



Soybean seed physiology, quality, and chemical composition under soil moisture stress

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ABSTRACT

Soybean seed quality is often determined by its constituents which are important to sustain overall nutritional aspects. The objective of this study was to investigate the effects of soil moisture stress during reproductive stage on seed quality and composition. Plants were subjected to five levels of soil moisture stresses at flowering, and yield and quality traits were examined at maturity. Seed protein, palmitic and linoleic acids, sucrose, raffinose, stachyose, N, P, K, and Ca significantly decreased whereas oil, stearic, oleic and linolenic acids, Fe, Mg, Zn, Cu, and B increased in response to soil moisture deficiency. The relationship between seed protein and oil was negatively correlated. The changes in seed constituents could be due to changes in nutrient accumulation and partitioning in soybean seeds under water stress. This information suggests the requirement of adequate soil moisture during flowering and seed formation stages to obtain the higher nutritional value of soybean seeds.

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is an important grain legume and one of the most valued oilseed crops in the world. Due to its unique chemical composition, it is widely grown as a valuable agricultural commodity throughout the world mainly in the United States (117 mmt), Brazil (114 mmt), Argentina (58 mmt), China (13 mmt), and India (12 mmt) (USDA, 2018). Soybean is mainly produced for soymeal and vegetable oil. The seeds provide approximately 60% of the world supply of vegetable protein and are considered to be a good substituent for animal protein (Liu, 1997). Also, soybeans are processed into several types of non-fermented (soy milk, tofu, soy cake) and fermented (soy sauce, natto, tempeh) food products. Recently, soy foods and its bioactive compounds received significant attention because of their human health benefits by lowering the risks of several heart diseases and cancers, including endometrial, breast, prostate, colon, lung, and bladder cancers (Sun et al., 2002).

Environmental factors, which vary spatially and temporally during the growing season and among years, play a major role in controlling soybean seed yield, quality, and its chemical composition. Among many of these environmental factors, soil moisture stress is undoubtedly one of the most important abiotic stresses causing a significant yield and quality reductions in soybean. Soybean yield reduction due to soil moisture stress depends on the severity and duration of the stress,

genotype, and the growth stage (Piper & Boote, 1999). The yield and quality losses during the early vegetative stage have reported being less obvious than reproductive stage (Mertz-Henning et al., 2018). However, some studies, have reported a yield reduction due to the reduced number of mainstem nodes and branches at early vegetative stages (Brevedan & Egli, 2003). The effect of drought stress at different soybean growth stages was significant on seed oil content, and the lowest oil percentage was obtained when drought stress applied at the V5 stage (Dornbos & Mullen, 1992). During the reproductive stage, water deficit accelerated leaf senescence, shortened the period of seed-filling, and reduced the seed number and seed size (DeSouza, Egli, & Bruening, 1997). Soybean seed yield is related to the number of seed-bearing pods produced per unit area which also could be related to the number of flowers produced by the plant and the proportion of flowers that develop into pods. Many studies have reported that flowering, pod development, and seed formation stages are the most sensitive stages to soil moisture stress during soybean reproductive stage which could affect in reducing yield through seed number and size reduction (Dennis & Bruening, 2000). Therefore, the post-flowering phases are considered to be the most critical periods of soybean development requiring optimum soil moisture for the yield determination and seed quality improvement.

Due to the multiplicity of growing conditions, it is expected that soybeans produced under numerous environmental conditions across

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the crop growing areas would have varying seed quality and nutrient composition. Therefore, a better understanding of environmental effects on seed composition is needed to improve management to optimize seed nutrient profiles depending on their use in industry or human consumption (Bellaloui, Smith, Ray, & Gillen, 2009; Reddy, Patro, Lokhande, Bellaloui, & Gao, 2016). Soybean seeds contain about 36% protein, 19% of oil, 30% carbohydrates, 5% crude fiber, and 5% of ash (Liu, 1997). Also, seeds contain both macro- and micro-nutrients such as N, P, K, Ca, Mg, Fe, Cu, Mn, Zn, Co, and several other components, including vitamins B1, B2, and B6, as well as bioactive compounds like isoflavones (Liu, 1997). Protein and oils are the two most important constituents in the soybean seed, and their synthesis and deposition take place over a long period during pod-filling. The two main storage proteins in soybean seeds are glycinin (7S), which accounts for about 60% of storage proteins and β -conglycinin (11S) for the remaining 40% (Thanh & Shibasaki, 1978). These two types of storage proteins are known to be deficient in sulphur amino acids, even though glycinin contains more (4%) than the latter (< 1%). Protein accumulation begins in the developing seeds 10–12 days after flowering, while oil starts to build up 15–20 days after flowering (Yazdi-Samadi, Rinne, & Seif, 1977). The deposition of both these components continues until 70 days after flowering with the rapid deposition occurring 20–40 days after flowering (Rose, 1988). Therefore, changing environmental conditions, such as soil moisture stress during pod-filling period may have a considerable effect on soybean seed composition (Rotundo & Westgate, 2009).

Many studies have demonstrated variability in seed protein, oil, and fatty acid content of soybean exposed to elevated temperature (Thomas, Boote, Allen, Gallo-Meagher, & Davis, 2003), high UV-B radiation (Reddy et al., 2016), and drought (Dornbos & Mullen, 1992; Rotundo & Westgate, 2009; Rotundo, Borrás, Westgate, & Orf, 2009; Specht et al., 2001). Some studies have reported a negative correlation between seed oil and protein (Reddy et al., 2016). The seed fatty acids play a major role in oil stability, which is an important factor for end users of soybean oils. Soybean oil contains about 16% saturated fatty acids, 23% monounsaturated fatty acids, and 58% polyunsaturated fatty acids at its maturity stage (Reddy et al., 2016). Major saturated fatty acids include palmitic (10–12%), and stearic (2.2–7.2%), and major unsaturated fatty acids include oleic (24%), linolenic (8%) and linoleic (54%) acid (Bellaloui, Mengistu, Fisher, & Abel, 2012). Studies conducted with soybean cultivars under controlled environment conditions have documented that soybean plants exposed to high UV-B doses during seed development have reduced protein, palmitic, and oleic and increased linoleic and linolenic acid and oil contents (Reddy et al., 2016). Gibson and Mullen (1996) found that protein content was slightly affected with increasing temperature, but oil content increased with an optimum at 25–28 °C, above which it started to decline. While many studies concerning temperature and UV-B effect on soybean seed composition exist, the effect of soil moisture stress has not been fully resolved. In some earlier studies it has been reported that severe drought can lead to a decrease in protein concentrations (Specht et al., 2001), but in some studies, 4.4% increase in protein and 2.9% decrease in oil content have been reported (Dornbos & Mullen, 1992). Rotundo et al. (2009) indicated a negative correlation between seed protein concentration and yield and reported a positive linkage between leaf area and protein content, where they observed a high seed protein content under higher leaf area index values.

On the other hand, Dornbos and Mullen (1992) have observed a little effect of soil moisture stress on the fatty acid composition, while high temperature caused a significant reduction in the polyunsaturated components. These studies, however, failed to provide a complete analysis of whole nutrient profile under varying soil moisture stress levels during soybean reproductive stage. Although some of the previous studies identified relationships between environmental variables and variables of different seed components, little work has been reported about the effect of soil moisture stress on mineral composition,

sugar content, and other chemical composition at different water availabilities in soybean. Therefore, the objectives of this study were to investigate the effect of soil moisture stress during the reproductive stage on seed protein, oil, fatty acids, sugars, and macro- and micro-nutrients in two soybean cultivars with distinct growth habits and to provide soil moisture-seed quality functional algorithms that could be used to develop new modules in soybean crop models.

2. Materials and methods

2.1. Facilities and growth conditions

An experiment was conducted at the Rodney Foil Plant Science Research facility of Mississippi State University, Mississippi State, MS utilizing sunlit environmental growth chambers. Initially, seeds from two soybean cultivars, Asgrow AG5332 (indeterminate type) and Progeny 5333RY (determinate type), representing the same maturity Group V, were planted in pots (15.2 cm diameter by 30.5 cm high) outdoors filled with 3:1 sand: top soil classified as sandy loam (87% sand, 2% clay, and 11% silt). Plants were irrigated with full-strength Hoagland's nutrient solution, and 30 days after planting pots were moved inside the growth chambers. Inside the chambers, pots were organized in a completely randomized design with twelve replications per cultivar arranged in 6 rows with two pots per row. In total, one hundred and twenty pots were used for the five soil moisture stress treatments. More details of the arrangement of the pots and the treatments have previously been described by Wijewardana, Alsajri, Irby, Krutz, and Golden (2018). The treatments included five levels of irrigation, 100, 80, 60, 40, and 20%, which were maintained, based on percent evapotranspiration (ET) values recorded on the previous day. Treatments were imposed 41 days after planting (DAP) and continued until harvests at 126 DAP. Initially, all plants were irrigated with the same water volume as in the 100% ET treatment until the time that each treatment was imposed. The ET measured on a ground area basis (Ld^{-1}) throughout the treatment period as the rate at which condensate was removed by the cooling coils at 900-s intervals (Timlin, Fleisher, Kim, Reddy, & Baker, 2007) by measuring the mass of water in collecting devices connected to a calibrated pressure transducer. Throughout the experimental period (starting from 41 DAP to 126 DAP), soil moisture contents were monitored using soil moisture sensors (5TM Soil Moisture and Temperature Sensor, Decagon Devices, Inc., Pullman, WA) inserted at a depth of 15 cm in every five random pots of each soil moisture treatment. Season-long mean ET values for each treatment, mean values of day/night temperature, chamber CO₂ concentrations, and vapor pressure deficit (VPD) are provided in Table S1. Plants were harvested 126 DAP and leaves, and all the plants were sampled to take total dry weight (TD) number of seeds, individual seed weight, and seed yield. About 25 g of seed from pooled samples from each cultivar under each treatment was ground using a Laboratory Mill 3600 (Perten, Springfield, IL) and the ground material was analysed for protein, oil, fatty acids, sugar, and minerals.

2.2. Measurement of seed protein, oil, and fatty acids

Seed protein and oil were determined using near infrared (NIR) spectroscopy (Wilcox & Shibbes, 2001) using a diode array feed analyser AD 7200 (Perten, Springfield, IL) at USDA research facility in Stoneville, MS, USA. Perten's Thermo Galactic Grams PLS IQ software was used (Bellaloui et al., 2012) to produce calibration equations and the curves were established according to AOAC (1990a, 1990b) methods. Analyses of fatty acids were performed based on an oil basis (Bellaloui et al., 2009; Wilcox & Shibbes, 2001).

2.3. Measurement of seed carbohydrates

Seed carbohydrate concentrations were determined using near-

infrared reflectance (AD 7200, Perten, Springfield, IL) according to the procedure described by Bellaloui et al. (2009) and Wilcox and Shibbes (2001). Seed sugars such as sucrose, raffinose, and stachyose were analysed based on a seed dry matter basis (Boydak, Alpaslan, Hayta, Gercek, & Simsek, 2002; Wilcox & Shibbes, 2001).

2.4. Measurement of seed minerals

Twenty-five grams of the seeds from each cultivar and each soil moisture stress treatment were ground using Wiley Mill (Wiley Mill, Thomas Scientific, NJ, USA) by passing through a 1 mm screen. The concentrations of seed mineral nutrients nitrogen (N), phosphorus (P), potassium (K), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Copper (Cu), Boron (B), Iron (Fe), and Manganese (Mn) were determined in the ground seed materials at the nutrient testing laboratory, Mississippi State University, MS, USA using standard protocols (Plank, 1992).

2.5. Data analysis

Analysis of variance (ANOVA) was performed to determine the significance of cultivar, soil moisture stress treatment, and interaction sources of variation using SAS (SAS Institute 2011, Cary, NC). When the F ratio is significant, the post-hoc analysis using Tukey's procedure was performed to uncover the source of the significance. Means were compared using Tukey's honest significant difference (HSD) at a $P < 0.05$ significance level. The regression analyses were carried out using SigmaPlot version 13 (Systat Software Inc., San Jose, CA). The relationships among the soil moisture content and measured seed parameters were tested for linear and sigmoidal functions, and the best-fit regressions were selected.

3. Results and discussion

3.1. Environmental characterization

The soil moisture contents affecting soybean seed development and composition were monitored throughout the experimental period using soil moisture sensors and were varied among the treatments (Table S1). On an average, the measured soil moisture content regulated through evapotranspiration-based irrigation showed $0.15 \text{ m}^3 \text{ m}^{-3}$ for the control treatment (100% ET), followed by 0.14, 0.13, 0.12, and $0.11 \text{ m}^3 \text{ m}^{-3}$ for 80, 60, 40, and 20% ET treatments, respectively. In the study period (41–126 DAP and 85 days of treatment), the total evapotranspiration recorded were 1372 L for 100% ET, 1187 L for 80% ET, 1099 L for 60% ET, 751 L for 40% ET, and 562 L for 20% ET-based irrigation treatments. The average day and night temperatures, VPDs, and CO_2 concentrations, however, were not significantly different among the soil moisture treatments and cultivars (Table S1).

3.2. Growth and yield attributes

Soil moisture stress had significant effects on total dry weight, seed number, individual seed dry weight, and seed yield (Table S2). Lack of soil moisture during the reproductive stage decreased the total dry weight at all the moisture stress levels compared with the control (100% ET), but the reduction was more at 20% ET level (Fig. S1). The total dry weight for Asgrow AG5332 decreased by 43% when the soil moisture content varied from 0.15 to $0.11 \text{ m}^3 \text{ m}^{-3}$ whereas Progeny 5333RY exhibited a 54% reduction under the same soil moisture variation (Fig. S1A). This is in agreement with the previous studies (DeSouza et al., 1997) where increased water stress (60 and 30% field capacity) caused a significant reduction in total biomass of soybean compared to well-watered treatment (100% field capacity).

Seed number was not significantly different among the cultivars (Table S2); however, moisture stress treatments reduced the seed number and increased the production of shriveled, wrinkled, and

shrunken seed (Fig. S2) in the seed lot. Based on the visual screening, under the well-watered condition, seeds were large, full, and rounded in shape and with the gradual increase in the moisture stress, the shape was changed into long-oval with many of them were underdeveloped and misshapen. When the soil moisture varied from control ($0.15 \text{ m}^3 \text{ m}^{-3}$) to severe stress ($0.11 \text{ m}^3 \text{ m}^{-3}$), the total seed number was reduced by 45% for both the cultivars which could be due to the production of fewer pods per plant under moisture stressed condition (Fig. S1B). Our findings were in consistent with the previous findings of Samarah, Mullen, and Anderson (2009) in which they found that drought stress reduced seed quality by not only producing small, underdeveloped seed but also by decreasing the seed vigor and germination properties.

Typically, seed size is a function of the rate of seed growth and the amount and duration of biomass accumulation and partitioning towards seed and thereby the seed dry weight. Similar to seed number, individual seed weight was also negatively impacted by soil moisture stress treatments (Fig. S1C). The seed yield was reduced by 50 and 64% for Asgrow AG5332 and Progeny P5333RY cultivars respectively when the soil moisture content varied from optimum to severe moisture stress (Fig. S1D). Lower seed yield could be due to the disruption of carboxylation and remobilization of photosynthetic products during reproductive growth stages resulting seed abortion, lesser seed number, and lower individual seed weight (Egli & Yu, 1991).

3.3. Seed quality parameters

3.3.1. Seed protein and oil content

The percent protein content did not show a significant difference ($P > 0.05$) among the cultivars, and it increased linearly ($P = 0.038$) with increasing the soil moisture content (Fig. 1). The treatment 100% ET ($0.15 \text{ m}^3 \text{ m}^{-3}$), which achieved the highest yields, had the highest protein content, followed by soil moisture treatments 80% ET ($0.14 \text{ m}^3 \text{ m}^{-3}$) and 60% ET ($0.13 \text{ m}^3 \text{ m}^{-3}$), suggesting that maintaining a high level of soil moisture during the reproductive stage was beneficial to acquire a higher protein content from soybean seed. Recently, endoplasmic reticulum (ER) which is the key organelle involved in the activation of possible stress responses has been identified as a key player in regulating unfolded protein response (UPR) in dicotyledonous plants like soybean (Reis et al., 2016). Upon dysfunction of ER due to stress like soil moisture, UPR is activated to cope with the disruption of ER hemostasis which results in the accumulation of misfolded or unfolded proteins leading to less protein content in soybean seeds. Lower

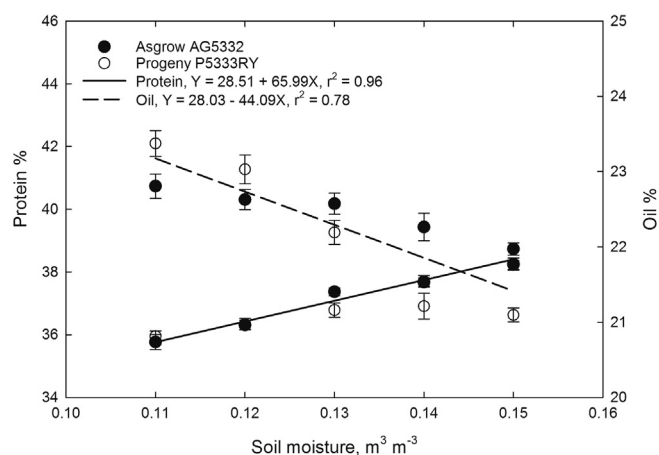


Fig. 1. Soil moisture stress effects on soybean seed protein and oil content for plants harvested at 126 d after planting. The treatments were imposed at the early flowering stage, 41 days after planting. Each data point is mean of four replications, and standard errors are shown if the values are larger than the symbols.

protein content in soybean seed is undesirable for soymilk as well as tofu yield. The protein content under well-watered condition ($0.15 \text{ m}^3 \text{ m}^{-3}$) was 37–39% of seed dry mass, which was consistent with the range reported in the previous studies (Gao, Hao, Kurt, & Robertson, 2009; Wilcox & Shibbes, 2001). Similar to the present finding, Specht et al. (2001) found that irrigation had a significant effect on the protein content of soybean and reported increased protein content in multiple years. Contrary, Rotundo, and Westgate (2009) found that water stress during soybean seed filling increased protein content and concluded that the increase in protein content did not necessarily due to the stimulation of protein synthesis rather from a concentration effect due to lower biomass production under the stress condition. However, Angra et al. (2010) proposed a mechanism to explain the decrease in protein content under unfavorable environmental conditions in which lower protein content under water stress conditions could be due to the hydrolysis and degradation of chaperones that were synthesized as a protection against drought.

Oil has considerable importance to the soybean industry because of its high economic value as a source of edible oil and a major renewable feedstock for biodiesel production. Soybean seed oil mostly from photosynthetic carbon assimilation of leaves and later carbohydrates are converted into triacylglycerol (TAG) through a metabolic pathway occurring in the endoplasmic reticula, plastid, and cytosol (Li et al., 2013). TAG biosynthesis is catalyzed by different enzymes, and recent studies suggest that phospholipid diacylglycerol acyltransferase (PDAT) plays a key role in plant storage lipid accumulation. A recent study on exploring the mechanisms of *Camelina sativa* oil biosynthesis identified five genes encoding PDAT in which drought treatment enhanced the expression of one of the genes (CsPDAT2-A) by twofold than that in control. In the present study, regardless of the cultivar effect, there was a significant increase in oil content due to water deficit, or in other words, the higher oil content was observed under low soil moisture content. Under optimum soil moisture condition, oil content ranged from 21 to 22% (Fig. 1), which is above the minimum value of 20% required by the industry (Wilson, 2004). Seed from severely water-stressed plants, Asgrow AG5332, and Progeny 5333RY contained 3.4% and 10.4% more oil respectively compared to the well-watered condition. Similar to our findings, Bellaloui et al. (2012) also documented that severe drought can increase the oil content in soybean seed. Due to the correlations with other seed composition traits such as seed yield and protein content improving seed oil concentration while maintaining higher protein content is complicated. In accordance with the previous studies (Bellaloui et al., 2009), soybean protein and oil content exhibited an inverse relationship (Fig. 1). An increase of 1% seed protein content has resulted in a 1.5% decrease in oil content. The negative correlation between oil and protein content could be due to (i) a single pleiotropic effect of QTLs (Quantitative Trait Locus) with two alleles of protein and oil or due to (ii) a pair of tightly linked QTLs (Specht et al., 2001; Sun, 2011). Due to a single pleiotropic QTL effect, two alleles could have inverse effects on both oil and protein; one allele simultaneously causes low protein, and another high allele oil or one allele concurrently causes high protein and other low oil. Due to the pair of tightly linked QTLs, high protein allele at the protein QTL and the low oil allele at the oil QTL are locked into a repulsion phase giving rise to a low oil-high protein or low protein-high oil (Sun, 2011). In the present study, a linear regression model as for protein and oil might explain the response that continues upward or downward in a straight line within the range of tested soil moisture levels. However, these model equations were given only to correlate within the data set, and there could be some minimum and maximum protein and oil of those components outside the scope of the experiment.

3.3.2. Seed fatty acids

The soil moisture stress treatments significantly affected the soybean seed fatty acid contents (Fig. 2). Oleic acid increased when higher the stress and decreased linearly ($P = 0.016$) when increasing the soil

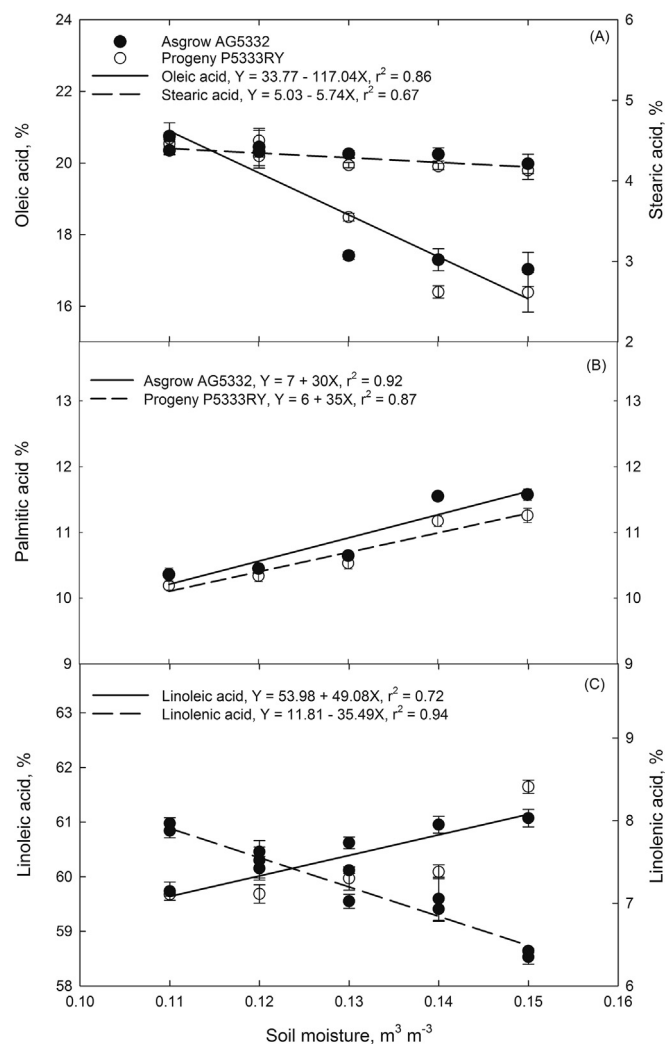


Fig. 2. Soil moisture stress effects on soybean seed fatty acid content for plants harvested at 126 d after planting, (A) oleic and stearic acids, (B) palmitic acid, and (C) linoleic and linolenic acids. The treatments were imposed at the early flowering stage, 41 days after planting. Each data point is mean of four replications, and standard errors are shown if the values are larger than the symbols.

moisture content (Fig. 2A). Stearic acid exhibited a slight increase with increasing soil moisture stress. Conversely, palmitic acid exhibited a linear decrease ($P < 0.0001$) when increasing the stress (Fig. 2B). The results of the analysis of variance show that the two soybean cultivars were significantly different for palmitic acid, but not for oleic, stearic, linoleic, and linolenic acids. The percent contents of polyunsaturated fatty acids such as linoleic and linolenic acids exhibited an inverse relationship (Fig. 2C). Under optimum soil moisture condition, the level of stearic, palmitic, and linolenic acids were in line with the previously reported values. However, oleic acid was lower, and linoleic acid was little higher. According to Henry (2010), optimal soybean fatty acid contents such as oleic, stearic, palmitic, linoleic, and linolenic acids are 17.7–28, 2.5–5.4, 8–13, 49.8–59, and 5–11%, respectively. In the present study, the most common monounsaturated fatty acid; oleic was in the range of 16–17% and the major polyunsaturated fatty acid, linoleic was 61–61.5%, correspondingly, under optimum soil moisture content. The fatty acid composition could vary during seed formation due to some factors, such as genotype and environment factors. In our study, the oleic acid content was increased by 10% (Fig. 2A) and linoleic acid was decreased by 2.5% (Fig. 2C) when the soil moisture content varied from control (100% ET) to severe stress (20% ET). As reported for some

other oil seed crops, such as sunflower, flax, and rape seed, biosynthesis of oleic acid stimulates under stress condition due to the enzymatic processes governed by genes coding for oleoyl D-9 desaturase and oleoyl D-12 desaturase which are being the main enzymes implicated in metabolic processes related to oleic and linoleic acid biosynthesis (Martinez-Force, Alvarez-Ortega, Cantisan, & Garces, 1998; Mertz-Henning et al., 2018).

Moreover, the activity of oleic acid desaturase enzyme has been reported to inhibit by stress conditions in soybean, giving rise to increased quantities of oleic acid and reduced quantities of polyunsaturated fatty acids such as linoleic and linolenic acids. Because linoleic and linolenic acids are synthesized by the successive desaturation of oleic acid, when the desaturation rate of oleic acid decreases, it hinders the synthesis of linoleic acids (Mertz-Henning et al., 2018). Together with this phenomenon, an increase in oleic and a decrease in linoleic acids were recorded. Linoleic and linolenic can be reduced by hydrogenation producing several types of unhealthy trans isomers (Willett & Ascherio, 1994) leading to poor flavor, lower oil stability, and health risks, while oleic acid is less susceptible to oxidative changes. Stearic acid has a neutral effect on low-density-lipoprotein (LDL) cholesterol level on plasma which is one of the risk factors for coronary heart disease, while palmitic acid strongly increases LDL-cholesterol (Briggs, Petersen, & Kris-Etherton, 2017). Producing soybean seed oils with high oleic acid content (> 70% 18:1), which is a result of a shift of the polyunsaturated fatty acids (PUFAs) to 18:1, is considered the best approach (Bilyeu et al., 2018) to improve the functionality of soybean oil for food uses in the absence of trans-fatty acids. Oils with a high proportion of oleic acid and low PUFA, offer many advantages for all kinds of applications such as reduced rancidity, increased oxidative stability, and shelf life (Huth, Fulgoni, & Larson, 2015).

3.3.2.1. Seed sugars. Soil moisture stress during soybean reproductive stage also impacted the sugar content of the soybean seed. Two soybean cultivars were significantly different for sucrose ($P = 0.036$), raffinose ($P = 0.0043$), and stachyose ($P = 0.0001$) (Fig. 3). The control plants of Asgrow AG5332 grown at 100% ET level produced seed with 50% more sucrose (Fig. 3A), 1.7% more raffinose (Fig. 3B), and 18% less stachyose (Fig. 3C) than those grown at 20% ET, whereas Progeny P5333RY exhibited 45% more sucrose (Fig. 3A), 4.8% more raffinose (Fig. 3B), and 12% less stachyose (Fig. 3C), correspondingly. Among the analyzed soluble sugars, sucrose was the most abundant sugar followed by stachyose and raffinose. The sucrose and raffinose contents linearly decreased, and the percent stachyose content linearly increased with decreasing soil moisture deficit. Similar to our observation, Bellaloui (2012) has reported a positive correlation between sucrose and raffinose with water stress, and a negative correlation between water stress and stachyose. Previous studies have reported that activity of the sucrose synthase enzyme involved in sucrose synthesis under drought conditions in soybean leading to a several-fold decline than the other enzymes important for raffinose and stachyose synthesis (Gonzalez, Gordon, James, & Arrese-Igor, 1995). Our findings were in line with their observations where percent sucrose content decreased much greater than raffinose and stachyose when higher the soil moisture stress suggesting a higher sensitivity of sucrose to soil moisture stress than other sugars. Sucrose is the principal end product of photosynthesis, which translocates from leaves (source) to the developing seed (sink) through the phloem. The drought stress has been reported to affect the translocation of sucrose and other sugars due to impaired photosynthesis process and the associated enzymes (Bellaloui, 2012). Sugar content is an important quality attribute for animal feed and soyfood. Therefore, higher sugar content; mainly sucrose is important as it increases the sweet taste and digestibility in soyfood products such as tofu, soymilk, and natto (Hou, Chen, Shi, Zhang, & Wang, 2009). Raffinose and stachyose are considered to be an important source of energy during seed germination; however, they contain anti-nutritional factors such as tannins, trypsin inhibitors, and

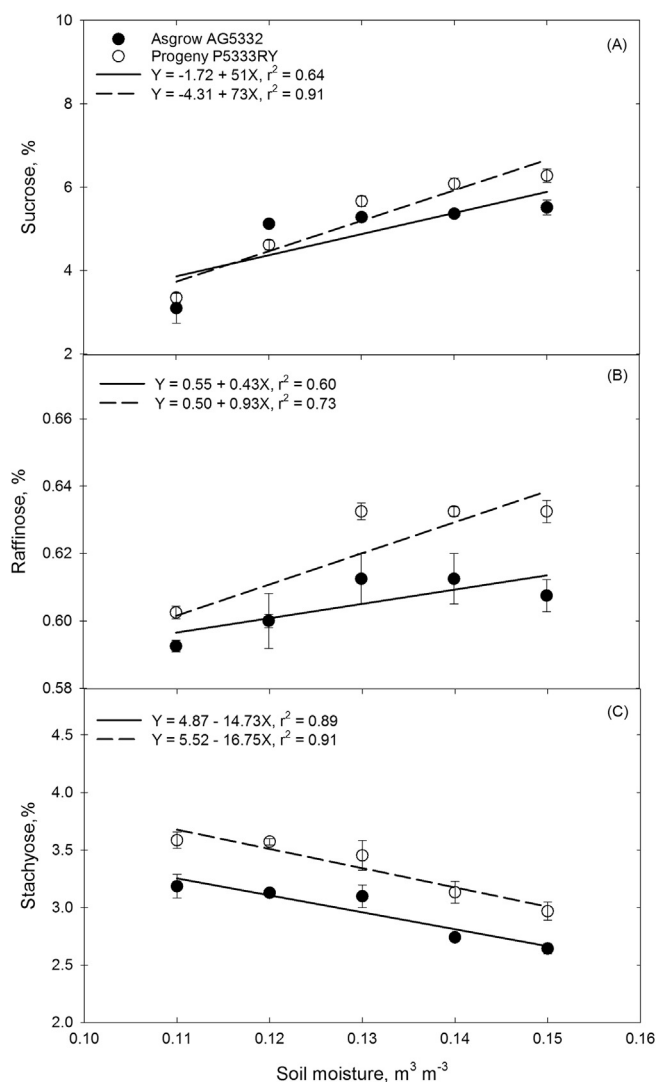


Fig. 3. Soil moisture stress effects on soybean seed sugar content for plants harvested at 126 d after planting, (A) sucrose, (B) raffinose, and (C) stachyose. The treatments were imposed at the early flowering stage, 41 days after planting. Each data point is mean of four replications, and standard errors are shown if the values are larger than the symbols.

phytate complexes (Aviles-Gaxiola, Chuck-Hernandez, & Saldivar, 2017), which reduce nutrient utilization in animals contributing to, reduced gastrointestinal performance (Arendt & Zannini, 2013). Therefore, a higher stachyose content that was observed in our study under soil moisture stress may not be desirable because it is indigestible and can cause flatulence and diarrhea in non-ruminants.

3.4. Seed mineral composition

Minerals are essential for plant growth, development, reproduction, and seed quality. Deficiencies in mineral uptake and transport due to abiotic stress such as drought especially at reproductive stage result in yield loss and poor seed quality (Bellaloui et al., 2012). Lowered absorption of the minerals could be due to reduced transpiration flow, limited availability of energy for assimilation, and interference in the unloading mechanism (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009). According to Knoblauch and Peters (2010), carbohydrates needed for storage travel from source (leaves) to sink (seeds) through a conduit system consisting of sieve cells. Once solutes are loaded into the conduits, it increases the osmotic potential leading to attract water from the surroundings through the semi-permeable cell walls. This pressure

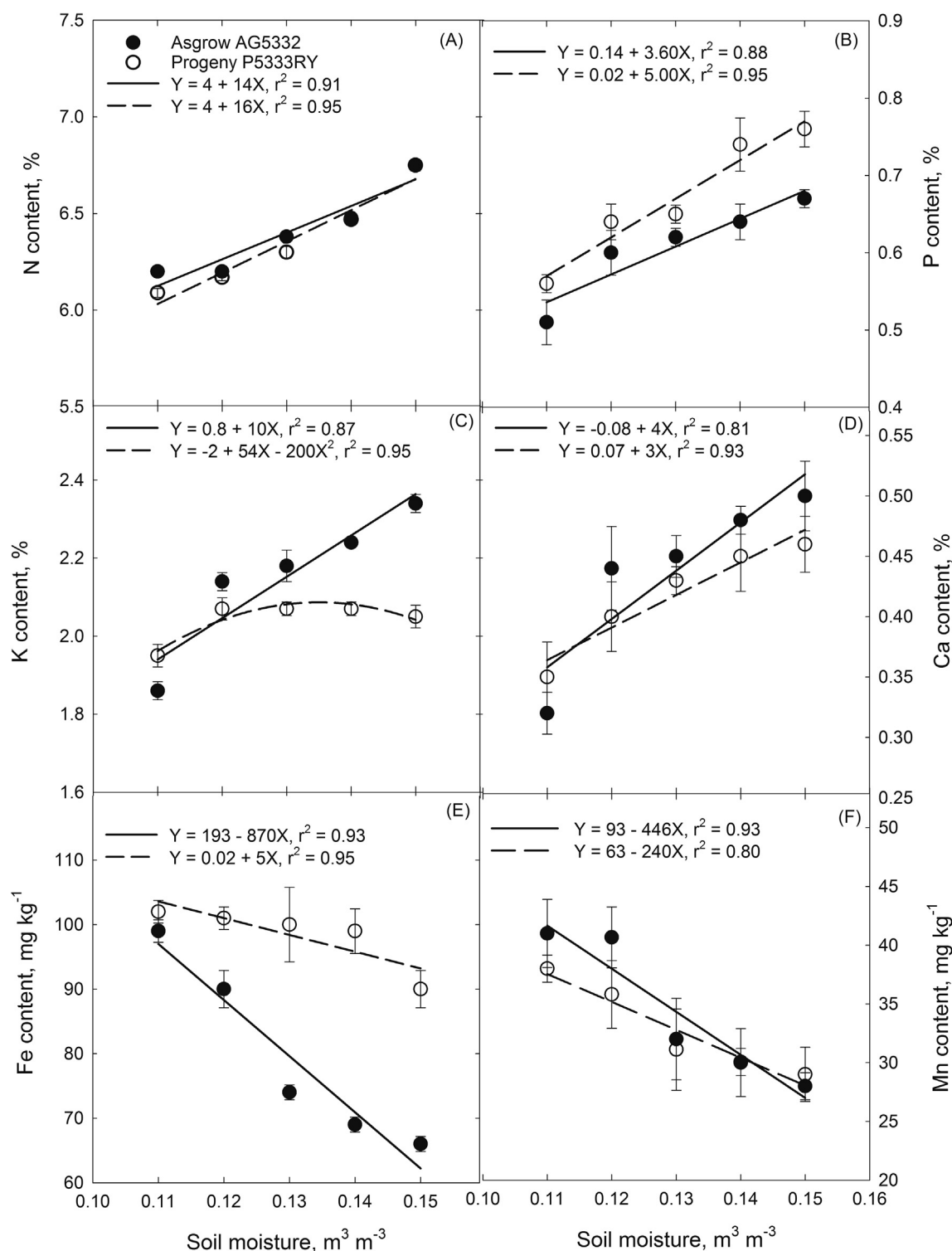


Fig. 4. Soil moisture stress effects on soybean seed mineral content for plants harvested at 126 d after planting, (A) nitrogen, (B) phosphorous, (C) potassium, (D) calcium, (E) iron, and (F) manganese. The treatments were imposed at the early flowering stage, 41 days after planting. Each data point is mean of four replications, and standard errors are shown if the values are larger than the symbols.

difference between two tissues (source and sink) drives the bulk flow of solutes between source and sink. Different cultivars of the same species may have different capacities to absorb, translocate, and accumulate nutrients such as Fe, Zn, and P that could be influenced by environmental factors, genetics, and the adaptability of the particular cultivar to a given environment (White & Broadley, 2009). In the present study, the two soybean cultivars showed a significant difference for N

($P = 0.0006$), P ($P = 0.0005$), K ($P = 0.0099$), Ca ($P = 0.0386$), Fe ($P < 0.0001$), and Mn ($P = 0.003$) (Fig. 4). Under well-watered condition (100% ET), seed N was significantly higher for both the cultivars and decreased linearly when increasing the soil moisture stress (Fig. 4A). This is consistent with the observed relationship between protein and soil moisture (Fig. 1) in which lower protein content under soil moisture deficit could be due to lower N availability, assimilation,

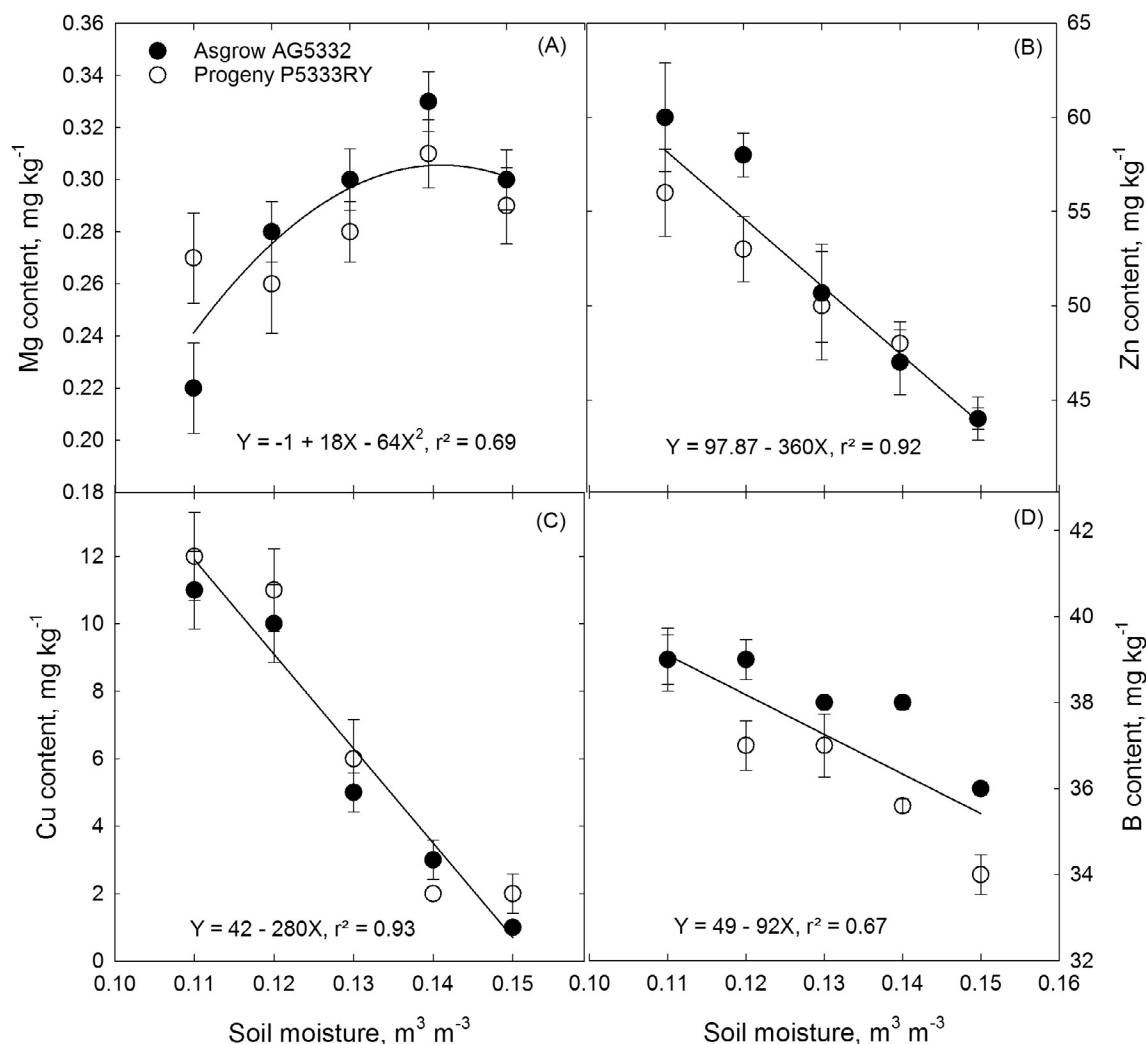


Fig. 5. Soil moisture stress effects on soybean seed mineral content for plants harvested at 126 d after planting, (A) magnesium, (B) zinc, (C) copper, and (D) boron. The treatments were imposed at the early flowering stage, 41 days after planting. Each data point is mean of four replications, and standard errors are shown if the values are larger than the symbols.

and partitioning under the stressed condition. Since N is the major component of amino acids; its availability could affect crude protein content within the soybean seed. Overall, the soybean cultivar Asgrow AG5332 showed higher N (Fig. 4A), K (Fig. 4C), Ca (Fig. 4D), and Mn (Fig. 4F) contents compared to Progeny P5333RY. The determinate type Progeny P5333RY showed higher P and Fe contents compared to indeterminate Asgrow AG5332 cultivar. The P accumulation was lower under soil moisture stress (Fig. 4B). The percent P content varied from 0.78 and 0.68% to 0.62 and 0.54% for Asgrow AG5332 and Progeny P5333RY cultivars, respectively, when the soil moisture varied from 100 to 20% ET. Even though the degree of influence of soil moisture stress reliant on the severity of the stress, many studies reported that drought inhibits P uptake, absorption, translocation, and accumulation (Bellaloui et al., 2012; Pinkerton & Simpson, 1986). Percent K content showed a linear correlation with soil moisture content for Asgrow AG5332; however, Progeny P5333RY cultivar exhibited a quadratic response (Fig. 4C). The maximum K content (2.1%) was observed under 0.13 and 0.14 m³ m⁻³ moisture levels for Progeny P5333RY where as 2.4% was the maxima for Asgrow AG5332 that was observed under well-watered condition (100% ET). The decrease in K mobility could be the possible reason not to having adequate K content in the soybean seed under water deficit for both the cultivars. Both the cultivars showed a positive linear correlation for the percent Ca content with soil moisture (Fig. 4D). The control plants of Asgrow AG5332 grown at

100% ET level produced seed with 38% more Ca (Fig. 4D) than those grown at 20% ET, whereas Progeny P5333RY exhibited 26% more Ca. Phosphorus, K, and Ca are typically not readily soluble in water, therefore; those may decrease with the amount of water supplied during the seed growth stage. The percent Fe and Mn contents had inverse linear relationships with soil moisture. When the soil moisture varied from 0.15 to 0.11 m³ m⁻³, Fe (Fig. 4E) and Mn (Fig. 4F) contents were increased by 54 and 43% for Asgrow AG5332 and 9 and 35% for Progeny P5333RY, respectively. The higher increase of Fe and Mn under soil moisture stress conditions may indicate the possible involvement of these nutrients in stomatal opening and osmoregulation which in turn increase the allocation of those nutrients towards seed (Bellaloui et al., 2012).

The Mg concentration showed a quadratic decline with increasing the soil moisture stress (Fig. 5A) which could be due to a decrease in its uptake under stress. Soybean seed came from 80% ET (0.14 m³ m⁻³) showed the maximum Mg content (0.32 mg kg⁻¹), and the least (0.22 mg kg⁻¹) was observed under 20% ET level (Fig. 5A). Soil moisture stress significantly affected the concentration of micro-nutrients of the soybean seed. The Zn (Fig. 5B), Cu (Fig. 5C), and B (Fig. 5D) concentrations increased by 33, 50, and 11% when the moisture content varied from control (100% ET) to 20% ET. In contrast to our findings, Hu and Schmidhalter (2005) reported decreased B uptake under drought stress due to low mineralization and limited

mobility.

In summary, observed differences among the cultivars for palmitic acid, seed sugars, N, P, K, Ca, Fe, and Mn could be due to their different growth habits (indeterminate and determinate) and inherited genotypic variations. The findings of the study suggest that maintaining an optimum soil moisture content during soybean reproductive stage is important for the accumulation of enhanced levels of protein, sucrose, oleic, and macro nutrients in soybean seed, which are desirable traits for processing soy products of improved quality. Also, seed quality and soil moisture stress functional algorithms could be used to develop new modules for seed quality in many existing crop models (Boote, Jones, White, Assend, & Lizo, 2013) that could be used to optimize management during the growing season.

4. Conclusion

The results of this study showed that soybean yield and its components were significantly affected by soil moisture stress during the reproductive growth stage. The two soybean cultivars have marked variations in plant growth characters, yield- and yield-attributes under soil moisture deficit. Asgrow AG5332 has shown greater yields due to the increased node, pod, and seed number, and seed dry weight compared to the Progeny P5333RY, probably due to its indeterminate growth habit and genetics. Soil moisture stress caused a reduction in soybean seed protein, palmitic and linoleic acids, sucrose, raffinose, stachyose, N, P, K, and Ca; however it increased oil, stearic, oleic and linolenic acids, Fe, Mg, Zn, Cu, and B. The relationship between seed protein and oil was negatively correlated, and it will provide a challenge for breeders to increase these two traits under stressful conditions. The changes in seed constituents could be due to changes in nutrient accumulation and partitioning in soybean seeds under water-stressed condition. The findings of the study suggest that maintaining optimum soil moisture content during soybean reproductive stage is important for the accumulation of enhanced levels of protein, sucrose, oleic, and macro nutrients in soybean seed, which are desirable traits for processing soy products of improved quality.

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Appendix A. Supplementary data

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