixation (BNF) under drought has been documented as a tolerant trait for legumes. In fact, using different physiological techniques, crop biologists and breeders have been able to introgress this trait in soybean elite lines with high BNF and yield under drought (Sinclair, 2000; Chen et al., 2007; King et al., 2014). Peanut BNF has been deemed more tolerant to drought than other crops such as soybean and cowpea (Devi et al., 2013), but variations in cultivar responses to drought have been found between commercial and breeding lines resulting in higher yields (Sinclair et al., 1995; Pimratch et al., 2008; Devi et al., 2013). Previous studies have documented some peanuts with high BNF are able to provide high yields in water limited conditions (Sinclair et al., 1995; Pimratch et al., 2008b; a, 2010; Devi et al., 2013). Other than these efforts, there has been no report in the literature in which a large number of peanut cultivars have been screened for BNF. In soybean and common bean, tolerance of BNF to drought has been screened in diverse populations using the ^{15}N natural abundance method (Hardarson and Danso, 1993) with the objective of finding new breeding lines and performing quantitative trait loci (QTL) mapping (Dhanapal et al., 2015; Steketee et al., 2019; Oladzad et al., 2020).

Another well-known drought tolerant mechanism in crops is the combination of high biomass accumulation and high harvest index (Vadez and Ratnakumar, 2016). The drought tolerant mechanisms listed before may result in an increase in shoot biomass accumulation. However, if the biomass is not properly partitioned into pods due to a low harvest index, the final yield and therefore the consideration of drought tolerance may be tainted (Polania et al., 2016; Vadez and Ratnakumar, 2016).

Although there is some literature discussing the effect of these characteristics on peanut yield under drought conditions, there are no reports which test these mechanisms together in a sizable number of cultivars. The objectives of this research were: 1) Selecting the best drought tolerant peanut cultivars based on their yields and drought indices in rainout shelters. 2. Quantify physiological variation in photosynthesis, water use efficiency, water use, nitrogen fixation, and final biomass partitioning between cultivars to differentiate between the four drought tolerance mechanisms.

Materials and Methods

Field Site, Experiment Design, Plant Material, and Crop Management

This experiment was conducted in the rainout shelters facility at the USDA-ARS National Peanut Research Laboratory in Dawson, GA to prevent precipitation on plants during the rain events (Blankenship et al., 1980) of the 2019 and 2020 growing seasons. Each metal shelter covers a ground area of 5.5m ×12.2m and is connected to a rain detector (Agrowtek IR Digital Rain Sensor, Agroetek, Brookfiled, Wisconsin) that automatically closes the shelters when a drop of water touches the sensor. Drought treatments were implemented during July and August to simulate mid-season drought stress coinciding with pod filling stage. The weather data in both years was collected by an on-site weather station by College of Agricultural & Environmental Sciences from University of Georgia and is summarized in Table 1. The soil in the rain-out shelters was Tifton sandy loam (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults).

In 2019, 36 peanut genotypes previously described as drought tolerant and/or sensitive were planted on May 7th in 6 rainout shelters (Table 2). Of those, five shelters were treated as drought stress plots and one shelter was maintained as a well water control. Each cultivar was hand planted in a 4 feet single row separated by a 2 feet alley to avoid contamination between cultivars. In 2020, 18 peanut genotypes previously described as drought tolerant and/or sensitive and selected from the 2019 experiment (except AP-3) were planted on May 12th in four rainout shelters (Table 2). Of those, three shelters were treated as drought plots and one shelter was maintained as a well water control. Each cultivar was hand planted in an 8 feet single row separated by a 2 foot alley to avoid contamination between cultivars.

Each shelter had a set of Water Mark (Irrometer, Riverside, CA, USA) soil moisture sensors placed at the center of the shelter and at a depth of 10 and 20 cm. Before the drought treatment was implanted and for the well water treatment during the drought, every time that the mean of the two soil moisture sensors was below –60 kPa, the plots were irrigated with 12.7 mm of water. The drought treatment in 2019 and 2020 started 60 (July 7th) and 70 (July 21st) days after planting (DAP) respectively. In 2019 the drought lasted 4 weeks (28 days) until August 3rd, then the plants were watered again with 12.7 mm of water and left to mature until September 10th when they were harvested. In 2020 the drought lasted five weeks (35 days) until August 25th due to mild temperatures that delayed the apparition of physiological and visual drought symptoms. After August 25th plots were watered again with 12.7 mm and left to mature until September 19th when they were harvested. During the recovery period the shelters were not active, and irrigation was activated when the soil moisture sensors showed values lower than –60 kPa. At harvest, all tested entries were considered to have similar maturity requirements, thus harvest time was determined by the hull scrape method (Williams and Drexler, 1981) based on the cultivar Georgia Green in each shelter. Peanuts were harvested with a plot thresher (Kingaroy Engineering Works, Kingaroy, Queensland, AU). During the experiment, all agronomic management practices related to fertilization, weed and pest control were applied according to University of Georgia best management practices for peanut.

Physiological traits measurements

Leaf samples for specific leaf area (SLA) and leaf water content (LWC) calculations were collected weekly during the drought and recovery period. In 2019, samples were taken the day of drought imposition (0 Days after drought, 0 DAD, July 7th), 8, 14, 21, and 28 DAD. A last sampling of leaves was performed 2 weeks after the irrigation was stablished again (recovery). In 2020, samples were taken the day of drought imposition (0 Days after drought, 0 DAD, July 21st), 15, 22, and 29 DAD. A last sampling of leaves was performed 2 weeks after the irrigation was stablished again (recovery). For these measurements, one fully expanded tetrafoliate leaf, containing four elliptical leaflets per leaf, were collected from the main stem of 3 randomly selected plants in each plot at mid-morning (10-11 a.m.), put into zip bags, placed on ice and immediately transported to the laboratory. Fresh weight of plants was recorded with a precision scale and leaf water content (LWC) was calculated as in Ceccato et al. (2001).

$$\text{LWC (\%)} = [(\text{FW}-\text{DW}) / \text{FW}] \times 100$$

where FW is the fresh weight and DW is the oven dry weight of the leaf. The DW was obtained by drying the leaves in a forced oven at 70 °C for at least 72 hours. Before the leaves were dried, the leaf area of the leaves was measured using a LI-3100 area meter (Li-Cor Biosciences, Lincoln, NE, USA). Specific leaf area (SLA), was measured for each leaf as (Vile et al., 2005):

$$\text{SLA (cm}^2\text{ g}^{-1}\text{)} = \frac{\text{leaf area}}{\text{leaf DW}}.$$

Mid-day leaf photosynthesis and stomatal conductance was measured on the youngest fully expanded tetrafoliate in the top of the main stem during 10:30 a.m. – 2 p.m. using two or three sets of LI-6400XT Portable Photosynthesis System (LI-COR Biosciences, Lincoln NE, USA). In 2019, measurements were performed at 8, 14, and 21 DAD, as well as 2 weeks after the end of the drought period (recovery). In that year, due to the high number of cultivars in the experiment, one gas exchange measurement per plot was performed. In 2020, measurements were performed at 0 (0 DAD, before drought), 6, 10, 15, 22, 29 DAD as well as 2 weeks after the end of the drought period (recovery). In 2020 due to a lower number of cultivars, two measurements per plot were taken and averaged to get the plot mean values.

Yield, harvest index and stable isotope calculations

Harvest was performed by hand inversion of plants using shovels on September 10th in 2019 and September 19th in 2020. Before threshing each plot in a small plot thresher, three representative plants per plot were separated for harvest index and stable isotope analysis. These plants were separated in pods and shoot biomass and dried for at least 72h at 60°C then weighted to record dry weight. Harvest index calculated as:

$$(\mathrm{HI} = \frac{\text{pods DW}}{\text{pods DW} + \text{shoot DW}})$$

The pods collected from the peanut thresher were dried at 40°C, and the pod weight used for the HI calculation was added to the total weight of each plot in order to calculate yield that was estimated as Kg ha^{-1}.

Dry seeds and shoot biomass samples were ground separately to pass a one mm sieve, weighted in tin capsules and sent to University of California Davis Stable Isotopes Facility (Davis, CA) for stable isotope analysis (C and N) using an isotope ratio mass spectrometer (IsoPrime, Elementar France) connected with an element analyzer (EA3000, EuroVector).

The ratio (R) of $^{13}C/^{12}C$ was showing as $\delta^{13}C$ (‰) indicating the C isotope composition, relative to Vienna Pee Dee Belemnite calcium carbonate (V-PDB):

$$\delta^{13}C = (R_{samples}/R_{standard})-1$$

Later $\delta^{13}C$ (‰) values were standardized to C isotope discrimination ($\triangle^{13}C$, ‰) data calculated as:

$$\triangle^{13}C = (\delta^{13}C_{atm}- \delta^{13}C_{sample})/ (\delta^{13}C_{sample}+1)$$

Where $\delta^{13}C_{atm}$ is the C isotope composition of atmospheric CO_2 (-8‰; Farquhar et al., 1989) and $\delta^{13}C_{sample}$ is the C isotope composition of the plant sample (shoot biomass or seed). Seed and shoot biomass carbon isotope discrimination were reported as seeds $\Delta^{13}C$ and shoot $\Delta^{13}C$ respectively.

The natural ^{15}N isotopic ratio ($\delta^{15}N$) in seed and shoot biomass were calculated using the formula described by Shearer and Kohl (1986):

$$\delta^{15}N = \frac{R_{sample}}{(R_{air} - 1)} * 1000$$

where, R_{sample} and R_{air} are the isotope ratios ($^{15}N/^{14}N$) of the sample and air, respectively. Seed and shoot biomass $\delta^{15}N$ were reported as $\delta^{15}N$ SD and $\delta^{15}N$ AB respectively.

Statistical Analysis

Due to the use of unidentical genotypes and different measurements over two years, all the data was analyzed separately by year. As the well water treatment was only consisted of one replicate, there was a statistical comparison of means that included all the cultivars between drought and well water treatment. However, in order to corroborate the negative effects of drought over the plants, a one-way analysis of the variance (ANOVA) was performed. In this analysis we used the mean of each cultivar under drought as one replication, meanwhile the single value of each cultivar was used as a replication in the well-watered treatment. The analysis of the drought effect was only used to make sure that the drought treatment had a significant negative effect on the growth and physiology of the plants. To determine differences between genotypes under drought for each measured parameter, the mixed model ANOVA was conducted using PROC GLIMMIX in SAS 9.4 (SAS Institute) with genotypes as a fixed effect and replication as a random effect.

As physiological parameters (photosynthesis, stomatal conductance, SLA, and LWC) under drought were measured at different time points during the drought period, a repeated measures mix model analysis of variance PROC MIXED (SAS 9.4; SAS Institute) with cultivars as fixed effect, days after drought (DAD) as repeated measurement, and block (shelter) as random effect was performed to analyze the effect of DAD and cultivars over measured parameters.

Correlation between all measured parameters was analyzed using PROC CORR (SAS 9.4; SAS Institute). In order to show correlations in a more visual display, a correlation matrix was obtained using CORRPLOT in R.

On a plot basis, broad sense heritability was calculated as $H^2 = \sigma^2_g / [\sigma^2_g + \sigma^2_{ge} + \sigma^2_e]$, where σ^2_g is the genotypic variance, σ^2_{ge} is the genotype by environment interaction variance, σ^2_e is the variance due to experimental error. For measurements that were taken multiple times during the season two approached were taken: (1) we calculated H^2 with the variance value of the mean of all the time points taken per cultivar, and (2) we calculated H^2 with the variance of each cultivar in the time point that parameter had a higher correlation with yield.

Results

Yield and agronomic traits

In 2019, pod yield under mid-season drought conditions was significantly different between cultivars (Table 3). Some of the previously identified drought tolerant varieties (PI 502120, PI 493329, TifNV-High O/L, Tifrunner, Line-8, and G06G) showed the highest yields with significant differences compared to moderate tolerant and drought sensitive cultivars (Table 3). There were not significant differences between these top yielding cultivars under drought, however, cultivars PI 502120 and PI 493329 showed the highest yields, which were 3,055 Kg/ha and 2,666 Kg/ha, respectively (Table 3). This is a similar value to the average yield of one replicate of all the cultivars (2,777 Kg/ha) under well water conditions grown in the rain out shelters (Table 3). In addition, the implementation of mid-season drought in this experiment seemed effective at reducing growth and yield ($P<0.0001$) as the mean of all the cultivars under drought (1,180 Kg/ha) was significantly lower than the mean under irrigation (2,777 Kg/ha; Table 3). The most drought susceptible cultivar, PI 372305 produced 117 Kg/ha which was 27 times lower than the highest yielding cultivar PI 502120. In 2020, pod yield under drought was significantly different between cultivars (Table 4). The drought treatment imposed by the rain-out shelters was significant as the mean all cultivars under drought was lower (2,063 Kg/ha) than the mean of cultivars grown under irrigated conditions (3,183 Kg/ha; Table 4). Previously identified drought tolerant varieties showed higher yields than drought sensitive ones with the exception of TifNV-High O/L, a documented drought tolerant cultivar, that have similar yield to PI 268755, a drought sensitive one. Between the high yielding cultivars under drought, AU 16-28, G06G, PI 493329, PI 502120, AU 18-35, AU-17, Tifrunner and Line-4 showed similar pod yields. AU 16-28 showed the highest yield under drought, 3,415 Kg/ha, which was very similar to the average of the yield of all the tested cultivars under irrigated conditions, 3,183 Kg/ha. The cultivar PI 390428 was the most drought susceptible cultivar producing 623.8 Kg/ha which was 5.5 times lower than the highest yielding cultivar, AU 16-28 (Table 4).

In 2019, shoot biomass under drought was significantly different between genotypes (p<0.0001; Table 3). In general, previously described drought tolerant cultivars showed higher shoot biomass (3,709 Kg/ha) than drought sensitive ones (2,335 Kg/ha), which was a little lower than the average biomass of all the cultivars grown under irrigated conditions (4,570 Kg/ha). The mean of cultivars grown under drought showed a significantly lower shoot biomass value (2,803 Kg/ha) than the ones grown under irrigated conditions (4,570 Kg/ha; Table 3). For example, TifNV-High O/L and SPT06-6 under drought had the highest shoot biomass 4,756 and 4,571 Kg/ha respectively; meanwhile the cultivar showing the lowest yield, PI 325943, showed the lowest biomass accumulation (940 Kg/ha). However, some low-yielding varieties such as PI 274193 and PI 290594 showed a high shoot biomass of 4,411 Kg/ha and 4,255 Kg/ha, respectively that was comparable with TifNV-High O/L and SPT06-6 (Table 3). Despite of this disparity between high yielding cultivars and biomass accumulation, the relationship between yield and biomass accumulation was significantly positive (Table 5). In 2020 shoot biomass under drought was significantly different between cultivars (Table 4). In general, previously described drought tolerant cultivars showed higher shoot biomass (3,264 Kg/ha) than drought sensitive ones (1,679 Kg/ha), which was very similar to the average biomass of all the cultivars grown under irrigated conditions (3,578 Kg/ha). However, the mean of all cultivars grown under irrigated conditions was significantly higher than the mean of cultivars grown under drought conditions (2688Kg/ha, Table 4). Of the top yielding cultivars under drought, AU 16-28 showed the highest shoot biomass 4,746 Kg/ha followed by Line-4 with 3,936 Kg/ha (Table 4). However, some low-yielding lines such as

PI 325943 showed high shoot biomass (2,540 Kg/ha). The lowest shoot biomass was 1,068 Kg/ha found in the lowest yielding cultivar PI 290560 (Table 4). In 2020 biomass accumulation was positively correlated with yield, even in higher degree than in 2019 (Table 5 and Table 6).

In 2019, harvest index (HI) under drought was significantly different between genotypes (p<0.0001). In general, previously identified drought tolerant cultivars showed similar HI (0.36) to the average of all cultivars under irrigated conditions (0.37) which was also higher than the drought sensitive ones (0.19). Overall, the HI mean of all cultivars grown under drought (0.27) was significantly lower than the mean of all cultivars grown under irrigated conditions (0.37; Table 3). Within the drought tolerant cultivars, PI 502120 had the highest HI (0.49) followed by G06G and PI 493329 (HI=0.4) (Table 3). PI 372305, a drought sensitive cultivar showed the lowest HI which was 0.07 under drought. However, not all drought sensitive cultivars had low HI, for example, PI 155107 showed a HI of 0.34 that was similar to the mean of high yielding cultivars (0.37; Table 3).

In 2020, HI was significantly different between genotypes (p<0.0001; Table 4). In general, previously identified drought tolerant cultivars showed similar HI (0.46) to the average of all cultivars under irrigated conditions (0.46) which was also higher than drought sensitive ones (0.32). Within the drought tolerant cultivars, G06G had the highest HI= 0.52 followed by AU 17 with 0.5 and C76-16 with 0.5 (Table 4). In the other hand, PI 325943 showed the lowest HI under drought conditions which was 0.21. In this experiment, some low yielding cultivars had acceptable HI such as PI 290560 which had a HI of 0.45 (Table 4). Drought rate was significantly different between genotypes. PI 325943 had the most server drought and AP-3 had the least drought stress. Drought treatment significantly affected HI in 2019 (P =0.0008, Table 3) but not in 2020 (Table

4). In both years HI was positively and significantly correlated with yield, with 2019 showing a higher correlation than in 2020 (Table 5; 6).

In 2019 and 2020, drought rate reading was significantly different between genotypes (Table 3, Table 4). In both years, higher yielding cultivars, the drought tolerant ones, had the lowest rate in comparison with drought sensitive ones resulting in a negative relationship between yield and drought rate in 2019 (Table 5) but not in 2020 (Table 6). In 2019, PI 502120 had the highest drought and PI 325943 had the least drought stress. In 2020, some of the higher yielding cultivars, AU16-28 and G06G had the highest drought, by the contrary, PI 325943, one of the lowest yielding cultivars showed the lowest drought rate of all cultivars (Table 4). Low drought stress did not result in high yield, therefore producing the lack of correlation between drought rates and yield in 2020 (Table 6).

Photosynthetic and water use efficiency traits

In 2019 and 2020, leaf water content (LWC) showed a significant effect of cultivars and days after drought (DAD) but did not showed interaction between cultivars and DAD (Table 11). In 2019, LWC decreased from 79% to 72% at 28 DAD, and increasing up to 76% after 2 weeks of recovery (Fig. 1). In 2020, LWC decreased from 77% to 74% at 28 DAD to recover to 75.8% after 2 weeks of re-watering. In both years, the drought treatment showed lower LWC than the irrigated treatment during the drought (Fig. 1). When the average of each cultivar was performed across the measured time points, there was not a clear distinction between drought tolerant and drought sensitive cultivars in both years (Fig. 2). However, the LWC mean of the irrigated treatments in 2019 and 2020 (77.65 and 77.13, respectively) was significantly (P=0.0013) higher than the mean of all cultivars under drought (75.5 and 75.65, respectively). In 2019 cultivars PI 502120, a drought tolerant cultivars and PI 497648 a medium drought tolerant cultivar showed the highest LWC. On the other hand, C76-16, a drought tolerant cultivar and PI 325943, a drought sensitive one showed significantly low LWC values. In 2020, AU-17 and Ga Green, both drought tolerant cultivars, showed the highest values and PI 390428 and AP-3, both drought sensitive cultivars, showed the lowest ones (Fig. 2). In both years, the LWC parameter did not show significant correlation with yield when measured at time points where there was no drought stress (0 DAD and recovery); however, it did show a low significant positive correlation with yield under drought when this parameter was measured during the drought period (Table 5; 6) indicating that cultivars that maintain a high LWC during drought usually show high yields.

In 2019 and 2020, SLA showed significant effects of cultivars and DAD but did not show interaction between cultivars and DAD (Table 11). In both years, SLA of drought plants tended to be slightly higher than the SLA of irrigated ones (Fig. 1). In 2019, SLA tended to increase at the beginning of drought then decreased after the second week of drought (Fig. 1). In 2020, SLA of drought treated plants increased at the beginning of drought then decreased after one week of drought and recovered after re-irrigation (Fig. 1). When the average of each cultivar was performed across the measured time points, there was not a clear distinction between drought tolerant and drought sensitive cultivars in both years (Fig. 2). In 2019, PI 337406, a moderate drought tolerant cultivar, showed the highest SLA; and PI 155107, drought sensitive cultivar, showed the lowest SLA (Fig. 2C). In 2020 AU-17 showed the highest SLA and there was no significant difference between AU-17 and PI 493329, both drought tolerant cultivars. Meanwhile, AU 16-28, the highest yielding cultivar under drought in 2020 showed the lowest SLA (Fig. 2D). SLA did not show any correlation with yield at any of the measured time points (Table 5; 6).

In 2019, there was significant cultivar effect on photosynthesis, meanwhile DAD showed no effect (Table 11). There was also a significant interaction effect between cultivars and DAD

(Table 11). In 2020, photosynthesis showed a significant effect of cultivars and DAD by individually but there was no the interaction (Table 11). In 2019, when the cultivar values where averaged across the drought period and recovery, the cultivar PI 502120 and AU-17, maintained the highest mean photosynthesis over the season in comparison with other classified drought tolerant cultivars such as TifNV High O/L, Tifrunner, Line-8, C76-16, Line-4 and AU 16-28. By the contrary, drought sensitive cultivars such as PI 325943, PI 372305, PI 268755, PI 339960 showed some of the lowest photosynthetic values (Fig. 3A). The highest photosynthesis was 5 times higher than the lowest photosynthesis under drought. The mean of photosynthesis of all cultivars and time points under drought conditions (7.2 μmol CO_2 mol^{-1} s^{-1} m^{-2}) was lower than the irrigated cultivars (13.72 μmol CO_2 mol^{-1} s^{-1} m^{-2}) in 2019 (Fig. 3A). As there was an interaction between cultivars and DAD, the photosynthesis values of each cultivar over the whole drought period was studied (Fig. 3B). Drought tolerant cultivars showed relative higher photosynthesis than drought sensitive cultivars during the drought period, especially for PI 502120 and AU-17. Photosynthesis increased for 2 weeks after drought started for most genotypes, declined in the 3rd week of drought, and recovered a little after re-irrigation. However, high yield cultivar PI 493329 decreased photosynthesis after drought started, increased in the 3rd week of drought and reduced photosynthesis after recovery. In 2020 when photosynthesis cultivar values were averaged across DAD, it was observed that photosynthesis decreased with the application of drought having a small peak 15 DAD and falling again until the irrigation recovered the photosynthesis values to moderate levels (Fig. 3D). When the cultivar values where averaged across the drought period and recovery, all the drought tolerant cultivars showed higher photosynthesis than the drought sensitive ones, with the exception of cultivar AU 18-35, a drought tolerant cultivar, that showed similar values to drought sensitive ones, and PI 325943, a drought sensitive cultivar showed similar photosynthesis as the other drought tolerant cultivars (Fig. 3C). Of the drought tolerant cultivars, G06G showed the highest photosynthesis of all, and PI 339960 showed the lowest photosynthesis among the drought sensitive cultivars. The photosynthesis means of all cultivars under drought (15.91 μmol CO_2 mol^{-1} m^{-2} s^{-1}) was significantly lower than the mean under irrigation (24.8 μmol CO_2 mol^{-1} m^{-2} s^{-1}). In both years all photosynthesis measurements at different time points were positively correlated with yield, being the average of all time points (DAD) the one that showed higher R^2 with yield (Table 5; 6).

In 2019 stomatal conductance (g_s) was not affected by DAD and the interaction of DAD x cultivar, however it was significantly affected by the cultivar effect (Table 9); as a result, the data were presented as the average of each time point for each cultivar (Fig. 4A). PI 502120 and AU-17 maintained the highest g_s over the season in comparison with other classified drought tolerant cultivars such as PI 493329, Tif-runner, C76-16 and Line-4. However, all of these cultivars showed higher stomatal conductance than drought sensitive ones such as PI 337406, PI 339960, PI 290560, PI 390428, PI 493581, PI 268755, PI 372305 and PI 325943 (Fig. 4A). Between the drought tolerant cultivars, TifNV High O/L, Line-8, and AU 16-28 showed lowest g_s that was similar to drought sensitive ones. Under irrigated conditions, g_s for all genotypes was 0.15 mmol H_2O $m^{-2}s^{-1}$ which was the highest g_s under drought showed by PI 502120 and AU-17 and was significantly higher than the average g_s of all cultivars under drought (0.06 mmol H_2O $m^{-2}s^{-1}$). In 2020, g_s showed significant effects of cultivar, DAD and the cultivar x DAD (Table 9). When the cultivar values where averaged across the drought period and recovery, G06G had the highest g_s under drought and PI 268755 showed the lowest one (Fig. 4B). The lowest stomatal conductance observed on PI 268755 (0.17 mmol H_2O $m^{-2}s^{-1}$) under drought in 2020 was very similar to the highest g_s observed under drought in 2019 for PI 502120 (0.16 mmol H_2O $m^{-2}s^{-1}$) which indicted that the drought stress in 2019 was higher than in 2020. Between the high yielding cultivars, AU 16-28 and Line-8 showed the lowest g_s which was similar to some of the drought sensitive cultivars including PI 325943, PI 339060, PI 390428 (Fig. 4B). Under irrigated conditions, g_s for all genotypes was similar to the highest stomatal conductance measured on some drought tolerant cultivars under drought (0.65 mmol H_2O $m^{-2}s^{-1}$), but much higher than the g_s mean of all the cultivars under drought (0.33 mmol H_2O $m^{-2}s^{-1}$). In 2020, there was an interaction of cultivars and DAD for g_s. In 2020, plants reduced stomatal conductance after drought started, increased around 15 DAD, and then declined until the end of the drought period (Fig. 4d). However, cultivars did not show the same performance under drought period. Highest yield cultivar AU 16-28 had relatively low g_s before drought but maintained a stable acceptable g_s instead of shutting down dramatically during the drought. Although the lowest yield cultivar PI 390428 had moderate g_s before drought, it had almost completely closed stomata after applying drought stress. G06G kept a high g_s during drought that resulted in high yields at the end of the season. In both years, g_s under drought was positively correlated with yield at every time point, with the average across time points showing the highest R^2 (Table 5; 6). This strong correlation between individual time points and its average with yield shows the importance of g_s and photosynthesis for yield determination under drought conditions.

In 2019 seeds $\Delta^{13}C$ was significantly affected by genotypes under drought (Table 7). The highest seeds $\Delta^{13}C$ value was found in PI 502120, followed by Tifrunner and PI 493938. The lowest $\Delta^{13}C$ value was performed by two of the lowest yielding cultivars, CG7-A and PI 442768 (Table 7). Among cultivars that showed the highest yields, not all showed high seed $\Delta^{13}C$. for instance, TifNV-High O/L, Line-8 and AU 16-28 showed relative low seeds $\Delta^{13}C$ which were the lowest within the drought tolerant cultivars. Most drought sensitive genotypes showed low seeds

$\Delta^{13}C$, however, a few drought sensitive cultivars such as PI 493938 had high seeds $\Delta^{13}C$. The mean of all the irrigated cultivars (18.5‰), was significantly higher than the mean of all cultivars grown under drought (17.4‰). Shoot $\Delta^{13}C$ did not showed significant differences between cultivars in 2019, and its value was higher than seed $\Delta^{13}C$ (Table 7). Shoot $\Delta^{13}C$ under irrigated conditions (19.9‰) was slightly higher than shoot $\Delta^{13}C$ under drought (19.2‰). In 2020, seeds $\Delta^{13}C$ was slightly affected by cultivars under drought conditions (P=0.078, Table 8). The highest seeds $\Delta^{13}C$ was 19.1‰ found in PI 493329 followed by Line-4 and AU-17; and the lowest in PI 390428 (Table 8). Of the high yielding cultivars, line-8 showed the lowest $\Delta^{13}C$ values equaling PI 390428. The mean of all cultivars under irrigated conditions (20.1‰) was higher than the mean of the ones grown under drought (18.4‰). Shoot $\Delta^{13}C$ was very similar to seed $\Delta^{13}C$ (Table 8). In 2020, there was a significant difference between cultivars for shoot $\Delta^{13}C$ under drought (Table 8). Line-4 showed the highest shoot biomass $\Delta^{13}C$ and PI 325943 showed the lowest shoot $\Delta^{13}C$ which were 20.5‰ and 18.9‰, respectively. Drought tolerant cultivars Line-8 and TifNV High O/L showed lower shoot $\Delta^{13}C$ values than Line-4. Water treatments had a significant effect on shoot $\Delta^{13}C$ ($p = 0.0008$). Irrigated plants showed a slightly higher shoot $\Delta^{13}C$ value (20.3‰) than plants grown under drought (19.8‰; Table 8). In 2019, seed and shoot $\Delta^{13}C$ were positively correlated with yield, with seed $\Delta^{13}C$ showing higher R^2 with yield than shoot $\Delta^{13}C$ (Table 5). In 2020, seed and shoot $\Delta^{13}C$ showed positive significant correlation with yield, but the R^2 was similar between the two organs and the R^2 between seed $\Delta^{13}C$ and yield was lower than in 2019 (Table 5;6).

It was worth noting that in both years, drought tolerant cultivars with medium photosynthetic rates such as Line-8 and AU 16-28 showed g_s and seed $\Delta^{13}C$ values as low as drought sensitive cultivars. However, these cultivars showed moderate to high photosynthesis and yields, with AU 16-28 being the highest yielder in 2020; indicating these cultivars may possess high water use efficiency (WUE). By the contrary, cultivars such as PI 502120, G06G, and AU-17 showed high yields at the same time that they showed high photosynthesis, g_s, and seed $\Delta^{13}C$ which may be associated with more efficient extraction and use of water from the soil.

Nitrogen and nitrogen fixation related traits

In both years seeds $\delta^{15}N$ under drought conditions was significantly affected by cultivar effect (Table 7; 8). In 2019, cultivar PI 502120 which was the highest yielder showed the lowest seeds $\delta^{15}N$, meanwhile PI 325943, the lowest yielding cultivar under drought showed the highest seeds $\delta^{15}N$. The mean of seed $\delta^{15}N$ for all cultivars under irrigation conditions was the same with the mean of all the cultivars under drought (Table 7). In 2019, shoot $\delta^{15}N$ was greater than seeds $\delta^{15}N$ under drought, however, there was not a significant difference between cultivars for shoot $\delta^{15}N$ (Table 7). The mean of shoot $\delta^{15}N$ for all cultivars was significantly lower (2.2‰) than the mean of all the cultivars under drought (2.5‰) (Table 7). In 2020, seeds $\delta^{15}N$ was significantly different between cultivars under drought. G06G, the second highest yielding cultivar this year, showed the lowest seeds $\delta^{15}N$ (0.91‰), meanwhile PI 390428, the lowest yielding cultivar, showed the second highest seeds $\delta^{15}N$ (2.57‰) which was 3 times the G06G value (Table 6). The mean of seed $\delta^{15}N$ for all cultivars under irrigation conditions was significant lower (1.15‰) than the mean of all the cultivars under drought (1.61‰) (Table 8). In 2020, shoot $\delta^{15}N$ did not show significant effect of cultivars, however the mean of irrigated cultivars (0.99‰) was significantly lower than the mean of cultivars grown under drought conditions (2.14‰) (Table 8). In 2019 seed $\delta^{15}N$ showed a negative relationship with yield meanwhile shoot $\delta^{15}N$ did not (Table 7). In 2020, seed $\delta^{15}N$ showed a marginal negative correlation with yield (p= 0.079), meanwhile shoot $\delta^{15}N$ did not show correlation (Table 6).

In 2019, seed and shoot N% showed a significant difference between genotypes under drought (Table 9). In both tissues PI 372305, a low yielding cultivar, had the highest seed and shoot N%; meanwhile Line-4, a high yielding cultivar under drought, showed the lowest N%. Seed and shoot N% was significantly lower under irrigated conditions in comparison with the mean of all cultivars under drought (Table 9). Seeds N% was greater than shoot N%. In 2020, seed N% under drought was significantly affected by the cultivar (Table 10). Mean seed N% of cultivars grown under drought (4.54%) was significantly higher than the ones grown under irrigated conditions (4.06%). Shoot N% was significant different between cultivars under drought (Table 10). PI 390428 showed the highest shoot N%, and Line-4 had the lowest. Mean shoot N% under drought conditions (1.53%), was significantly higher than under irrigation (1.28%; Table 10). In both years seed N% was negatively correlated with yield (Table 5; 6). In the other hand, shoot N% was only negatively correlated with yield on 2019 (Table 5).

In 2019 and 2020, seed total N content (Seed TN), Shoot total N content (Shoot TN) and plant total N content (Plant TN) were significantly affected by cultivars (Table 9; 10). Contrary to what happens in N% traits, in which low yielding cultivars showed high N%. Seed, shoot and plant total N content was higher in cultivars with high yield and shoot biomass accumulation such as the drought tolerant cultivars PI 502120, G06G and AU16-28. This was explained by the tight correlation that exist between total N content with yield and shoot biomass (Table 10; Figure 5; 6). The mean of all cultivars for seed, shoot and plant total N content was lower in plants grown under drought than in irrigated conditions in both years (Table 9; 10). The seed TN was higher than shoot TN in both years. In 2019 and 2020, seed TN, shoot TN, and plant TN were positively correlated with yield, with shoot TN showing the weakest correlation (Table 5; 6).

Relationship between physiological parameters

In both years photosynthesis and g_s under drought were significantly positively correlated with shoot biomass at any time point, with the average of all the time points showing a stronger correlation (Fig. 5; 6). Drought rate showed negative correlation with all photosynthetic parameters. Average photosynthesis and stomatal conductance showed a low positive correlation with LWC at different DAD in both years (Fig. 5; 6). However, SLA did not show any correlation with photosynthesis, g_s, or even with seed and shoot $\Delta^{13}C$. In 2019, seed $\Delta^{13}C$ showed significant positive correlation with photosynthesis and g_s at all time points; meanwhile shoot $\Delta^{13}C$ correlation with photosynthesis and g_s was still positive but much weaker (Fig. 5). In 2020, seed $\Delta^{13}C$ showed a weak positive correlation with photosynthesis and g_s at all points but there was no correlation between shoot $\Delta^{13}C$ and photosynthesis or g_s (Fig. 6). Seed $\delta^{15}N$ showed significant negative correlation with all photosynthesis and g_s time point measurements indicating the N_2-fixation dependency of the photosynthetic and water status of the plant.

Heritability

In both years heritability for yield and HI were moderate to high ranging from 0.53 to 0.71 (Table 12). Heritability was higher in 2020 in comparison with 2019 for these two parameters. Seed and shoot $\Delta^{13}C$ showed moderate low H^2 ranging from 0.14 to 0.24, which was higher than the $\delta^{15}N$ values for both tissues (Table 12). Photosynthesis and g_s, showed moderate H^2 values (0.48 and0.41 respectively) in 2020 but much lower values in 2019 (0.13 and 0.09 respectively) (Table 12). Seed N% showed the highest H^2 with 0.61 and 0.71 in 2019 and 2020 respectively.

Discussion

Two treatments were applied in this experiment, which were irrigated and drought treatment. The drought treatment was achieved by the utilization of the rainout shelters, causing mean yield losses of 57.5% and 35.2% in yield in 2019 and 2020, respectively, which is similar to other peanut trials under complete field conditions (Rucker et al., 1995; Balota, 2020). Some greenhouse studies indicated that mid-season drought, the same kind of drought that we implemented in our experiment, can cause a decrease on yield around 35% (Junjittakarn et al., 2014; Carvalho et al., 2017) while previous field experiments showed that there was a 53% yield loss under drought (Songsri et al., 2008). Therefore, the observed yield reductions caused by rainout shelters in our experiment can be considered normal and comparable to peanut field experiments.

The lower yield reduction in 2020 (35.2%) in comparison with 2019 (57.5%) was probably due to a more wet and cooler year (Table 1). In 2020 average maximum temperature was 1.2ºC lower than in 2019 with more than 110mm of precipitation. The fact that 2020 was a cooler and wetter year than 2019 probably also explains why in this year the differences in physiological parameters such $\Delta^{13}C$, photosynthesis and stomatal conductance between cultivars under drought is significant but smaller in comparison with 2019 (Table 7,8, 11). This smaller difference between cultivars has been shown before in common bean under irrigated conditions (Polania et al., 2016; Sanz-Saez et al., 2019) and is due to the fact that some cultivars only show significant differences when they are under extreme drought conditions (Tardieu et al., 2018).

Various peanut cultivars previously known by its drought tolerance and sensitiveness were tested in this experiment and showed significant differences on yield under drought conditions in 2019 and 2020 (Table 3 and 4). Between the drought tolerant cultivars, PI 502120 and PI 493329 in 2019, and AU 16-28 and G06G in 2020 were the highest yielding cultivars. Our results coincide with previous research that showed PI 493329 and G06G as drought tolerant (Otyama et al., 2019; Balota, 2020). On the other hand, cultivars PI 339960, PI 390428, and AP-3 showed the lowest yields under drought in both years (Table 3 and 4), therefore demonstrating that these cultivars are drought sensitive. Cultivar AP-3 has shown sensitivity to drought in previous research (Dang et al., 2012, 2013). Drought tolerant cultivars showed high HI under drought compared to drought sensitive ones (Fig. 8), suggesting high HI can be used as a drought tolerant indicator for breeding (Vadez and Ratnakumar, 2016).

Maintaining high yield under drought can be achieved by different physiological mechanisms (Condon et al., 2002; Blum, 2009; Vadez and Ratnakumar, 2016; Tardieu et al., 2018). Vadez et al., (2016) demonstrating that cultivars that showed high water use efficiency (WUE), that is, maintaining high yields while showing low transpiration can be a strategy for breeding for drought tolerance (Condon et al., 2002; Vadez and Ratnakumar, 2016;). High WUE can be achieved under severe drought conditions by reduced stomatal conductance during drought and remobilizing carbohydrates from biomass fixed before the drought period (Condon et al., 2002). Selecting for this trait using mini-lysimeters as Vadez et al., (2016) is very time and labor intensive. Therefore, in our research we used the $\Delta^{13}C$ that has been previously used by Wright et al., (1994) in peanut. With this technique, were able to identify Line-8 and AU 16-28 as high WUE cultivars because they showed low $\Delta^{13}C$ (Table 7 and 8). Low $\Delta^{13}C$ has been related with high WUE in previous research in other crops such as wheat, common bean and peanut cultivars (Passioura, 1977; Hubick et al., 1986; Wright et al., 1988, 1994; Rao et al., 1993; Condon et al., 2002; Blum, 2009). Additionally, we measured photosynthesis and stomatal conductance several times during the drought period in order to verify the lower transpiration of the cultivars that showed high WUE. We found that the cultivars showing low $\Delta^{13}C$ showed low stomatal conductance and that the relationship between the parameters was high (Fig. 5; 6). The relationship between $\Delta^{13}C$ and yield under drought has been used to screen for high WUE cultivars in the past (Polania et al., 2016; Sanz-Saez et al., 2019). In this experiment we found that this relationship is strong in peanut in both years (Table 5 and 6; Fig. 7). Using the same approach, we have found that Line-8 and AU 16-28 cultivars show low $\Delta^{13}C$ and high yield (Fig. 7), therefore showing high WUE (Wright et al., 1994; Polania et al., 2016; Sanz-Saez et al., 2019). Cultivars that show low stomatal conductance and therefore low transpiration have been named/classified as "Water Savers" (Polania et al., 2016). In our experiment cultivar AU-16-28 and Line 8, were considered water savers, and should be introgressed in breeding programs targeting environments with severe water stress.

High WUE and yields under drought can be achieved by reduced stomatal conductance. Some cultivars show the opposite mechanism of drought tolerance, maintaining a high efficient use of water (EUW, Blum, 2009). EUW refers to the amount of water used in transpiration under limited soil water condition to produce high biomass (Blum, 2009). Cultivars with high EUW are able to maintain high transpiration and therefore photosynthesis that results in higher biomass accumulation and yield (Blum, 2009; Polania et al., 2016). In common bean, it has been demonstrated that cultivars with high EUW also have high $\Delta^{13}C$ and that it results in higher yields (Polania et al., 2016; Sanz-Saez et al., 2019). The high transpiration in common bean in high EUW cultivars has been related with more deep and complex roots (Polania et al., 2017). In our study, we found that cultivars PI 502120 and AU 17 showed high yields with high $\Delta^{13}C$ (Tables 3, 4, 6, 7; Fig. 7) in both years which would classify them as high EUW cultivars according to Polania et al., (2016). In addition, we also showed a very significant correlation between stomatal conductance and yield (Fig. 7) in which these cultivars always show the higher values. According to the Blum, (2009) and Polania et al., (2016) classification, as these two cultivars showed high yields but used a lot of water regarding the Δ^{13}C and stomatal conductance measurements (Fig. 7), they should be classified as “Water Spender” cultivars.

The stable natural abundance carbon isotope discrimination (Δ^{13}C) technique was used in this experiment to determine the integral WUE and EUW for the whole growing season in the whole plant as it has been used before (Condon et al., 2002; Blum, 2009; Polania et al., 2016). Although this technique is useful, it’s expensive, \$9.5 per sample, and very time consuming as a lot of labor is needed to get a prepare the sample for isotope analysis. In addition, this technique is not very sensitive to small changes in transpiration in some points during the growing season due to changes in stomatal conductance (Nautiyal et al., 2012). For this reason, we performed an intense campaign of gas exchange measurements taken with a portable gas exchange analyzer (LI-COR6400) during the drought period in order to test if these measurements could be used to better detect cultivars with high WUE and EUW than with the Δ^{13}C. We found that the correlation between stomatal conductance and photosynthesis with seed Δ^{13}C was high (Fig. 5; 6) as it has been described before (Nautiyal et al., 2012). In addition, the correlation between yield and stomatal conductance and photosynthesis when these values where averaged through the drought period was higher that the correlation between yield and seed Δ^{13}C. This is surprising as in previous research the correlation between yield and gas exchange measurements has been reported as weak (Zheng et al., 2011; Dutra et al., 2017). The high correlation observed in our study is likely the result of abundant and repeated measurements during the drought period.

In this experiment we also studied the correlation between yield and photosynthetic traits with shoot and seed Δ^{13}C, to test which sample is better to study the effect of drought over the peanut physiology. In this study seed $\Delta^{13}C$ showed higher correlation with yield than shoot $\Delta^{13}C$ (Table 5 and 6). This indicates that seed $\Delta^{13}C$ is a better parameter than shoot $\Delta^{13}C$ to predict yield, however, studies in soybean have demonstrated that shoot and seed $\Delta^{13}C$ can be both used to predict yield (Dutra et al., 2017; Kaler et al., 2018). The low correlation between shoot $\Delta^{13}C$ and yield observed in our study (Table 5 and 6) was likely caused due to the fact that the samples were taken at the moment of harvest and at that point some of the ^{13}C fixed in the shoot may have been already transported to the reproductive organ (Kvien et al., 1986; Zhou et al., 2016).

Maintaining a high nitrogen fixation under drought is known as an indicator of drought tolerance. Negative correlation between seed $\delta^{15}N$ and N_2-fixation had been used for determining nitrogen fixation in legumes such as lentil (Hafeez et al., 2000), soybean (George et al., 1993), and peanuts (Boddey et al., 1990; Tu et al., 2009) growing under field conditions. A few early publications mentioned that lentil cultivars with low $\delta^{15}N$ value showed more N_2 fixation in plants and then produced a higher yield (Hafeez et al., 2000). In peanut, cultivars that show high nitrogen fixation under drought gave higher yields (Pimratch et al., 2008a, 2010; Dinh et al., 2013). In this experiment, drought tolerant cultivars showed lower seed $\delta^{15}N$ and higher yield than drought sensitive cultivars under drought, especially high yield cultivars considered as “water spenders” such as PI 502121 and AU 17 (Fig. 8). Seed $\delta^{15}N$ was slightly negatively correlated with seed $\Delta^{13}C$ and also showed a strong negative correlation with all the photosynthesis and stomatal conductance parameters (Fig. 5 and 6). This indicates that the cultivars that show higher transpiration and therefore photosynthesis were able to maintain a higher BNF. This phenomenon has been previously described in soybean, in which cultivars that are able to maintain high photosynthesis under drought also show higher N_2 fixation (Cerezini et al., 2014; Furlan et al., 2017). However, Line 8 and AU16-28, also showed high yields and low $\delta^{15}N$, therefore, cultivars with lower stomatal conductance and acceptable photosynthesis could also show high BNF. Therefore, cultivars that are able to maintain an adequate water status no matter the drought tolerance mechanism, water saver or water spender, are able to maintain a high BNF. As it was observed for the $\Delta^{13}C$,shoot $\delta^{15}N$ had very weak correlation with yield under drought in both years resulted from remobilization and redistribution of N from shoot to pods over time in peanut (Kvien et al., 1986), as well as wheat (Zhou et al., 2016), therefore it may not recommended to test in drought tolerance selection.

Conclusion

We have demonstrated that the rain-out shelter facility is able to implement mid-season drought comparable to the one suffered under field conditions. This study has shown how drought tolerance in peanut can be achieved by selecting cultivar for four hypothesized mechanisms tested in other crops but not in peanut at the same time: high water use efficiency (WUE), high effective use of water (EUW), high nitrogen fixation, and high HI. Of these, high EUW had never been described in peanut. In this study we found two cultivars that showed signs of high EUW also called "Water spender" cultivars, PI 502120 and AU 17, which showed high $\Delta^{13}C$, photosynthesis and stomatal conductance. In the other hand, we also found two cultivars, Line-8 and AU16-28, that showed the contrary mechanisms, high WUE or also called "Water Savers", represented by low $\Delta^{13}C$, photosynthesis and stomatal conductance. all of these cultivars should be introgressed in elite lines and developed as drought tolerant lines. In this study it was also demonstrated that $\Delta^{13}C$ measurements could be substituted as a drought tolerance indicator by several measurements of photosynthesis and stomatal conductance during the drought period as it has higher correlation with yield and allows to differentiate better between "Water Savers" and Water expender" cultivars. The cultivars showing high yield under drought not matter if they were water savers or spenders, showed high BNF reveling that plants that maintain an equilibrate water status are able to maintain high BNF.

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Table 6. Correlation of yield with other measured parameters (see abbreviations list) under mid-season drought conditions in 2020. R-square and p-values are shown in the table for each parameter.

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Table 10. N percentage in seed (Seed N%), N percentage in shoot biomass (Shoot N%), total N in seed (Seed TN), total N in shoot biomass (Shoot TN), and plant total N (Plant TN) mean values ± standard error of 18 cultivars grown under mid-season drought conditions in 2020. Means followed by different letters were statistically different. In the bottom of the table the mean of all cultivars grown in one replicate irrigated conditions and the mean of all cultivars under drought conditions was shown for comparison using a one-way ANOVA. The table also shows the ANOVA result (p-value) for the effect of cultivars in the plants grown under mid-season drought conditions. Cultivars were grouped as drought resistant, drought moderate and drought susceptible based on previous studies.

Table 11. P-Values of the repeated measures ANOVA considering the cultivars as fixed effect and the days after drought (DAD) as a repeated measurement. The parameters measured were

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Figure 2. Leaf water content (LWC, %; A, B) and specific leaf area (SLA, mm^2 mg^{-1}; C, D) in 2019 and 2020. Bars represent the cultivar mean over the whole drought measurement period. Different letters between cultivar means that averages were statistically different. As there was only one replication in the well water treatment, there was not statistical analysis, and the mean (green) were shown as reference.

Figure 3. Leaf photosynthesis of 36 peanut cultivars grown under mid-season drought conditions on 2019 (A, B). A) Represents the mean photosynthesis of each cultivar averaged over the whole drought period and recovery in 2019. B) Represent leaf photosynthesis of each cultivar measured at different time points during the drought periods. Colored cultivars represent the most extreme cultivars for yield and photosynthesis. Leaf photosynthesis of 18 peanut cultivars grown under mid-season drought conditions on 2020 (C, D). C) Represents the mean photosynthesis of each cultivar averaged over the whole drought period and recovery in 2020. D) Represent the average leaf photosynthesis of all the cultivars measured at different time points during the drought period in 2020. Different letters between cultivar or time points averages were statistically different. As there was only one replication in the well water treatment, there was not statistical analysis, and the mean (green bar or line) were shown as reference.

Figure 4. Leaf stomatal conductance of 36 peanut cultivars grown under mid-season drought conditions on 2019 (A). A) Represents the mean stomatal conductance of each cultivar averaged over the whole drought period and recovery in 2019. Leaf stomatal conductance of 18 peanut cultivars grown under mid-season drought conditions on 2020 (B, C). B) Represents the mean stomatal conductance of each cultivar averaged over the whole drought period and recovery in 2020. C) Represent the average leaf stomatal conductance of all the cultivars measured at different time points during the drought period in 2020. Different letters between cultivar or time points means were statistically different. As there was only one replication in the well water treatment, there was not statistical analysis, and the mean (green) were shown as reference.

Figure 5. Correlation between all the parameters measured in the 2019 growing season (See abbreviation list for full understanding of parameter names). Blue and red colors indicate positive and negative correlation between parameters respectively. The size and intensity of the circle indicate higher correlation.

Figure 6. Correlation between all the parameters measured in the 2020 growing season (See abbreviation list for full understanding of parameter names). Blue and red colors indicate positive and negative correlation between parameters, respectively. The size and intensity of the circle indicate higher correlation.

Figure 7. Correlation between yield with average photosynthesis during the season (A, B), average stomatal conductance during the season (C, D), and seed $\Delta^{13}C$ (E, F) measured in 2019 and 2020. For photosynthesis, stomatal conductance and seed $\Delta^{13}C$ cultivars were divided in water saver and water spender cultivars according with the classification in Sanz-Saez et al., (2019). Black trend lines represent a significant correlation (R^2 and p-values) including all the cultivars. Grey trend line indicates significant correlation of only classified drought tolerant cultivars (red circles).

Figure 8. Correlation between yield $\delta^{15}N$ (A, B) and harvest index (HI) (C, D) in 2019 and 2020. Black trend lines represent a significant correlation (R^2 and p-values) including all the cultivars. Grey trend line indicates significant correlation of only classified drought tolerant cultivars (red circles).

Table 1.

Date	Avg. daily max. temperature (°C)	Avg. daily min. temperature (°C)	WW precipitation+ irrigation (mm)	WS Irrigation precipitation+ irrigation (mm)
May 7th -Sep 10th 2019	33.7 ± 0.2	20.7 ± 0.2	430.2	339.1
May 12th -Sep 19th 2020	32.5± 0.2	20.7 ± 0.2	547.1	414.8

WW: Well water, WS: water stress

Table 2.

Cultivar	Selection characteristic	Experiment year		Reported in publication or personal communication by breeder
		2019	2020	
PI 502120	Drought tolerant	X	X	Charles Chen (PC)
PI 493329	Drought tolerant	X	X	Charles Chen (PC)
TifNV-High O/L	Drought tolerant	X	X	Balkcom et al., 2019; Btillman, 2019
Tifrunner	Drought tolerant	X	X	Holbrook and Culbreath, 2007
Line-8	Drought tolerant	X	X	Charles Chen (PC)
G06G	Drought tolerant	X	X	Balkcom et al., 2019; Btillman, 2019
C76-16	Drought tolerant	X	X	Dang et al., 2012
AU-17	Drought tolerant	X	X	Balkcom et al., 2019; Btillman, 2019
Line-4	Drought tolerant	X	X	Charles Chen personal communication
AU-16-28	Drought tolerant	X	X	Balkcom et al., 2019
AU-18-35	Drought tolerant		X	Charles Chen (PC)
SPT06-6	Drought tolerant	X		Charles Chen (PC)
PI 196635	Drought tolerant	X		Charles Chen (PC)
PI 576636	Moderate drought tolerant	X		Rosas-Anderson et al., 2013
Ga Green	Moderate drought tolerant	X	X	Dang et al., 2013
Ga HI O/L	Moderate drought tolerant	X		Anco and Thomas, 2021
PI 497648	Moderate drought tolerant	X		Charles Chen (PC)
Line-2	Moderate drought tolerant	X		Charles Chen (PC)
PI 370331	Moderate drought tolerant	X		Charles Chen (PC)
PI 337406	Moderate drought tolerant	X		Charles Chen (PC)
PI 268996	Moderate drought tolerant	X		Charles Chen (PC)
PI 290566	Moderate drought tolerant	X		Kottapalli et al., 2009
CG7-A	Moderate drought tolerant	X		Subrahmanyam et al., 2000
AT 3085RO	Drought susceptible	X		Anco and Thomas, 2021
PI 290594	Drought susceptible	X		Charles Chen (PC)
PI 155107	Drought susceptible	X		Charles Chen (PC)
PI 493938	Drought susceptible	X		Charles Chen (PC)
PI 339960	Drought susceptible	X	X	Charles Chen (PC)
PI 290560	Drought susceptible	X	X	Charles Chen (PC)
PI 274193	Drought susceptible	X		Kottapalli et al., 2009;
PI 390428	Drought susceptible	X	X	Charles Chen (PC)
PI 442768	Drought susceptible	X		Charles Chen (PC)
PI 493581	Drought susceptible	X		Charles Chen (PC)
PI 268755	Drought susceptible	X	X	Charles Chen (PC)
PI 288210	Drought susceptible	X		Charles Chen (PC)
PI 372305	Drought susceptible	X		Kottapalli et al., 2009
PI 325943	Drought susceptible	X	X	Charles Chen (PC)
AP-3	Drought susceptible		X	Dang et al., 2012

Table 3.

Cultivar	**Yield (Kg/ha)**	**Shoot Biomass (Kg/ha)**	**HI**	**Drought Index**
PI 502120	3055.2 ± 414.3 a	3223.5±466.7 abcdefg	0.49±0.01 a	2±0.4 h
PI 493329	2666.5±513 ab	4007.8±816.5 abcd	0.4±0.03 abc	3±0.7 defgh
TifNV-High O/L	2385.6±217.2 abc	4756.3±548.4 a	0.34±0.03 bcdefghi	3±0.4 defgh
Tifrunner	2281.9±210.6 abcd	4260.8±1037.8 abc	0.37±0.04 bcdef	2.7±0.5 efgh
Line-8	2221.1±440 abcd	3636.7±422.4 abcde	0.37±0.02 bcdef	3.3±0.6 bcdefg
G06G	2192.2±377 abcd	3239.7±472.8 abcdefg	0.4±0.04 ab	3.5±0.5 bcdef
C76-16	2009.6±543.5 bcde	3062.8±588.8 abcdefg	0.39±0.03 abcde	3±0.6 defgh
AU-17	1942.3±530.4 bcdef	3604.1±461.4 abcde	0.33±0.06 bcdefghi	2.1±0.7 gh
Line-4	1843.5±485.3 bcdefg	3798.5±1215.7 abcde	0.34±0.02 bcdefghi	3.4±0.7 bcdef
AU16-28	1800.4±189 bcdefg	2911.4±387 abcdefg	0.39±0.01 abcd	3.1±0.6 cdefgh
SPT06-6	1790.8±380.6 cdefg	4571.8±661.4 a	0.28±0.03 defghijk	2.5±0.7 fgh
PI 196635	1421.3±350.3 defgh	3444.2±795.1 abcdef	0.29±0.04 cdefghijk	2.7±0.4 efgh
PI 576636	1247.6±488.1 efghi	2968.1±641.2 abcdefg	0.28±0.04 efghijk	3.3±0.6 bcdefg
AT 3085RO	1219.8±472 efghi	3242.7±641.6 abcdefg	0.24±0.05 mhijkl	4.1±0.5 abcd
Ga Green	1143.9±277.2 efghij	2032.3±514.3 defg	0.36±0.01 bcdefg	3.7±0.3 bcdef
Ga HI O/L	1117.6±261.2 fghij	2367.8±518.6 bcdefg	0.32±0.06 bcdefghij	3.1±0.5 cdefgh
PI 497648	1106.2±232.6 fghij	1734.3±201.3 efg	0.36±0.04 bcdefg	3±0.3 defgh
Line-2	1090.6±314.3 fghij	1927.4±469.1 defg	0.37±0.07 bcdefg	3.9±0.7 abcde
PI 370331	1064.1±379.1 ghijk	3124.5±923.7 abcdefg	0.23±0.04 mijkln	4.1±0.3 abcd
PI 337406	1021.2±253.8 ghijkl	2078.8±634.8 cdefg	0.35±0.02 bcdefgh	3.8±0.3 abcde
PI 268996	819.7±264.8 hijklm	3175.6±665.7 abcdefg	0.19±0.04 mklno	3.7±0.4 bcdef
PI 290594	808.6±242 hijklm	4254.9±2483.2 abc	0.21±0.05 mjkln	4.2±0.3 abcd
PI 155107	782.3±381.9 hijklm	1556±556.1 efg	0.34±0.04 bcdefghi	4.1±0.4 abcd
PI 493938	747.5±174 hijklm	2601±770.3 abcdefg	0.28±0.08 efghijk	3.4±0.4 bcdef
PI 339960	661.4±244.4 hijklm	1643.7±499.3 efg	0.26±0.05 ghijkl	4.3±0.3 abc
PI 290566	651.3±137.8 hijklm	2990.2±1044.6 abcdefg	0.2±0.03 mklno	4±0.3 abcd
PI 290560	618.8±128.8 hijklm	1763.2±284.9 efg	0.27±0.05 fghijkl	3.7±0.3 bcdef
PI 274193	584.6±270.7 hijklm	4411.1±1167.1 ab	0.12±0.03 nop	4±0.4 abcd
PI 390428	484.5±169.4 ijklm	2644.8±970.2 abcdefg	0.16±0.02 mlnop	3.9±0.5 abcde
PI 442768	377.1±87.3 ijklm	4080.7±1201.5 abcd	0.1±0.02 op	3.4±0.2 bcdef
PI 493581	355.6±189.7 ijklm	1246.7±129.8 fg	0.19±0.08 mklno	3.9±0.4 abcde
PI 268755	325.5±83.3 jklm	1387.6±371.3 fg	0.2±0.04 mklno	4.3±0.3 abc
PI 288210	205.7±63.8 klm	1660.6±381.7 efg	0.12±0.03 nop	4.1±0.3 abcd
CG7-A	183.7±29.1 lm	1294.5±288.9 fg	0.13±0.02 mnop	3.6±0.2 bcdef
PI 372305	121.1±54.6 m	1268.6±366.5 fg	0.07±0.02 p	4.5±0.2 ab
PI 325943	117.1±49.1 m	940.5±280.9 g	0.1±0.03 op	5±0 a
Cultivar (P-Value)	<.0001***	<.0001***	<.0001***	0.0009***
		Water Stress		
DS mean	1179.6	2803.1	0.27333	3.5389
WW mean	2777.2	4570.8	0.36583	1
Water Stress (P-Value)	<.0001***	<.0001***	0.0008***	<.0001***

Table 4.

Cultivar	Yield (Kg/ha)	Shoot Biomass (Kg/ha)	HI	Drought Index
AU 16-28	3415.6±408.4 a	4746±564.5 a	0.42±0.02 bc	2±0.6 de
G06G	3111.9±64.1 ab	2915.5±234.5 bcde	0.52±0.02 a	2±0.6 de
PI 493329	2927.7±53.2 abc	3014.6±116.8 bcde	0.49±0.01 abc	2.7±0.6 cde
PI 502120	2861.8±181.4 abc	3118.2±133.8 bcd	0.48±0.01 abc	2.3±0.6 de
AU 18-35	2816.3±504.3 abc	3793.5±489.1 ab	0.42±0.01 abc	4.3±0.6 abc
AU-17	2767.8±508.8 abcd	2838.6±586.2 bcdef	0.5±0.01 ab	3±0.6 bcde
Tifrunne	2733.8±230.3 abcd	3351.1±277.4 bc	0.45±0.02 abc	2.5±0.6 de
Line-4	2581.5±211 abcde	3936.1±330.1 ab	0.4±0.03 cd	3.2±0.6 bcde
Line-8	2485.5±287.9 bcde	3093.1±860.7 bcd	0.47±0.05 abc	2.3±0.6 de
C76-16	2197.4±457.1 cdef	2258.3±599.5 cdefg	0.5±0.01 ab	3.7±0.6 abcd
TifNVHig	1918.1±60.6 defg	2820.3±410.3 bcdef	0.41±0.03 bcd	2.7±0.6 cde
Ga Green	1824.8±232.5 efg	2002.7±242.7 defg	0.48±0.02 abc	4.5±0.6 ab
AP-3	1432.9±360.3 fgh	2107.1±230.8 cdefg	0.4±0.05 cd	5±0.6 a
PI 268755	1148.8±413 gh	1683.3±642.9 efg	0.4±0.05 cd	3.5±0.6 abcde
PI 290560	854±85.3 h	1067.6±213.3 g	0.45±0.02 abc	3.5±0.6 abcde
PI 325943	714.7±356 h	2539.6±978 efg	0.21±0.02 f	1.8±0.6 e
PI 339960	711.4±343.5 h	1501.8±764 g	0.32±0.07 de	3.7±0.6 abcd
PI 390428	623.8±289.3 h	1606.7±288.4 fg	0.25±0.08 ef	4.5±0.6 ab
Cultivar (P-Value)	<.0001***	<.0001***	<.0001***	0.0098**
		Water Stress		
DS mean	2062.64 b	2688.57 b	0.42	3.18 a
WW mean	3183.15 a	3578.76 a	0.46	1.00 b
Water Stress (P-Value)	0.0047**	0.0163*	0.2125NS	<.0001***

Table 5.

Parameter 2019	R^2	P (value)	Parameter	R^2	P (value)
HI	0.7145	<.0001***	LWC (8DAD)	0.0235	0.3724
Shoot biomass	0.3919	<.0001***	Photosynthesis (14DAD)	0.4008	<.0001***
Drought Rate	-0.5915	<.0001***	g_s (14DAD)	0.1894	0.008**
Seed $\Delta^{13}C$	0.4318	<.0001***	SLA (14DAD)	0.0485	0.1969
Seed $\delta^{15}N_{air}$	-0.3971	<.0001***	LWC (14DAD)	0.1548	0.0176*
Seed N%	-0.4746	<.0001***	Photosynthesis (21DAD)	0.3958	<.0001***
Shoot $\Delta^{13}C$	0.1619	0.015*	g_s (21DAD)	0.3526	0.0001***
Shoot $\delta^{15}N_{air}$	0.0318	0.2977	SLA (21DAD)	0.084	0.0865
Shoot N%	-0.2042	0.0057**	LWC (21DAD)	0.1986	0.0065**
Seed TN (Kg/ha)	0.9879	<.0001***	SLA (28DAD)	0.0414	0.2341
Shoot TN	0.177	0.0106*	LWC (28DAD)	0.1354	0.0273*
Plant TN (Kg/ha)	0.8374	<.0001***	Photosynthesis recovery	0.4725	<.0001***
SLA (0DAD)	0	0.9802	g_s recovery	0.4686	<.0001***
LWC (0DAD)	-0.0136	0.4981	SLA recovery	-0.015	0.4767
Photosynthesis (8DAD)	0.2202	0.0039**	LWC recovery	-0.0121	0.5238
g_s (8DAD)	0.3222	0.0003***	Photosynthesis Average	0.5773	<.0001***
SLA (8DAD)	0	0.9702	g_s Average	0.4926	<.0001***

Table 6.

Parameter 2020	**R^2**	**P (value)**	**Parameter**	**R^2**	**P (value)**
HI	0.5007	0.001**	g_s (10DAD)	0.4249	0.003**
Shoot biomass	0.6985	<.0001***	Photosynthesis (15DAD)	0.4276	0.003**
Drought Rate	-0.1958	0.065	g_s (15DAD)	0.3796	0.006**
Seed $\Delta^{13}C$	0.2421	0.038*	SLA (15DAD)	0.0009	0.907
Seed $\delta^{15}N$	-0.1799	0.079	LWC (15DAD)	0.2342	0.041*
Seed N%	-0.4577	0.002**	Photosynthesis (22DAD)	0.2959	0.019*
Shoot $\Delta^{13}C$	0.2695	0.027*	g_s (22DAD)	0.2029	0.060
Shoot $\delta^{15}N$	0.0095	0.700	SLA (22DAD)	0.0003	0.947
Shoot N%	-0.0874	0.233	LWC (22DAD)	0.3031	0.017*
Seed TN	0.9878	<.001***	Photosynthesis (29DAD)	0.0592	0.330
Shoot TN	0.5696	0.001***	g_s (29DAD)	0.0639	0.311
Plant TN	0.9548	<0.001***	SLA (29DAD)	-0.0077	0.729
Photosynthesis (0DAD)	0.3185	0.014*	LWC (29DAD)	0.015	0.628
g_s (0DAD)	0.2431	0.037*	Photosynthesis recovery	0.3697	0.007**
SLA (0DAD)	0.0922	0.220	g_s recovery	0.3676	0.007**
LWC (0DAD)	0.1115	0.175	SLA recovery	-0.0476	0.384
Photosynthesis (6DAD)	0.6097	0.001***	LWC recovery	-0.0025	0.844
g_s (6DAD)	0.4945	0.001**	Photosynthesis Average	0.6135	0.7158
Photosynthesis (10DAD)	0.6229	<.001***	g_s Average	0.5124	0.0008***

Table 7.

Cultivar	Δ^{13}C Seed (‰)	Δ^{13}C Shoot (‰)	δ^{15}N Seed (‰)	δ^{15}N Shoot (‰)
PI 502120	18.5±0.4 a	20±0.3	0.49±0.3 g	3.11±0.16
PI 493329	17.9±0.4 abcde	20±0.1	1.58±0.32 cdef	2.85±0.27
TifNV-High O/L	17.4±0.2 cdefghijk	19.3±0.2	1.22±0.23 defg	2.11±0.37
Tifrunner	18.2±0.1 ab	19.8±0.2	1.42±0.35 cdefg	2.16±0.15
Line-8	17.6±0.2 bcdefghij	19.6±0.1	1.16±0.33 defg	2.15±0.39
G06G	18±0.2 abcd	19.8±0.2	1.85±0.24 bcde	3.13±0.21
C76-16	18.1±0.3 abc	19.9±0.2	1.63±0.41 bcdef	2.15±0.36
AU-17	17.7±0.3 bcdefghi	19.1±0.4	1.03±0.38 efg	2.91±0.31
Line-4	17.8±0.2 abcdefg	19.7±0.3	1.39±0.22 defg	2.24±0.33
AU16-28	17.4±0.2 cdefghijk	19.3±0.1	1.61±0.26 cdef	2.82±0.33
SPT06-6	17.5±0.2 bcdefghijk	19.1±0.2	1.86±0.48 bcde	2.47±0.62
PI 196635	17.5±0.2 bcdefghij	19.3±0.1	1.14±0.18 defg	2.42±0.44
PI 576636	17.2±0.4 efghijkl	18.8±0.4	1.2±0.28 defg	2.98±0.25
AT 3085RO	17.2±0.3 defghijkl	19.3±0.3	1.52±0.29 cdef	2.33±0.3
Ga Green	17±0.3 ghijkl	18.8±0.3	0.78±0.34 fg	2.58±0.15
Ga HI O/L	17.1±0.3 efghijkl	19.4±0.2	1.74±0.27 bcdef	2.69±0.23
PI 497648	17.6±0.1 bcdefghij	19.8±0.1	1.69±0.3 bcdef	2.43±0.3
Line-2	17.1±0.3 fghijkl	19.2±0.3	1.67±0.42 bcdef	2.76±0.25
PI 370331	17.7±0.2 bcdefghi	19.8±0.4	1.63±0.36 bcdef	2.5±0.53
PI 337406	17.8±0.3 abcdefgh	19.5±0.2	1.74±0.12 bcdef	2.65±0.12
PI 268996	17.1±0.1 efghijkl	19.2±0.1	1.65±0.66 bcdef	2.23±0.28
PI 290594	17.3±0.3 cdefghijk	19.7±0.2	1.68±0.27 bcdef	2.06±0.3
PI 155107	18±0.5 abcde	19.6±0.2	1.99±0.45 bcde	2.64±0.26
PI 493938	18.2±0.6 ab	19.4±0.5	2.6±0.42 ab	2.94±0.32
PI 339960	17.7±0.5 bcdefghi	19.6±0.2	2.08±0.4 bcd	2.24±0.34
PI 290566	17±0.2 hijkl	19.3±0.2	1.55±0.25 cdef	1.97±0.28
PI 290560	17.8±0.4 abcdef	19.5±0.3	1.68±0.22 bcdef	2.39±0.23
PI 274193	17.1±0.2 efghijkl	19.3±0.3	1.9±0.4 bcde	1.99±0.31
PI 390428	17.3±0.2 cdefghijk	15.5±0.3	1.66±0.52 bcdef	2.07±0.4
PI 442768	16.4±0.2 l	18.8±0.1	1.82±0.25 bcdef	2.7±0.23
PI 493581	17.3±0.5 cdefghijk	18.9±0.7	2.45±0.4 abc	2.91±0.68
PI 268755	16.9±0.2 ijkl	19.3±0.2	2.11±0.59 bcd	2.64±0.36
PI 288210	17±0.3 ghijkl	18.7±0.2	1.97±0.2 bcde	2.42±0.25
CG7-A	16.7±0.2 kl	18.9±0.1	2.08±0.19 bcd	2.53±0.28
PI 372305	17.1±0.2 fghijkl	19.1±0.1	1.73±0.33 bcdef	2.07±0.27
PI 325943	16.8±0.2 jkl	19.1±0.3	3.4±0.69 a	2.87±0.44
Cultivar (P-Value)	<.0001***	0.3637	0.0042**	0.4808
		Water Stress		
DS mean	17.43 b	19.2619 b	1.69	2.5031 a
WW mean	18.53 a	19.9794 a	1.85	2.1992 b
Water Stress (P-Value)	<.0001***	<.0001***	0.3928NS	0.0467*

Table 8.

Cultivar	**Δ^{13}C Seed (‰)**	**Δ^{13}C Shoot (‰)**	**δ^{15}N Seed (‰)**	**δ^{15}N Shoot (‰)**
AU 16-28	18.4±0.4 abc	19.9±0.2 abc	1.22±0.36 def	2.41±0.29
G06G	18.8±0.1 abc	20±0.1 ab	0.91±0.14 f	2.33±0.48
PI 493329	19.1±0.4 a	20±0.3 abc	2.1±0.62 abcde	2.27±0.13
PI 502120	18.4±0.2 abc	19.7±0.2 bc	1.48±0.43 cdef	2.43±0.57
AU 18-35	18.3±0.4 abc	19.8±0.3 abc	2.71±0.31 a	2.93±0.36
AU-17	18.9±0.4 ab	20±0.1 abc	1.2±0.34 def	1.88±0.17
Tifrunne	18.6±0.3 abc	20±0.2 abc	1.05±0.33 f	1.55±0.33
Line-4	19±0.2 ab	20.5±0.2 a	1.26±0.25 cdef	1.98±0.28
Line-8	18±0.4 cd	19.7±0.1 bc	1.12±0.1 ef	1.97±0.32
C76-16	18.3±0.3 abc	19.9±0.1 abc	1.58±0.41 bcdef	2.06±0.2
TifNVHig	18.2±0.2 bcd	19.4±0.1 bc	1.23±0.43 cdef	1.9±0.38
Ga Green	18.4±0.2 abc	20.2±0.3 ab	1.4±0.45 cdef	1.94±0.31
AP-3	18.1±0.1 bcd	19.9±0.2 abc	1.88±0.07 abcdef	1.89±0.21
PI 268755	18.4±0.3 abc	19.6±0.1 bc	2.12±0.15 abcd	2.25±0.21
PI 290560	18.7±0.5 abc	19.8±0.5 abc	1.56±0.38 bcdef	2.22±0.62
PI 325943	17.9±0.3 bcd	18.9±0.3 d	1.41±0.18 bcdef	1.89±0.5
PI 339960	18.6±0 abc	19.9±0.4 abc	2.22±0.35 abc	1.69±0.38
PI 390428	17.4±0.2 d	19.3±0.4 cd	2.57±0.61 ab	2.95±0.65
Cultivar (P-Value)	0.078	0.0374*	0.0157*	0.4162NS
		Water Stress		
DS mean	18.43 b	19.80 b	1.61 a	2.14 a
WW mean	20.05 a	20.30 a	1.15 b	0.99 b
P-Value	<.0001***	0.0008***	0.0291*	<.0001***

Table 9.

Cultivar	Seed N%	Shoot N%	Seed TN (Kg/ha)	Shoot TN (Kg/ha)	Plant TN (Kg/ha)
PI 502120	4.65±0.2 lmno	1.38±0.15 fg	144.8±22.1 a	43.7±8.1 a	188.5±29.7 a
PI 493329	5.09±0.11 ghijk	1.3±0.11 g	137.3±28.6 ab	31.6±8.5 abcde	149.4±35.1 abc
TifNV-High O/L	5.17±0.09 ghijk	1.86±0.1 abcd	124±12.9 abc	43.6±2.7 ab	167.6±15 ab
Tifrunner	5.03±0.04 ghijkl	1.49±0.1 defg	114.7±10.1 abc	34.1±3.9 abc	148.8±13.7 abc
Line-8	4.86±0.04 jklmn	1.32±0.06 g	108±21.6 abcd	28.7±4.7 cdef	136.7±26.2 abcde
G06G	4.84±0.08 klmn	1.48±0.05 defg	105.8±17.6 abcde	32.1±5.4 abcd	137.9±22.8 abcd
C76-16	4.52±0.11 no	1.32±0.14 g	91.1±25.7 cdef	27.2±7.7 cdefg	118.2±33.2 bcdefg
AU-17	4.87±0.06 jklmn	1.91±0.21 abc	94.2±25.3 bcdef	36.1±11.9 abc	130.3±36.3 bcdef
Line-4	4.43±0.06 o	1.26±0.17 g	81.8±21.9 cdefg	20.7±4.4 defghi	102.5±25.8 cdefgh
AU16-28	4.85±0.08 klmn	1.43±0.06 efg	87.6±10.1 cdef	25.8±2.8 cdefgh	113.3±12.7 bcdefg
SPT06-6	5.07±0.05 ghijk	1.8±0.12 abcde	90.8±19.8 cdef	30.9±4.8 bcde	121.8±24.3 bcdefg
PI 196635	4.94±0.16 ijklm	1.48±0.16 defg	68.3±15.4 defgh	19.6±3.7 defghij	87.9±18.8 defghi
PI 576636	5.19±0.15 fghijk	1.78±0.14 abcdef	62.1±22.4 efghij	20.6±6.5 defghi	82.8±28.7 efghij
AT 3085RO	5.32±0.08 defghi	1.48±0.12 defg	64.8±24.8 defghi	16±4.9 ghijkl	80.9±29.7 fghij
Ga Green	4.64±0.23 mno	1.76±0.08 abcdef	52.1±12.8 fghijk	20.2±4.7 defghi	72.3±17.4 ghijk
Ga HI O/L	5.24±0.09 efghij	1.58±0.12 bcdefg	58.3±13.5 fghij	17.3±3.7 fghijk	75.6±17 fghijk
PI 497648	4.86±0.04 jklmn	1.49±0.14 defg	53.6±11.1 fghijk	16.8±4.3 fghijk	70.5±15.1 ghijk
Line-2	4.92±0.14 jklm	1.47±0.13 defg	52.5±14 fghijk	15.7±4 ghijkl	68.1±17.7 ghijkl
PI 370331	4.95±0.13 ijklm	1.65±0.2 bcdefg	52±18.5 fghijk	18±5.7 efghijk	77.4±27.4 fghijk
PI 337406	5.64±0.06 cd	1.47±0.04 defg	57±13.4 fghij	14.6±3.1 ghijklm	71.7±16.5 ghijk
PI 268996	4.99±0.2 hijklm	1.64±0.09 bcdefg	40.3±12.2 ghijk	13.5±4.5 hijklm	53.8±16.6 hijklm
PI 290594	5.04±0.16 ghijkl	1.52±0.14 cdefg	41.7±13.2 ghijk	12.5±4.4 ijklm	54.3±17.5 hijklm
PI 155107	5.75±0.26 bc	1.42±0.07 efg	53.7±22.9 fghijk	10.2±4.8 ijklm	66.3±28.1 ghijklm
PI 493938	5.57±0.16 cdef	1.42±0.2 efg	40.8±8.8 ghijk	9.6±1.7 ijklm	50.4±10.2 hijklm
PI 339960	5.63±0.16 cd	1.58±0.15 bcdefg	37.2±13.2 hijk	9.4±3.1 ijklm	46.6±16.3 ijklm
PI 290566	5.39±0.16 cdefgh	1.87±0.12 abcd	35.2±7.9 hijk	12.6±3.1 ijklm	47.9±10.9 hijklm
PI 290560	5.42±0.05 cdefg	1.28±0.13 g	33.4±6.7 hijk	8.2±2 ijklm	41.6±8.6 ijklm
PI 274193	5.62±0.3 cde	1.79±0.24 abcdef	33.4±16.8 hijk	8.8±3.2 ijklm	43.1±20.8 ijklm
PI 390428	5.74±0.09 bc	1.48±0.07 defg	27.8±9.7 hijk	4.9±1.8 klm	32.6±9.2 jklm
PI 442768	5.4±0.17 cdefgh	1.88±0.08 abcd	21.6±5.8 ijk	7.2±1.8 jklm	29.2±8 jklm
PI 493581	6.28±0.3 a	1.47±0.19 defg	21.2±10.6 ijk	4.2±1.7 klm	25.5±12.3 jklm
PI 268755	5.63±0.09 cd	1.59±0.09 bcdefg	18.5±4.9 jk	5.1±1.3 klm	23.7±6.2 klm
PI 288210	5.19±0.14 fghijk	2.11±0.07 a	10.5±3.1 k	4.4±1.4 klm	14.9±4.5 lm
CG7-A	5.39±0.23 cdefgh	1.86±0.11 abcd	9.7±1.4 k	3.3±0.5 lm	13.1±1.8 lm
PI 372305	6.12±0.09 ab	2.17±0.22 a	7.2±3.2 k	2.3±0.9 m	9.5±4.1 m
PI 325943	5.62±0.13 cde	1.99±0.25 ab	8.1±3.3 k	2±0.7 m	10.4±4.1 lm
Cultivar (P-Value)	<.0001***	<.0001***	<.0001***	0.002**	<.0001***
			Water Stress		
DS mean	5.22 a	1.605 a	59.48 b	44.327 b	103.34 b
WW mean	4.72 b	1.3025 b	130.23 a	61.433 a	191.68 a
Water Stress (P-Value)	<.0001***	<.0001***	<.0001***	0.0071**	<.0001***

Table 10.

Cultivar	**Seed %N**	**Shoot %N**	**Seed TN (Kg/ha)**	**Shoot TN (Kg/ha)**	**Plant TN (Kg/ha)**
AU 16-28	4.54±0 de	1.39±0.09 cdef	155±18.7 a	27.9±4.9 a	182.9±22.9 a
G06G	4.39±0.07 def	1.68±0.1 abc	136.7±1.2 ab	25.3±2.5 abcd	161.9±3.5 ab
PI 493329	4.13±0.13 fg	1.31±0.08 ef	120.9±5.1 abcd	19.5±1.5 cdef	140.4±6.3 bcd
PI 502120	4.39±0.12 def	1.59±0.09 bcde	125.7±9.2 abc	23.6±0.5 abcd	149.2±9.5 abc
AU 18-35	4.45±0.03 de	1.66±0.18 abcd	125.6±23.5 abc	26.4±4.1 ab	152±27.2 ab
AU-17	4.45±0.13 de	1.49±0.03 cde	121.8±19.2 abcd	21±4.2 bcde	142.8±23.4 bcd
Tifrunner	4.49±0.03 de	1.52±0.11 cde	122.8±10.7 abcd	22.8±2.6 abc	145.6±12.3 abc
Line-4	4.02±0.11 g	1.16±0.07 f	104.1±11.1 bcd	18.1±1.9 bcd	122.2±13 bcde
Line-8	4.38±0.05 ef	1.46±0.06 cde	108.8±12.2 bcd	19.8±4 bcd	128.6±16.2 bcd
C76-16	4.14±0.01 fg	1.46±0.09 cde	90.8±18.5 cde	16±3.4 cdefg	106.8±21.8 cdef
TifNVHig	4.69±0.08 cd	1.82±0.04 ab	89.9±3.5 cde	20.6±1.3 abc	110.5±4.7 bcde
Ga Green	4.65±0.17 cde	1.56±0.06 bcde	84.7±9.9 de	14.7±1.3 defg	99.4±11.2 def
AP-3	4.45±0.03 de	1.52±0.02 cde	64±16.5 ef	12.6±2.2 cdefg	76.6±18.7 efg
PI 268755	5.04±0.09 ab	1.39±0.19 cdef	58±21.2 efg	9.2±3.9 efg	67.2±24.8 fg
PI 290560	4.87±0.18 bc	1.37±0.11 def	41.8±5.5 fg	6.6±1.5 g	48.4±7 g
PI 325943	4.43±0.23 cde	1.9±0.14 a	30.5±13.8 g	9.9±4.3 cdefg	40.4±18.1 g
PI 339960	5.19±0.12 a	1.4±0.09 cdef	37.8±18.9 fg	6.6±3.5 fg	44.3±22.3 g
PI 390428	5±0.34 ab	1.92±0.11 a	29.3±12.6 fg	7.8±3.1 defg	37.1±15.7 g
Cultivar (P-Value)	<.0001***	0.0004***	<.0001***	0.0003***	<.0001***
Water Stress					
DS mean	4.54	1.53	91.55	40.80	132.35
WW mean	4.06	1.28	127.19	46.20	173.39
Water Stress (P-Value)	0.0003***	0.0002***	0.0224*	0.3078NS	0.0293*

Table 11.

Year		A	g_s	SLA	LWC
2019	**Cultivar**	<.0001***	<.0001***	<.0001***	<.0001***
	DAD	0.6334	0.4647	<.0001***	<.0001***
	Cult*DAD	0.0276*	0.23	0.3378	0.9022
2020	**Cultivar**	<.0001***	<.0001***	<.0001***	0.0217*
	DAD	<.0001***	<.0001***	<.0001***	0.0016**
	Cult*DAD	0.1949	<.0001***	0.8303	0.7423

Table 12.

Heritability	**2019**	**2020**
Yield	0.51	0.73
Shoot	0.15	0.49
HI	0.54	0.64
Seed $\Delta^{13}C$	0.24	0.22
Seed $\delta^{15}N$	0.15	0.33
Seed N%	0.61	0.71
Shoot $\Delta^{13}C$	0.14	0.20
Shoot $\delta^{15}N$	0.00	0.02
Shoot N%	0.30	0.50
Seed TN	0.48	0.71
Shoot TN	0.15	0.43
Plant TN	0.36	0.65
Photosynthesis	0.13	0.48
g_s	0.09	0.41
SLA	0.17	0.44
LWC	0.00	0.11
Photo Recovery	0.37	0.17
g_s Revocery	0.25	0.15
SLA Recovery	0.20	0.24
LWC Recovery	0.15	0.02

Figure 1.


2019
2020
WW
WS
LWC
SLA
a)
b)
c)
d)
80
78
76
74
72
180
170
160
150
140
0 DAD
8 DAD
14 DAD
21 DAD
28 DAD
15 DAD
22 DAD
29 DAD
Recovery
a
b
c
d
ab
bc

Figure 2.

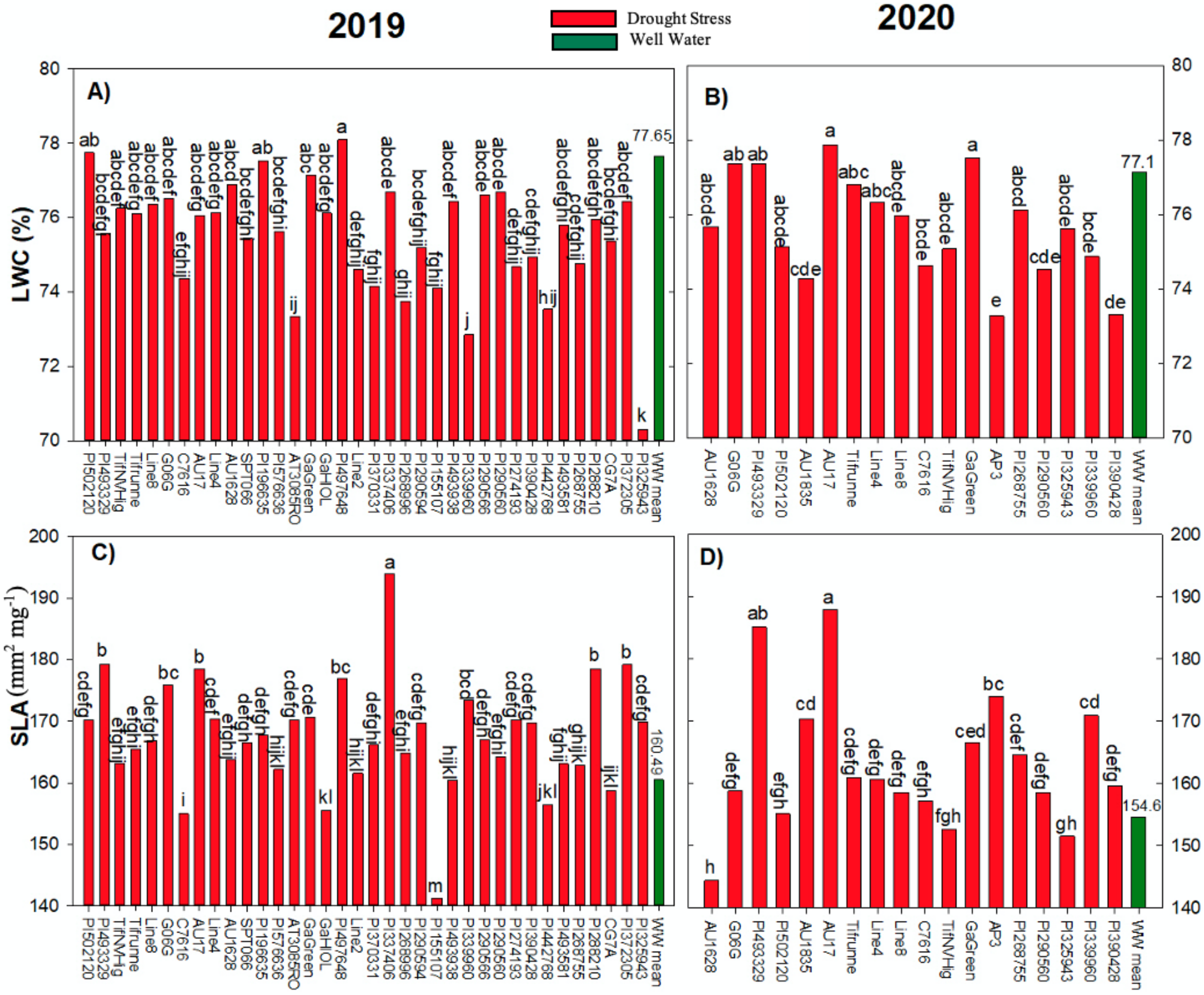

2019
2020
🟥 Drought Stress
🟩 Well Water
A)
B)
C)
D)
LWC (%)
SLA (mm^2 mg^{-1})
80
78
76
74
72
70
200
190
180
170
160
150
140
PI502120 ab
PI493329 bcdefg
TifNVHig abcdef
Tifrunne abcdefg
Line8 abcdef
G06G abcdef
C7616 efghij
AU17 abcdefg
Line4 abcdefg
AU1628 abcd
SPT066 bcdefgh
PI196635 ab
PI576636 bcdefghi
AT3085RO ij
GaGreen abc
GaHIOL abcdefg
PI497648 a
Line2 defghij
PI370331 fghij
PI337406 abcde
PI268996 ghij
PI290594 bcdefghij
PI155107 fghij
PI493938 abcdef
PI339960 j
PI290566 abcde
PI290560 abcde
PI274193 defghij
PI390428 cdefghij
PI442768 hij
PI493581 abcdefghi
PI268755 cdefghij
PI288210 abcdefgh
CG7A bcdefghi
PI372305 abcdef
PI325943 k
WW mean 77.65
AU1628 abcde
G06G ab
PI493329 ab
PI502120 abcde
AU1835 cde
AU17 a
Tifrunne abc
Line4 abc
Line8 abcde
C7616 bcde
TifNVHig abcde
GaGreen a
AP3 e
PI268755 abcd
PI290560 cde
PI325943 abcde
PI339960 bcde
PI390428 de
WW mean 77.1
PI502120 cdefg
PI493329 b
TifNVHig efghij
Tifrunne efghi
Line8 defgh
G06G bc
C7616 i
AU17 b
Line4 cdef
AU1628 efghij
SPT066 defgh
PI196635 defgh
PI576636 hijkl
AT3085RO cdefg
GaGreen cde
GaHIOL kl
PI497648 bc
Line2 hijkl
PI370331 defghi
PI337406 a
PI268996 efghi
PI290594 cdefg
PI155107 m
PI493938 hijkl
PI339960 bcd
PI290566 defgh
PI290560 efghi
PI274193 cdefg
PI390428 cdefg
PI442768 jkl
PI493581 fghij
PI268755 ghijk
PI288210 b
CG7A ijkl
PI372305 b
PI325943 cdefg
WW mean 160.49
AU1628 h
G06G defg
PI493329 ab
PI502120 efgh
AU1835 cd
AU17 a
Tifrunne cdefg
Line4 defg
Line8 defg
C7616 efgh
TifNVHig fgh
GaGreen ced
AP3 bc
PI268755 cdef
PI290560 defg
PI325943 gh
PI339960 cd
PI390428 defg
WW mean 154.6

Figure 3.

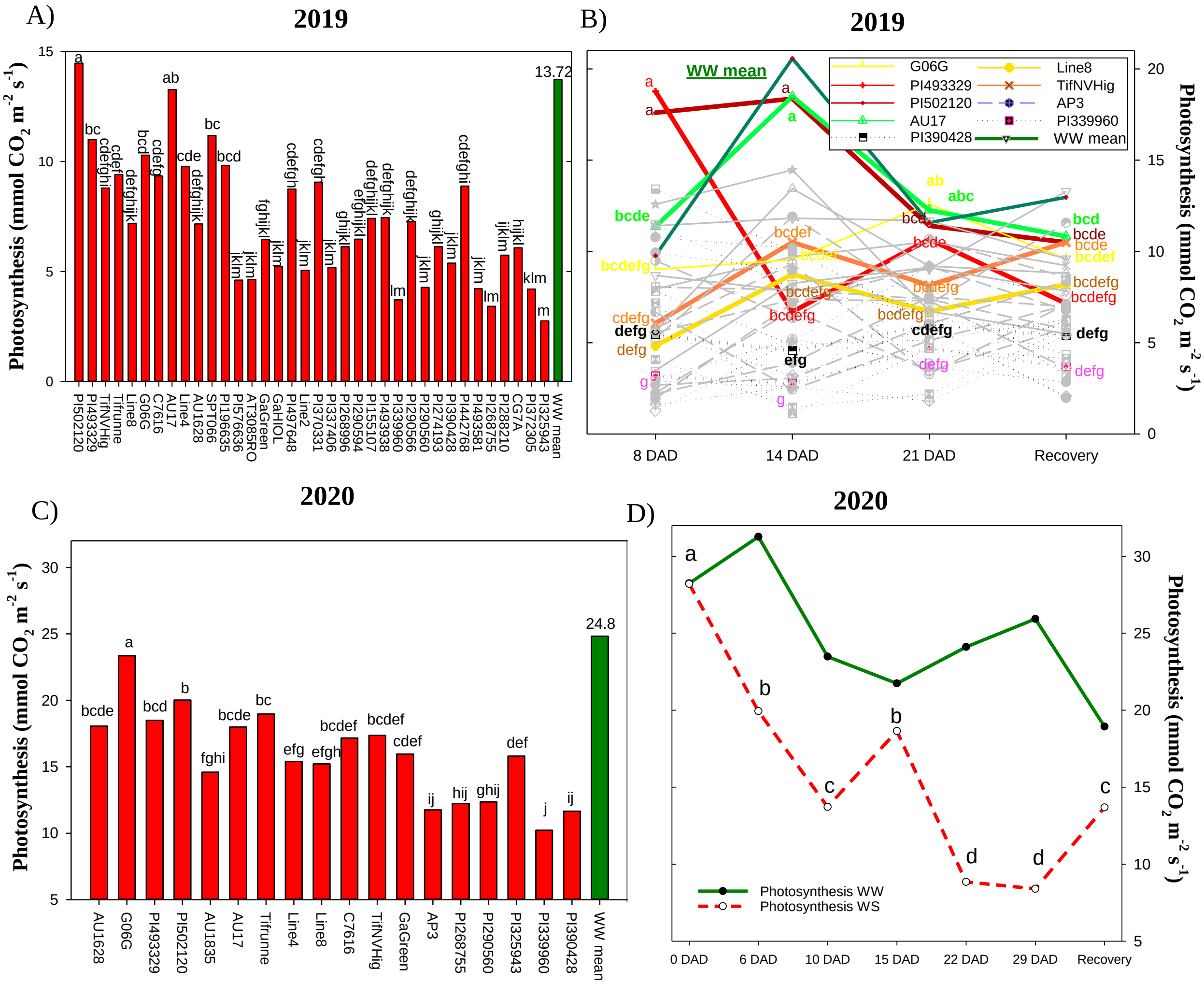

A)
2019
Photosynthesis (mmol CO$_2$ m^{-2} s^{-1})
0
5
10
15
a
bc
cdef
cdefghi
defghijk
bcd
cdefg
ab
cde
defghijk
bc
bcd
klm
jklm
fghijkl
ijklm
cdefgh
ijklm
cdefgh
ijklm
ghijkl
efghijkl
defghijkl
defghijk
lm
defghijk
jklm
ghijkl
jklm
cdefghi
jklm
lm
ijklm
hijkl
klm
m
13.72
PI502120
PI493329
TifNVHig
Tifrunne
Line8
G06G
C7616
AU17
Line4
AU1628
SPT066
PI196635
PI576636
AT3085RO
GaGreen
GaHIOL
PI497648
Line2
PI370331
PI337406
PI268996
PI290594
PI155107
PI493938
PI339960
PI290566
PI290560
PI274193
PI390428
PI442768
PI493581
PI268755
PI288210
CG7A
PI372305
PI325943
WW mean
B)
2019
WW mean
G06G
PI493329
PI502120
AU17
PI390428
Line8
TifNVHig
AP3
PI339960
WW mean
a
a
a
a
bcde
bcdefg
cdefg
defg
defg
g
bcdef
bcdef
bcdefg
bcdefg
efg
g
ab
abc
bcd
bcde
bcde
bcdefg
bcdefg
cdefg
defg
bcd
bcde
bcde
bcdef
bcdefg
bcdefg
defg
defg
8 DAD
14 DAD
21 DAD
Recovery
0
5
10
15
20
Photosynthesis (mmol CO$_2$ m^{-2} s^{-1})
C)
2020
Photosynthesis (mmol CO$_2$ m^{-2} s^{-1})
5
10
15
20
25
30
bcde
a
bcd
b
fghi
bcde
bc
efg
efgh
bcdef
bcdef
cdef
ij
hij
ghij
def
j
ij
24.8
AU1628
G06G
PI493329
PI502120
AU1835
AU17
Tifrunne
Line4
Line8
C7616
TifNVHig
GaGreen
AP3
PI268755
PI290560
PI325943
PI339960
PI390428
WW mean
D)
2020
a
b
c
b
d
d
c
Photosynthesis WW
Photosynthesis WS
0 DAD
6 DAD
10 DAD
15 DAD
22 DAD
29 DAD
Recovery
5
10
15
20
25
30
Photosynthesis (mmol CO$_2$ m^{-2} s^{-1})

Figure 4.

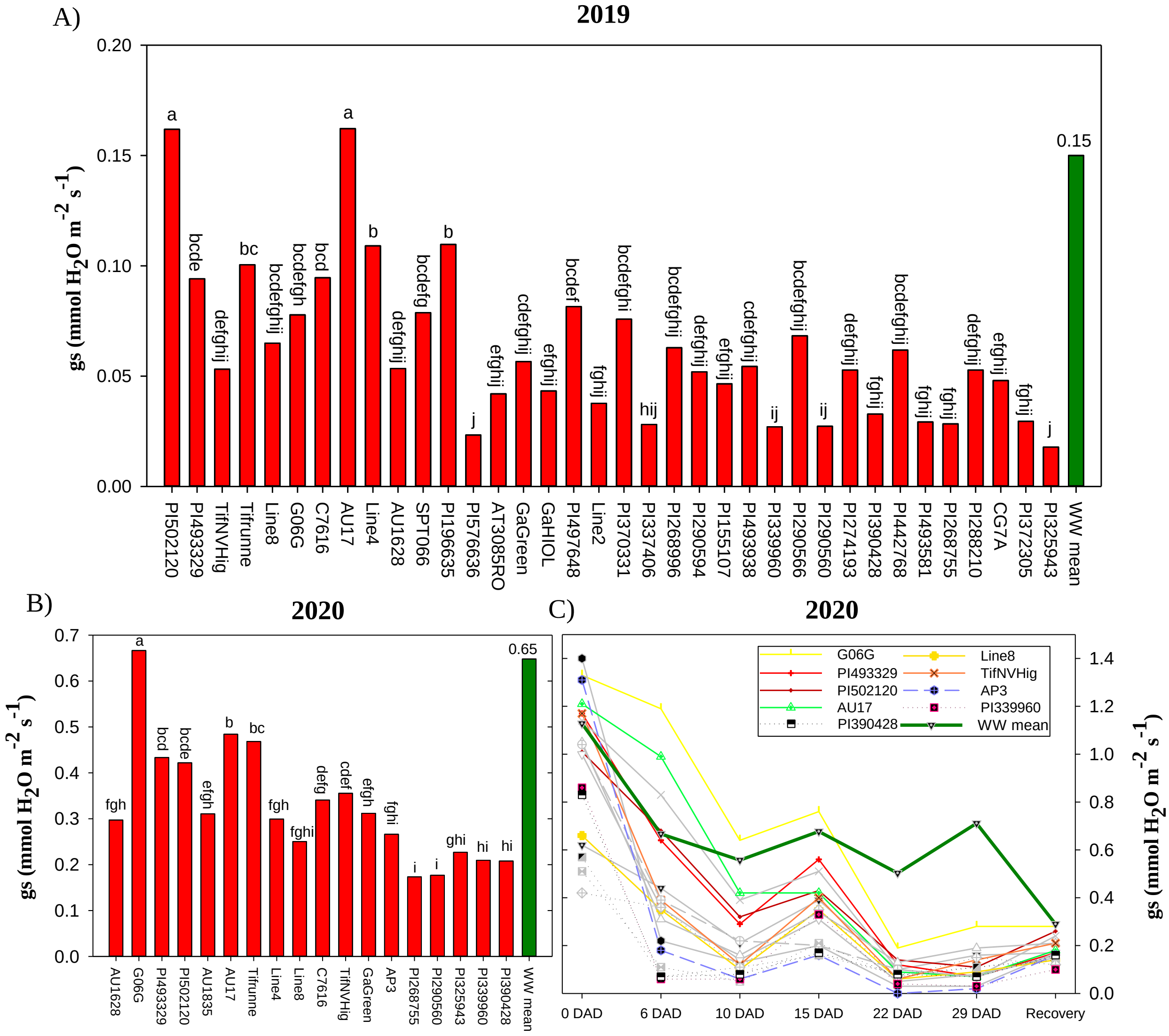

A)
2019
gs (mmol H_2O m^{-2} s^{-1})
0.00
0.05
0.10
0.15
0.20
a
bcde
defghij
bc
bcdefghij
bcdefgh
bcd
a
b
defghij
bcdefg
b
j
efghij
cdefghij
efghij
bcdef
fghij
bcdefghi
hij
bcdefghij
defghij
efghij
cdefghij
ij
bcdefghij
ij
defghij
fghij
bcdefghij
fghij
fghij
defghij
efghij
fghij
j
0.15
PI502120
PI493329
TifNVHig
Tifrunne
Line8
G06G
C7616
AU17
Line4
AU1628
SPT066
PI196635
PI576636
AT3085RO
GaGreen
GaHIOL
PI497648
Line2
PI370331
PI337406
PI268996
PI290594
PI155107
PI493938
PI339960
PI290566
PI290560
PI274193
PI390428
PI442768
PI493581
PI268755
PI288210
CG7A
PI372305
PI325943
WW mean
B)
2020
gs (mmol H_2O m^{-2} s^{-1})
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
fgh
a
bcd
bcde
efgh
b
bc
fgh
fghi
defg
cdef
efgh
fghi
i
i
ghi
hi
hi
0.65
AU1628
G06G
PI493329
PI502120
AU1835
AU17
Tifrunne
Line4
Line8
C7616
TifNVHig
GaGreen
AP3
PI268755
PI290560
PI325943
PI339960
PI390428
WW mean
C)
2020
G06G
PI493329
PI502120
AU17
PI390428
Line8
TifNVHig
AP3
PI339960
WW mean
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
gs (mmol H_2O m^{-2} s^{-1})
0 DAD
6 DAD
10 DAD
15 DAD
22 DAD
29 DAD
Recovery

Figure 5.

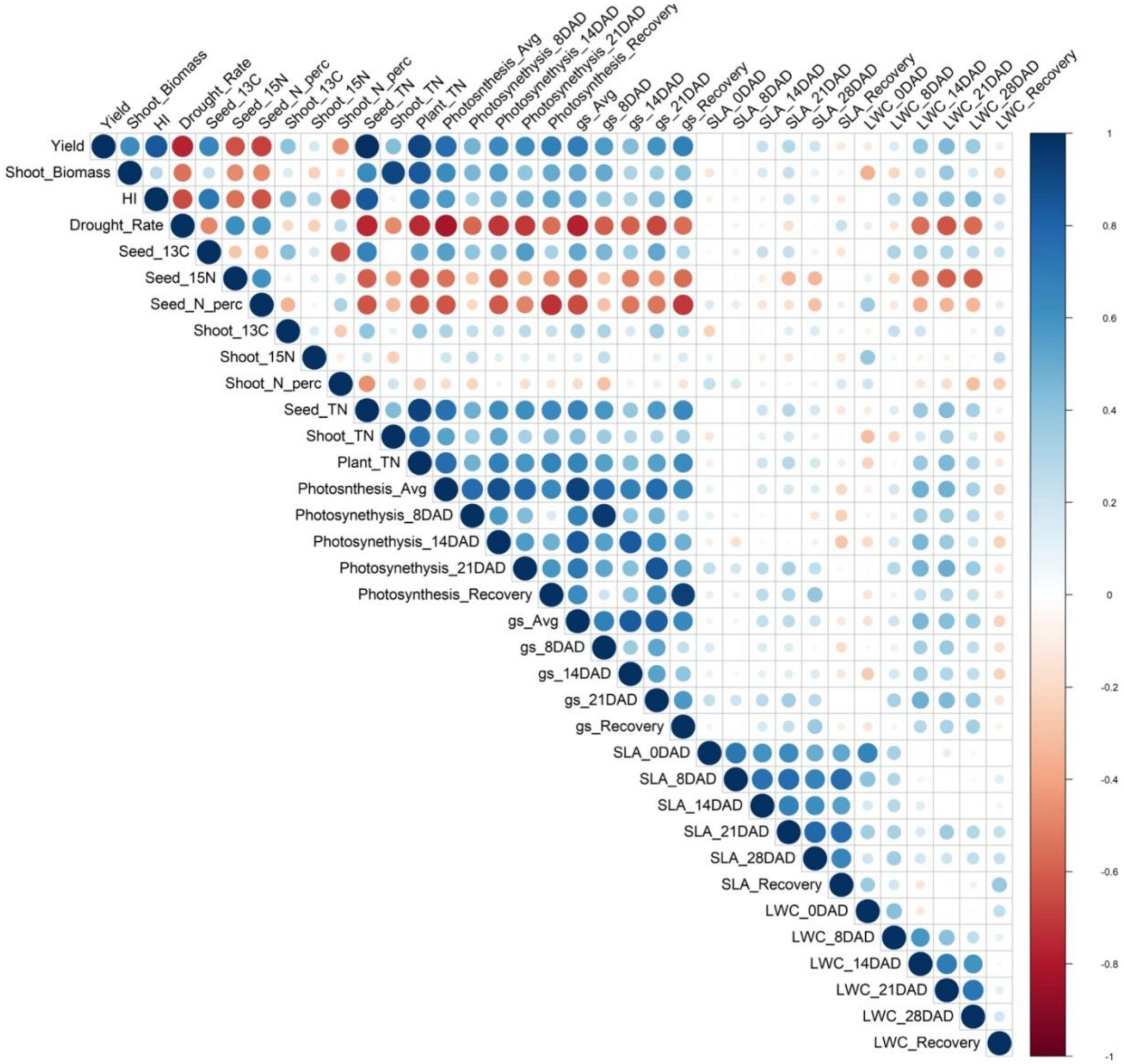

Yield
Shoot_Biomass
HI
Drought_Rate
Seed_13C
Seed_15N
Seed_N_perc
Shoot_13C
Shoot_15N
Shoot_N_perc
Seed_TN
Shoot_TN
Plant_TN
Photosnthesis_Avg
Photosynethysis_8DAD
Photosynethysis_14DAD
Photosynethysis_21DAD
Photosynthesis_Recovery
gs_Avg
gs_8DAD
gs_14DAD
gs_21DAD
gs_Recovery
SLA_0DAD
SLA_8DAD
SLA_14DAD
SLA_21DAD
SLA_28DAD
SLA_Recovery
LWC_0DAD
LWC_8DAD
LWC_14DAD
LWC_21DAD
LWC_28DAD
LWC_Recovery
Yield
Shoot_Biomass
HI
Drought_Rate
Seed_13C
Seed_15N
Seed_N_perc
Shoot_13C
Shoot_15N
Shoot_N_perc
Seed_TN
Shoot_TN
Plant_TN
Photosnthesis_Avg
Photosynethysis_8DAD
Photosynethysis_14DAD
Photosynethysis_21DAD
Photosynthesis_Recovery
gs_Avg
gs_8DAD
gs_14DAD
gs_21DAD
gs_Recovery
SLA_0DAD
SLA_8DAD
SLA_14DAD
SLA_21DAD
SLA_28DAD
SLA_Recovery
LWC_0DAD
LWC_8DAD
LWC_14DAD
LWC_21DAD
LWC_28DAD
LWC_Recovery
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure 6.

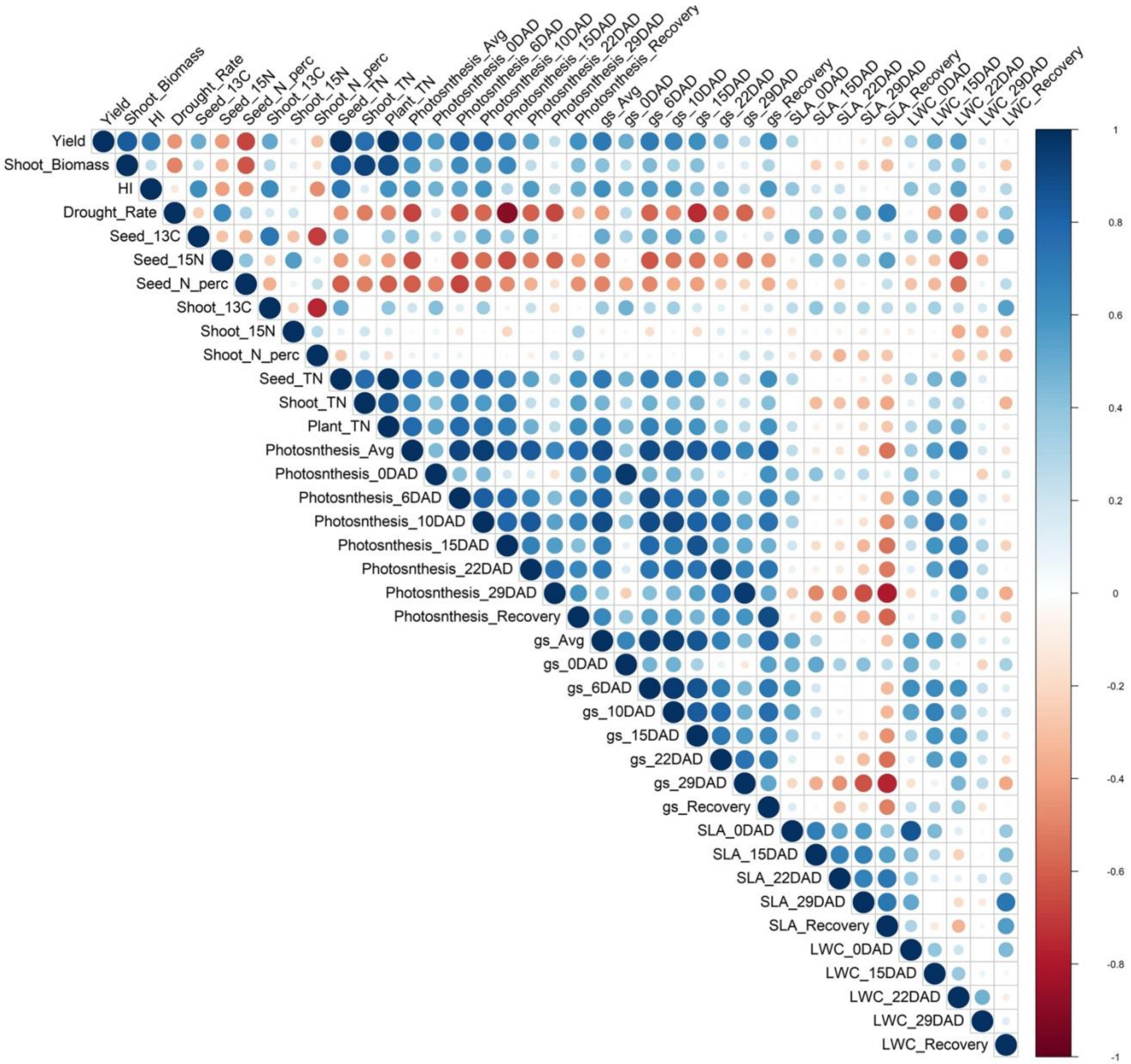

Yield
Shoot_Biomass
HI
Drought_Rate
Seed_13C
Seed_15N
Seed_N_perc
Shoot_13C
Shoot_15N
Shoot_N_perc
Seed_TN
Shoot_TN
Plant_TN
Photosnthesis_Avg
Photosnthesis_0DAD
Photosnthesis_6DAD
Photosnthesis_10DAD
Photosnthesis_15DAD
Photosnthesis_22DAD
Photosnthesis_29DAD
Photosnthesis_Recovery
gs_Avg
gs_0DAD
gs_6DAD
gs_10DAD
gs_15DAD
gs_22DAD
gs_29DAD
gs_Recovery
SLA_0DAD
SLA_15DAD
SLA_22DAD
SLA_29DAD
SLA_Recovery
LWC_0DAD
LWC_15DAD
LWC_22DAD
LWC_29DAD
LWC_Recovery
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure 7.

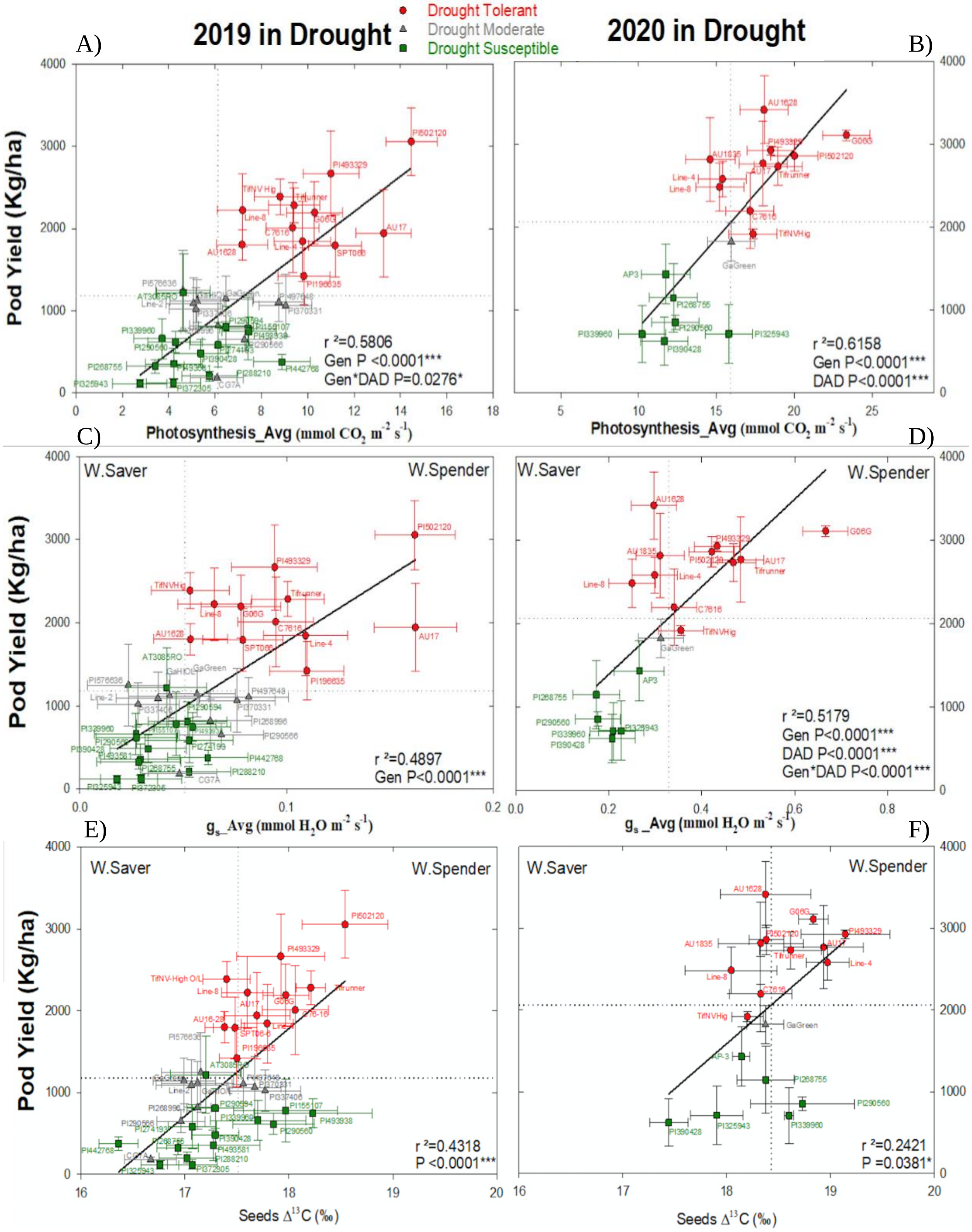
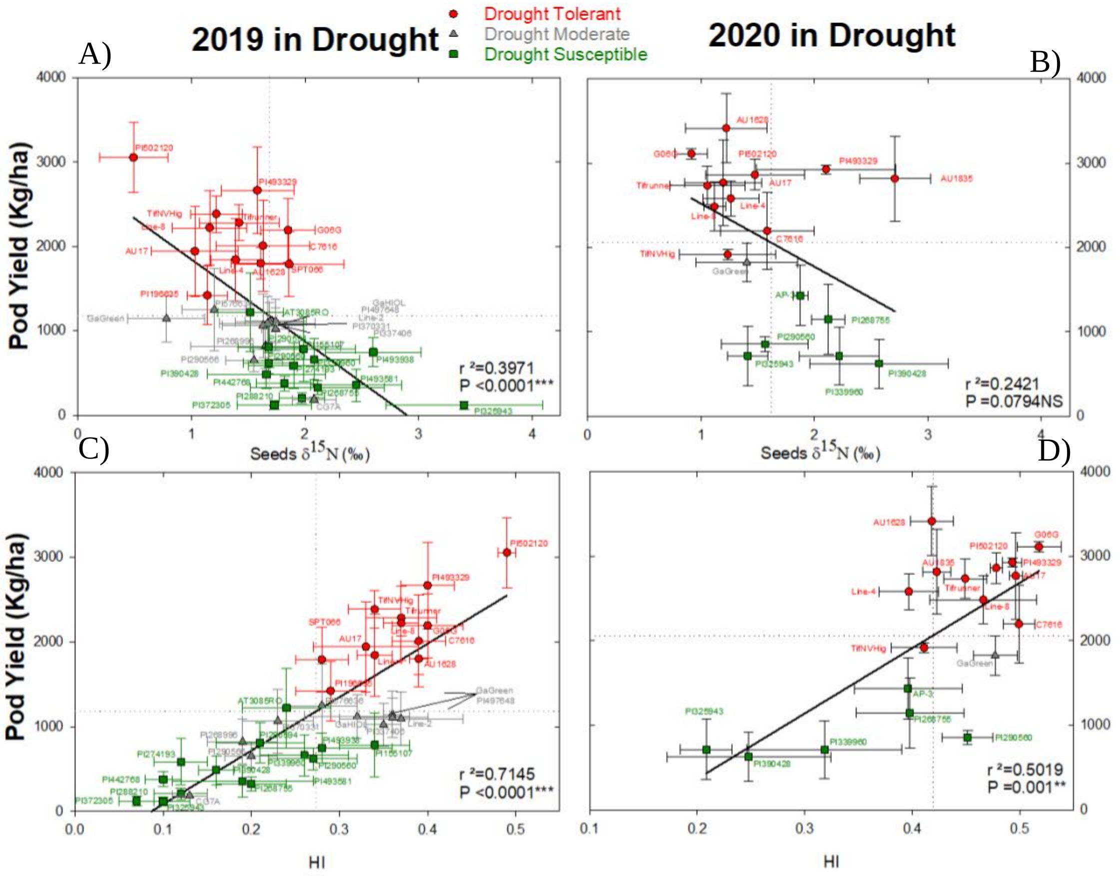

2019 in Drought
2020 in Drought
● Drought Tolerant
▲ Drought Moderate
■ Drought Susceptible
A)
B)
C)
D)
E)
F)
Pod Yield (Kg/ha)
0
1000
2000
3000
4000
Photosynthesis_Avg (mmol CO$_2$ m^{-2} s^{-1})
0
2
4
6
8
10
12
14
16
18
5
10
15
20
25
r ^{2}=0.5806
Gen P <0.0001***
Gen*DAD P=0.0276*
r ^{2}=0.6158
Gen P<0.0001***
DAD P<0.0001***
PI502120
PI493329
TifNV Hig
Tifrunner
Line-8
G06G
C7616
AU1628
AU17
Line-4
SPT06
PI196635
PI576636
AT3085RO
Line-2
GaGreen
PI497648
PI370331
PI339960
PI290594
PI155107
PI493938
PI250560
PI274193
PI290566
PI390428
PI268755
PI493581
PI442768
PI288210
PI325943
PI372305
CG7A
AU1835
PI493329
Line-4
Line-8
Tifrunner
C7616
TifNVHig
GaGreen
AP3
PI268755
PI290560
PI339960
PI325943
PI390428
W.Saver
W.Spender
g$_s$_Avg (mmol H$_2$O m^{-2} s^{-1})
0.0
0.1
0.2
0.0
0.2
0.4
0.6
0.8
r ^{2}=0.4897
Gen P<0.0001***
r ^{2}=0.5179
Gen P<0.0001***
DAD P<0.0001***
Gen*DAD P<0.0001***
TifNVHig
GaHIOL
PI337406
PI288996
CG7A
Seeds Δ^{13}C (‰)
16
17
18
19
20
r ^{2}=0.4318
P <0.0001***
r ^{2}=0.2421
P =0.0381*
TifNV-High O/L
AU16-28
SPT06-6
C76-16
AP-3
PI290560

Figure 8.


A)
2019 in Drought
● Drought Tolerant
▲ Drought Moderate
■ Drought Susceptible
2020 in Drought
B)
Pod Yield (Kg/ha)
0
1000
2000
3000
4000
PI502120
PI493329
TifNVHig
Tifrunner
Line-8
G06G
C7616
AU17
Line-4
AU1628
SPT066
PI196635
GaGreen
GaHIOL
PI497648
AT3085RO
Line-2
PI370331
PI337406
PI268996
PI290566
PI390428
PI442768
PI288210
PI372305
PI493938
PI493581
PI268755
CG7A
PI325943
r ²=0.3971
P <0.0001***
AU1628
G06G
PI502120
PI493329
AU1835
Tifrunner
AU17
Line-4
Line-8
C7616
TifNVHig
GaGreen
AP-3
PI268755
PI290560
PI325943
PI339960
PI390428
r ²=0.2421
P =0.0794NS
Seeds δ¹⁵N (‰)
0
1
2
3
4
C)
D)
PI502120
PI493329
TifNVHig
Tifrunner
SPT066
Line-8
C7616
AU17
AU1628
PI196635
GaGreen
PI497648
AT3085RO
PI268996
PI370331
PI337406
GaHIOL
Line-2
PI274193
PI442768
PI288210
PI372305
PI325943
CG7A
PI390428
PI339960
PI290560
PI493938
PI493581
PI155107
PI268755
r ²=0.7145
P <0.0001***
0.0
0.1
0.2
0.3
0.4
0.5
HI
AU1628
G06G
PI502120
PI493329
AU1835
Line-4
Tifrunner
AU17
Line-8
C7616
TifNVHig
GaGreen
AP-3
PI268755
PI290560
PI325943
PI339960
PI390428
r ²=0.5019
P =0.001**
0.1
0.2
0.3
0.4
0.5
HI