Chapter 8

Effects of Drought and Elevated Atmospheric Carbon Dioxide on Seed Nutrition and ^{15}N and ^{13}C Natural Abundance Isotopes in Soybean Under Controlled Environments

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Additional information is available at the end of the chapter

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Abstract

The objective of the current research was to evaluate the effects of drought and elevated CO_2 on seed production and seed nutrition under controlled conditions in soybean. Soybean plants were subjected to ambient and elevated CO_2 and under irrigated and drought conditions. The results showed that drought or drought with elevated CO_2 resulted in high protein and oleic acid, but low in oil and linoleic and linolenic acids. Significant decrease of sucrose, glucose, and fructose concentrations was noticed, but high content of raffinose and stachyose was observed. Nutrients such as N, P, K, and some micro-nutrients were reduced under drought or drought with normal or elevated CO_2 concentrations. Seed δ ^{15}N ($^{15}N/^{14}N$ ratio) and δ ^{13}C ($^{13}C/^{12}C$ ratio) natural abundance isotopes were also altered under drought or drought with ambient or elevated CO_2 concentrations, reflecting nitrogen and carbon metabolism changes. The current research demonstrated that global climate changes may lead to changes in seed nutrition, and nitrogen and carbon metabolism. Efforts of breeders to select for these traits will sustain food source and food security for humans and livestock as soybean is a major source for protein and oil for human consumption and soymeal for animals.

Keywords: elevated carbon dioxide, climate change, seed composition, seed nutrition, drought, soybean

1. Introduction

Global climate changes due to elevated CO_2 are expected to lead to high heat and drought in some regions, affecting crop production and seed nutrition [1]. Climate change, whether is caused naturally or human-made, threatens crop production and food security. It was reported that food systems that include food availability, food access, and food utilization will suffer when food systems are stressed [1]. It is predicted that climate change will alter the food systems through its effects on crop production and rainfall, leading to drought or flooding or warmer or cooler temperatures [1]. Global warming resulted from climate change leads to high risk of drought and warmer temperatures, resulting in an increase of water demands and evaporation (http://www.c2es.org/science-impacts/extreme-weather/drought) [2]. The USA historically has suffered major droughts, and the living memories of the Dust Bowl of the 1930s or the drought of the 1950s and recently the droughts of 2011 in Texas and 2012 in the USA are examples, highlighting our vulnerabilities to drought as we move forward to warmer and drier climate (http://www.c2es.org/science-impacts/extreme-weather/drought) [2]. It was also reported that a doubling of CO_2 concentration could result in changes in global climate, including precipitation patterns, resulting in drought conditions in some areas of the world [3]. Generally, elevated CO_2 concentration during crop growth enhances CO_2 exchange rate and final yield [4] as growth at elevated CO_2 stimulates photosynthesis and increases carbon supply in C_3 crops and enhances nutrient supply to match the increase in C acquisition [4]. However, drought stress results in a decrease in CO_2 exchange rate. The mechanisms involved in the reduction of CO_2 exchange rate are still not well understood, although part of the reduction was attributed to stomatal closure [5], leaf water potentials [6], decreases in activities of Photosystem I and Photosystem II [7–9], effects on assimilation products, and photosynthetic enzyme activities [6].

The effects of climate changes on yield were previously reported [1], and it was shown that severe drought stress can lead to crop losses of up to 90% [10–12]. While there has been considerable progress in understanding the sensitivities of crop yield to climate change, evaluation of climate change factors such as CO_2, elevated temperature, or drought on seed nutrition remains rather limited [13]. Drought resulted from climate change is expected to alter the biochemistry and physiology of crops, impacting the nutritional value of the crop grains. Alteration of carbohydrates including starch and soluble sugars such as glucose, fructose, and sucrose occurs under stress conditions [14]. It is well known that carbohydrates are the major product of photosynthesis, which are transported to stems and leaves (important reserve of photo-assimilates for grain filling) and consequently to the sink (grains) during grain-filling stage [15]. The sugars, sucrose and raffinose, are also known to protect cells against oxidative damage and accumulate later during responses to stress [16, 17]. It was reported that the high levels of raffinose and stachyose were thought to play a role in the acquisition of desiccation tolerance due to over expression of galactinol synthase and accumulation of galactinol and raffinose improving drought tolerance [18, 19]. The mechanisms of involvement of these sugars in drought tolerance are still not fully understood [19–21]. The accumulation of proline, amino acids, and organic acid such as malic acid, fumaric acid, and citric acid is also a common biochemical indicator occurring at high level under drought stress [12]. The accumulation of

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these compounds is a part of the mechanism of osmotic adjustment to maintain water potential gradient under drought conditions and to protect subcellular structure from the damaging effects of drought stress [22]. The high level of proline accumulation under drought stress was previously identified as a mechanism for the protection of cytosolic proteins and organelles [12]. Therefore, these biochemical compounds are important to understand the relationship between drought stress and drought tolerance and for decision-making when breeding for drought tolerance [23]. Since drought stress results in limiting the growth and development of the plant and consequently the supply of the photosynthate to grain during grain filling [24], the chemical composition of the grain and its quality can be affected by drought stress [12, 25]. The effect of drought on seed quality could be due to the decrease of total soluble sugars especially in the grain under drought stress due to decreases in the levels of sucrose, stachyose, and verbascose. Sucrose plays an important role in the development of the grain and is sensitive to drought stress and involved in the synthesis of raffinose oligosaccharides including stachyose and verbascose, and these sugars play an important role in seed tolerance to desiccation and against oxidative effects of drought stress [18, 26]. Some sugars, for example, maltose, accumulate in the grain under drought stress, maybe due to starch degradation [12]. For example, during the remobilization phase, starch remobilized was significantly lower under drought stress, but the total amounts of soluble sugars and amino acids remobilized were greater [12], contrasting previous studies that showed higher carbohydrate accumulation under drought stress [27]. Therefore, the role of carbohydrates in drought tolerance is still not well understood, and further investigations are needed to reach the conclusive results.

Research on the possible effects of elevated CO_2 and drought resulted from climate changes on biochemical and chemical composition and mineral nutrition of major crops is limited. We chose soybean as a subject of our research because soybean is a major crop in the world; it is a major source of human nutrition because it contains protein (40%), oil (20%), fatty acids, amino acids, carbohydrates (30%), crude fiber (5%), and ash (5%) and it contains minerals (such as P, K, Ca, Mg, Fe, Cu, Mn, Zn, and Mo), vitamins (B1, B2, and B6), phytoestrogen, such as isoflavones, and phenolics. The objective of the current research was to investigate the possible effects of elevated CO_2 and drought on seed chemical composition (protein, oil, fatty acids, sugars, and minerals). A special attention was given to possible alteration of seed $\delta^{15}N$ ($^{15}N/^{14}N$ ratio) and δ ^{13}C ($^{13}C/^{12}C$ ratio) natural abundance isotopes as they reflect nitrogen and carbon metabolism.

2. Materials and methods

2.1. Growth conditions

The experiments were conducted under controlled environments of growth chambers. Soybean seeds were planted in vermiculite and grown under greenhouse conditions until V1 stage where similar plants were transported to pots filled with field soil. The soil texture was 8% sand, 31.6% silt, and 60.4% clay. Soil nutrient concentrations of macro- and

micronutrients were adequate to support the growth and development of plants till maturity. When plants reached R5 (beginning of seed-fill stage) [28], they were transferred to the growth chambers until full maturity. Two soybean cultivars, Freedom and Hutcheson of maturity group V, were used. The plants were subjected to the following four treatments (T): T1 = plants were grown irrigated and subjected to 360 μmol mol^{-1} CO_2 concentration; T2 = plants were grown irrigated and subjected to 700 μmol mol^{-1} CO_2 concentration; T3 = plants were subjected to drought and to 360 μmol mol^{-1} CO_2 concentration; and T4 = plants were subjected to drought and to 700 μmol mol^{-1} CO_2 concentration. Drought stress treatment was imposed by growing the plants at soil water potential of about −199 kPa. For irrigated plants, soil water potential was kept at about −15 to −20 kPa, which was considered to give the field water holding capacity. Soil water potential was monitored by using soil water potential sensors and read by Soil Moisture Meter (Watermark Company, Inc., Wisconsin, USA). For irrigated experiment, the plants were watered as needed. For CO_2 treatment, CO_2 concentration was supplied by CO_2 cylinder located outside the growth chamber, and the rate of CO_2 was controlled by a regulator, monitoring the CO_2 concentration flow. The CO_2 flowed through the tube to the growth chamber. The growth chamber was equipped with CO_2 sensor to read the concentration of CO_2 inside the growth chamber. The plants were grown under growth chamber conditions supplied with light source of photon flux density of about 1000 μmol m^{-2} s^{-1} and provided with a combination of 10,400 W high-pressure sodium and metal halide lights. The experiments were conducted under constant normal temperature of 26/16°C, day/night.

2.2. Determination of seed minerals, N, S, and C

Mature seeds were ground using a Laboratory Mill 3600 (Perten, Springfield, IL, USA), and the ground samples were analyzed for minerals, N, S, and C, by digesting 0.6 g of the ground-dried plant materials in HNO_3 in a microwave digestion system as detailed elsewhere [29, 30]. Potassium was determined by inductively coupled plasma spectrometry [29, 30]. Each content of N, C, and S was measured in a 0.25 g ground-dried sample which was combusted in atmospheric oxygen of 1350°C, and then the elements N, S, and C were converted to N_2, SO_2, and CO_2, respectively. The contents of N, S, and C in seed were measured by an elemental analyzer using thermal conductivity cells (LECOCNS-2000 elemental analyzer, LECO Corporation, St. Joseph, MI, USA) [29, 30].

2.3. Determinations of seed protein, oil and fatty acids

The mature seed samples of 25 g were ground using the Laboratory Mill 3600, and seed protein, oil, and fatty acids in the samples were analyzed by near-infrared reflectance [29–31] using a diode array feed analyzer AD 7200 (Perten, Springfield, IL USA). The calibration equation was developed using Preteen's Thermo Galactic Grams PLS IQ software, and the calibration curve was established using Association Official Analytical Chemists (AOAC) methods. The contents of protein and oil were determined based on a seed dry matter [29, 31]. The contents of palmitic, stearic, oleic, linoleic, and linolenic fatty acids were determined based on a total oil basis [29].

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2.4. Determinations of sucrose, raffinose, stachyose, glucose, and fructose

Seed sucrose, raffinose, and stachyose were determined as detailed elsewhere (Bellaloui et al. [29]) by near-infrared reflectance using the AD 7200 array feed analyzer. Sugars were determined based on a seed dry matter basis [29–31]. The concentration of glucose in mature seed was determined by an enzymatic reaction using a Glucose (HK) Assay Kit, Product Code GAHK-20 (Sigma-Aldrich Co., St Louis, MO, USA) as detailed elsewhere [29]. The concentrations were determined spectrophotometrically by reading the samples at absorbance of 340 nm using the Beckman Coulter DU 800 spectrophotometer. The concentration was expressed as mg g^{-1} dry weight. Fructose concentration in mature seed was determined based on an enzymatic reaction using a Fructose Assay Kit, Product Code FA-20 (Sigma-Aldrich Co., St. Louis, MO, USA) as detailed by Bellaloui et al. elsewhere [29]. Fructose concentration was determined spectrophotometrically by the Beckman Coulter DU 800 spectrophotometer by reading the samples at absorbance of 340 nm. Fructose concentration in seed was expressed as mg g^{-1} dry weight.

2.5. Boron determination

Boron concentrations in mature seeds were determined using the Azomethine-H method [32, 33] as reported elsewhere [29]. Briefly, 1.0 g of ground sample was ashed at 500°C, extracted with 20 ml of 2 M HCl at 90°C for 10 min, and then 4 ml of a buffer solution (containing 25% ammonium acetate, 1.5% EDTA, and 12.5% acetic acid) was added to a filtered 2-ml sample. An amount of 4 ml of fresh azomethine-H solution (0.45% azomethine-H and 1% of ascorbic acid) [34] was added. Boron concentration was determined spectrophotometrically by reading the samples at absorbance of 420 nm using a Beckman Coulter DU 800 spectrophotometer (Beckman Coulter, Inc., Brea, CA, USA). The concentration was expressed as mg kg^{-1} dry weight.

2.6. Iron determination

Iron concentration in mature seed was determined according to the method described elsewhere [35, 36]. Briefly, 2 g of dry ground samples were acid wet digested, extracted, and reacted with reduced ferrous Fe with 1,10-phenanthroline and as detailed elsewhere [29]. After the extraction, the soluble constituents were dissolved in 2 M HCl, and the phenanthroline solution of 0.25% (w/v) in 25% (v/v) ethanol and quinol solution (1% w/v) was used as a reagent. Standard curve of Fe was created by preparing a range of concentrations from 0.0 to 4 μg ml^{-1} of Fe in 0.4 M HCl. Iron concentration was determined spectrophotometrically by reading the samples at absorbance of 510 nm using the Beckman Coulter DU 800 spectrophotometer. The concentration was expressed as mg kg^{-1} dry weight.

2.7. Phosphorus determination

Phosphorus content in mature seeds was measured according to [37]. Phosphorus determination was based on the yellow phosphor-vanado-molybdate complex as detailed elsewhere [29]. Briefly, dry ground seed samples of 2 g were ashed. Then, 10 ml of 6MHCl was added,

and phosphorus was extracted using 2 ml of 36% v/v HCl under heat and filtration and 5 ml of 5 M HCl. A volume of 5 ml of reagent (ammonium molybdate-ammonium metavanadate) was added to 5 ml of the filtrate. The reagent ammonium molybdate-ammonium metavanadate was prepared by dissolving 25 g of ammonium molybdate and 1.25 g of ammonium metavanadate in distilled water. The standard curve solutions of P were prepared by using a range of concentrations from 0 to 50 μg ml^{-1} using dihydrogen orthophosphates. The concentration of P was determined spectrophotometrically by reading the absorbance at 400 nm using the Beckman Coulter DU 800 spectrophotometer.

2.8. Experimental design and statistical analysis

Two experiments were carried out in a split plot design with arrangement of treatments in a randomized complete block design (RCBD) with four replicates. Main plot was drought/CO_2 treatment and subplot was cultivar. Each experiment was considered as a replicate for the main plot. One growth chamber was used for each drought/CO_2 treatment. Treatments in each experiment were the following: T1 = plants were grown irrigated and subjected to 360 μmol mol^{-1} CO_2 concentration; T2 = plants were grown irrigated and subjected to 700 μmol mol^{-1} CO_2 concentration; T3 = plants were grown under drought and subjected to 360 μmol mol^{-1} CO_2 concentration; and T4 = plants were grown under drought and subjected to 700 μmol mol^{-1} CO_2 concentration. Two soybean cultivars were used. The analysis of variance of data was conducted using PROC MIXED in SAS [38]. Experiment (EXP), drought, CO_2 concentration (CO_2), and their interactions were considered fixed effects, and replication within EXP and replication × drought × CO_2 × CV within EXP were considered random effects. The level of significance was $P \leq 0.05$. Detailed design of the current experiments was similar to that previously reported by Bellaloui et al. [39].

3. Results and discussion

Analysis of variance showed that the main effects of experiment (EXP), drought, CO_2, and cultivar (CV) were the most significant factors affecting variability of seed protein, oil, fatty acids, and sugars (**Table 1**). There were no significant effects of the interactions between EXP and other factors, which were expected. Interactions between CO_2, CV, and drought showed significant effects for some seed constituents such as protein, oleic, glucose, fructose, and sucrose, indicating the different responses of cultivars to drought and CO_2 (**Table 1**). Since there were no significant interaction effects between EXP and the others factors, the results were combined across the two experiments. Analysis of variance showed that drought, CO_2, and CV were the major factors affecting the variability for macro- and micronutrients in seed (**Table 2**). There were no effects of EXP on nutrients, as expected, because the experiments were conducted under controlled environmental conditions of growth chambers. Both CO_2 and CV significantly interacted with drought, indicating that the effect of CO_2 was influenced by drought and the cultivars responded differently due to genotype and genetic background effect (**Table 2**).

Treatments	Protein	Oil	Oleic	Linolenic	Glucose	Fructose	Sucrose	Raffinose	Stachyose
EXP	ns[1]	ns	ns	ns	ns	ns	ns	ns	ns
Drought[2]	*	*	**	**	***	**	**	*	*
CO_2	**	*	*	*	***	**	***	*	*
CV	*	ns	*	ns	ns	*	*	ns	ns
EXP × drought	ns	ns	ns	ns	ns	ns	ns	ns	ns
EXP × CO_2	ns	ns	ns	ns	ns	ns	ns	ns	ns
EXP × CV	ns	ns	ns	ns	ns	ns	ns	ns	ns
Drought × CO_2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Drought × CV	ns	ns	*	ns	*	*	***	ns	ns
CO_2 × CV	*	ns	ns	ns	ns	*	*	ns	ns
EXP × drought × CO_2 × CV	ns	ns	ns	ns	ns	ns	ns	ns	ns

Treatments used were four: T1 = plants were grown irrigated and subjected to 360 μmol mol^{-1} CO_2 concentration; T2 = plants were grown irrigated and subjected to 700 μmol mol^{-1} CO_2 concentration; T3 = plants were grown under drought and subjected to 360 μmol mol^{-1} CO_2 concentration; and T4 = plants were grown under drought and subjected to 700 μmol mol^{-1} CO_2 concentration. Plants were grown in the greenhouse, and they were transferred to growth chambers at the beginning of seed-fill stage (R5) until full maturity (R8). The experiments were conducted under constant normal temperature of 26/16°C, day/night.

[1] * = significance at $P \leq 0.05$; ** = significance at $P \leq 0.01$; *** = significance at $P \leq 0.001$; ns = not significant.

[2] Drought treatment was imposed by growing the plants at soil water potential of about −199 kPa. For irrigated plant, soil water potential was kept at about −15 to −20 kPa.

Table 1. Analysis of variance for soybean cultivars (Freedom and Hutcheson, Maturity group V) for seed protein, oil, fatty acids (%), and sugars (glucose, fructose, sucrose, raffinose, and stachyose, mg g^{-1}) and their responses to the main factors of experiment (EXP, two experiments were conducted), drought, carbon dioxide (CO_2), cultivar (CV), and their interactions.

Treatments	N	P	K	Mg	Fe	B	Cu	Zn	Mn
EXP	ns[1]	ns	ns	ns	ns	ns	ns	ns	ns
Drought[2]	***	***	**	*	*	***	*	*	*
CO_2	*	*	*	*	*	*	*	**	**
CV	*	**	*	*	***	*	*	*	*
EXP × drought	ns	ns	ns	ns	ns	ns	ns	ns	ns
EXP × CO_2	ns	ns	ns	ns	ns	ns	ns	ns	ns
EXP × CV	ns	ns	ns	ns	ns	ns	ns	ns	ns
Drought × CO_2	*	*	*	*	*	***	*	**	*
Drought × CV	**	*	*	*	**	*	*	*	*
CO_2 × CV	**	ns	*	ns	ns	*	ns	*	ns
EXP × drought × CO_2 × CV	ns	ns	ns	ns	ns	ns	ns	ns	ns

Treatments used were four: T1 = plants were grown irrigated and subjected to 360 μmol mol^{-1} CO_2 concentration; T2 = plants were grown irrigated and subjected to 700 μmol mol^{-1} CO_2 concentration; T3 = plants were grown under drought and subjected to 360 μmol mol^{-1} CO_2 concentration; and T4 = plants were grown under drought and subjected to 700 μmol mol^{-1} CO_2 concentration. Plants were grown in the greenhouse, and they were transferred to growth chambers at the beginning of seed-fill stage until maturation (R8). The experiments were conducted under constant normal temperature of 26/16°C, day/night.

* = significance at $P \leq 0.05$; ** = significance at $P \leq 0.01$; *** = significance at $P \leq 0.001$; ns = not significant

Drought treatment was imposed by growing the plants at soil water potential of about −199 kPa. For irrigated plant, soil water potential was kept at about −15 to −20 kPa.

Table 2. Analysis of variance for soybean cultivars (Freedom and Hutcheson, maturity group V) for seed macro- and micronutrients (N, P, K, and Mg: %; Fe, B, Cu, Zn, and Mn: mg kg^{-1}) as affected by the main effect factors of experiment (EXP, two experiments were conducted), carbon dioxide (CO_2), cultivar (CV), and their interactions.

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Mean values of seed constituents showed that seed protein, oleic acid, raffinose, and stachyose were higher, and oil, linolenic acid, glucose, fructose, and sucrose were lower under drought or drought with elevated CO_2 conditions (**Table 3**). Seed N was higher under drought or drought with elevated CO_2. However, seed contents of P, K, Mg, Fe, B, Cu, Zn, and Mn were generally lower under drought or drought with elevated CO_2. Comparing with ambient CO_2, elevated CO_2 showed higher contents of seed protein, oil, glucose, sucrose, raffinose, and stachyose and lower contents of seed N, P, B, and Cu in Freedom cultivar only. Cultivar Hutcheson responded differently to ambient CO_2 or elevated CO_2. The different responses of cultivars to ambient CO_2 or elevated CO_2 are suggested to be due to genotype and/or genetic background (**Tables 1–4**). What is clear is that seed oleic acid was higher and linolenic acid was lower and most nutrients were lower under elevated CO_2 (**Tables 3–6**). δ ^{15}N ($^{15}N/^{14}N$ Ratio) and δ ^{13}C ($^{13}C/^{12}C$ Ratio) natural abundance isotopes showed alterations under drought and drought with elevated CO_2, indicating that there were changes of nitrogen and carbon metabolism under drought stress (**Figures 1** and **2**).

The higher contents of seed protein, oleic acid, raffinose, and stachyose under drought or drought with elevated CO_2 conditions could be due to drought effects and physiological and biochemical responses to stress. It was reported that drought resulted from climate change is predicted to alter the biochemistry and physiology of crops, impacting the nutritional value of the crop grains [12]. The effects of elevated CO_2 and drought on seed composition (protein, oil, fatty acids, and sugars) and minerals were previously reported. However, results of the effects of elevated CO_2 on seed composition are still conflicting. For example, small effects of elevated CO_2 on seed composition were found [40], whereas others found a significant effect of elevated CO_2 on soybean seed oil in cultivars Essex, Holladay, and NK6955 [41]. The authors also found that oleic fatty acid concentration was positively affected and protein concentration was not affected by CO_2. Recently, it was found that elevated CO_2 resulted in decreased content of protein and increased contents of oil and oleic acid [39].

It was reported that alteration of carbohydrates such as starch and soluble sugars such as glucose, fructose, and sucrose occurred under stress conditions [14] and sugars of sucrose and raffinose may play a role in protecting cells against oxidative damage and accumulate as a response to stress [16, 17]. Other researchers suggested that high levels of raffinose and stachyose contribute to the acquisition of desiccation tolerance due to overexpression of galactinol synthase and accumulation of galactinol and raffinose improving drought tolerance [18, 19]. Sucrose was reported to be involved in the synthesis of raffinose oligosaccharides including stachyose and verbascose, and these sugars play an important role in seed tolerance to desiccation and drought stress [18, 26]. Some sugars, for example, maltose, accumulate in the grain under drought stress, maybe due to starch degradation [12]. The mechanisms of the involvement of these sugars in drought tolerance are still not fully understood [19–21]. It was concluded that these biochemical compounds can be used as drought indicators and can be used in breeding program to select for drought tolerance [12, 23]. CO_2 elevation resulted in a decrease of seed Na, Ca, Mg, S, Fe, Zn, and Mn [42, 43]. The percentage decrease of nutrients by elevated CO_2 ranged from 0.7 to 19.5%, except for K and P [44]. The decrease of macro- and micronutrients by elevated CO_2 was due to the dilution effect induced by the increase of carbohydrates in seeds [12, 39, 42–44].

Treatments[1]	Protein	Oil	Oleic	Linolenic	Glucose	Fructose	Sucrose	Raffinose	Stachyose
T1	38.5 c	21.3 b	22.2 c	9.6 a	1.4 b	1.01 a	61 b	8.7 d	33.6 d
T2	39.0 c	22.1 a	27.5 b	8.7 b	2.1 a	0.98 a	67 a	9.6 c	34.9 c
T3	41.2 a	19.4 c	29.7 a	6.3 c	0.8 c	0.54 b	32 d	11.5 a	45.3 a
T4	40.3 b	19.6 c	29.5 a	6.8 c	0.9 c	0.52 b	39 c	10.3 b	40.7 b

Treatments used were four: T1 = plants were grown irrigated and subjected to 360 μmol mol^{-1} CO_2 concentration; T2 = plants were grown irrigated and subjected to 700 μmol mol^{-1} CO_2 concentration; T3 = plants were grown under drought and subjected to 360 μmol mol^{-1} CO_2 concentration; and T4 = plants were grown under drought and subjected to 700 μmol mol^{-1} CO_2 concentration. Plants were grown in the greenhouse, and they were transferred to growth chambers at the beginning of seed-fill stage (R5) until full maturity (R8). The experiments were conducted under constant normal temperature of 26/16°C, day/night.

[1]Means within a column of each water treatment followed by the same letter are not significantly different at the 5% level as determined by Fishers' least significant difference (LSD) test. Values are means of four replicates. Drought treatment was imposed by growing the plants at soil water potential of about −199 kPa. For irrigated plant, soil water potential was kept at about −15 to −20 kPa.

Table 3. Drought and elevated carbon dioxide effects on soybean (Freedom cultivar, maturity group V) seed protein, oil, fatty acids (%), and sugars (glucose, fructose, sucrose, raffinose, and stachyose, mg g^{-1}).

Treatments[1]	N	P	K	Mg	Fe	B	Cu	Zn	Mn
T1	6.5 a	0.64 a	1.9 a	0.34 a	75 a	64 a	12.4 a	51.2 a	42.5 a
T2	5.1 c	0.53 b	1.5 b	0.33 a	72 a	57 b	9.3 b	52.3 a	41.2 a
T3	6.7 a	0.47 c	1.1 c	0.23 b	65 b	41 d	8.2 c	46.5 c	37.6 c
T4	6.1 b	0.46 c	1.2 c	0.34 a	63 b	47 c	8.5 c	49.6 b	40.0 b

Treatments used were four: T1 = plants were grown irrigated and subjected to 360 μmol mol^{-1} CO_2 concentration; T2 = plants were grown irrigated and subjected to 700 μmol mol^{-1} CO_2 concentration; T3 = plants were grown under drought and subjected to 360 μmol mol^{-1} CO_2 concentration; and T4 = plants were grown under drought and subjected to 700 μmol mol^{-1} CO_2 concentration. Plants were grown in the greenhouse, and they were transferred to growth chambers at the beginning of seed-fill stage (R5) until full maturity (R8). The experiments were conducted under constant normal temperature of 26/16°C, day/night.

[1]Means within a column of each water treatment followed by the same letter are not significantly different at the 5% level as determined by Fishers' LSD test. Values are means of four replicates. Drought treatment was imposed by growing the plants at soil water potential of about −199 kPa. For irrigated plant, soil water potential was kept at about −15 to −20 kPa.

Table 4. Drought and elevated carbon dioxide effects on soybean (Freedom cultivar, maturity group V) seed macro- and micronutrients (N, P, K, and Mg: %; Fe, B, Cu, Zn, and Mn: mg kg^{-1}).

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Treatments[1]	Protein	Oil	Oleic	Linolenic	Glucose	Fructose	Sucrose	Raffinose	Stachyose
T1	39.8 c	21.3 a	21.4 c	10.5 a	2.4 a	1.23 a	68 a	11.2 c	41.4 c
T2	40.1 c	21.4 a	29.7 a	9.7 b	2.6 a	1.21 a	66 a	12.5 b	40.5 d
T3	43.5 b	18.5 b	29.6 a	7.5 c	0.7 c	0.73 c	51 c	13.5 a	47.6 a
T4	44.1 a	18.3 b	28.1 b	7.3 c	1.3 b	0.85 b	60 b	13.7 a	46.8 b

Treatments used were four: T1 = plants were grown irrigated and subjected to 360 μmol mol^{-1} CO_2 concentration; T2 = plants were grown irrigated and subjected to 700 μmol mol^{-1} CO_2 concentration; T3 = plants were grown under drought and subjected to 360 μmol mol^{-1} CO_2 concentration; and T4 = plants were grown under drought and subjected to 700 μmol mol^{-1} CO_2 concentration. Plants were grown in the greenhouse, and they were transferred to growth chambers at the beginning of seed-fill stage (R5) until full maturity (R8). The experiments were conducted under constant normal temperature of 26/16°C, day/night.

[1]Means within a column of each water treatment followed by the same letter are not significantly different at the 5% level as determined by Fishers' LSD test. Values are means of four replicates. Drought treatment was imposed by growing the plants at soil water potential of about −199 kPa. For irrigated plant, soil water potential was kept at about −15 to −20 kPa.

Table 5. Drought and elevated carbon dioxide effects on soybean (Hutcheson cultivar, maturity group V) seed protein, oil, fatty acids (%), and sugars (glucose, fructose, sucrose, raffinose, and stachyose, mg g^{-1}).

Treatments[1]	N	P	K	Mg	Fe	B	Cu	Zn	Mn
T1	6.9 a	0.72 a	2.7 a	0.31 a	76 b	67 a	11.3 a	48 a	43 a
T2	5.3 c	0.59 b	1.7 b	0.26 b	81 a	65 a	10.5 b	43 b	39 b
T3	6.3 b	0.42 c	1.2 c	0.20 c	60 c	51 b	8.1 d	31 d	31 c
T4	6.1 b	0.45 c	1.1 c	0.22 c	63 c	53 b	9.8 c	35 c	33 c

Treatments used were four: T1 = plants were grown irrigated and subjected to 360 μmol mol^{-1} CO_2 concentration; T2 = plants were grown irrigated and subjected to 700 μmol mol^{-1} CO_2 concentration; T3 = plants were grown under drought and subjected to 360 μmol mol^{-1} CO_2 concentration; and T4 = plants were grown under drought and subjected to 700 μmol mol^{-1} CO_2 concentration. Plants were grown in the greenhouse, and they were transferred to growth chambers at the beginning of seed-fill stage (R5) until full maturity (R8). The experiments were conducted under constant normal temperature of 26/16°C, day/night.

[1]Means within a column of each water treatment followed by the same letter are not significantly different at the 5% level as determined by Fishers' LSD test. Values are means of four replicates. Drought treatment was imposed by growing the plants at soil water potential of about −199 kPa. For irrigated plant, soil water potential was kept at about −15 to −20 kPa.

Table 6. Drought and elevated carbon dioxide effects on soybean (Hutcheson cultivar, maturity group V) seed macro- and micronutrients (N, P, K, and Mg: %; Fe, B, Cu, Zn, and Mn: mg kg^{-1}).

http://dx.doi.org/10.5772/67511

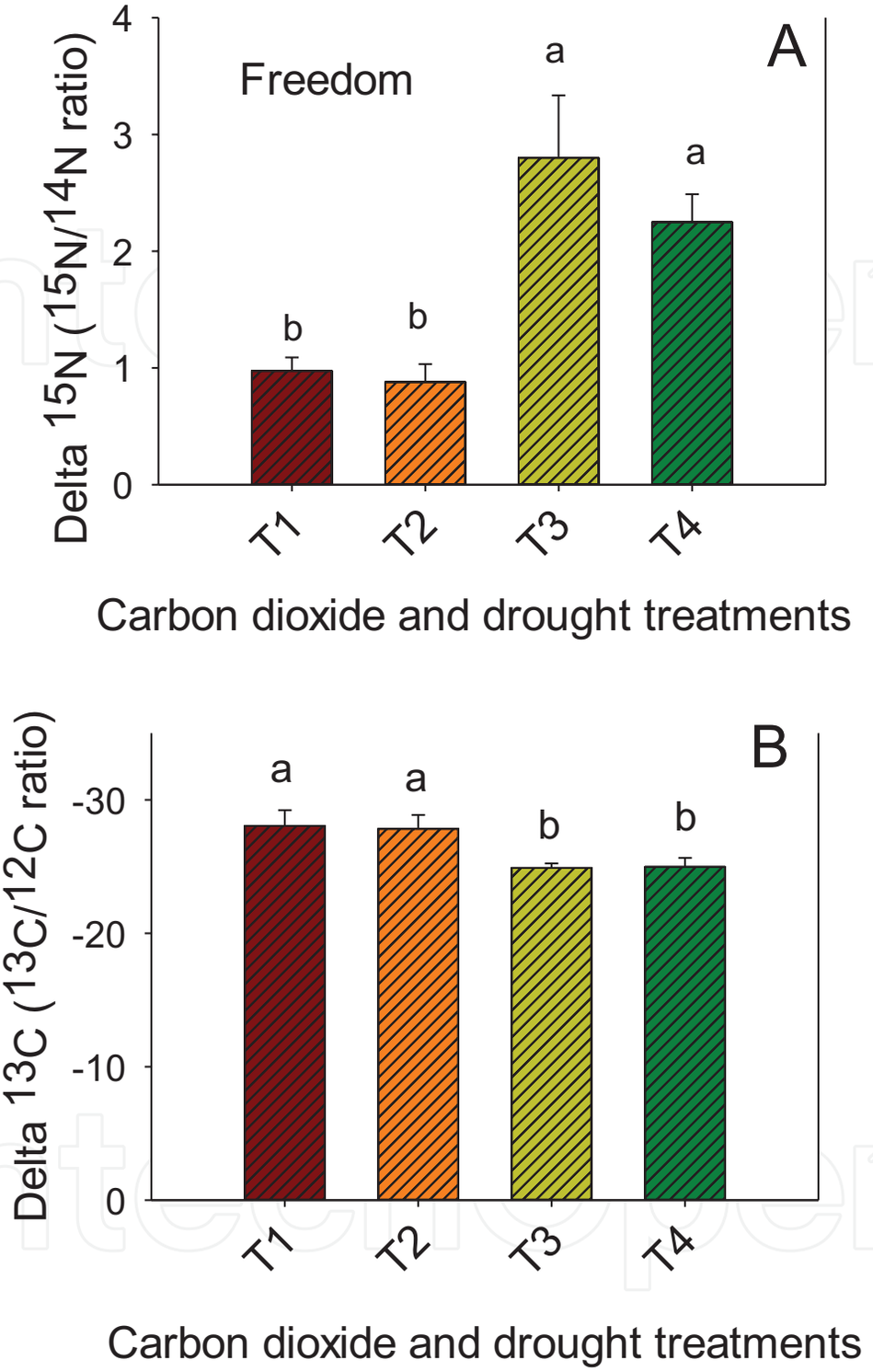

Figure 1. Cultivar Freedom (maturity group V). δ ^{15}N ($^{15}N/^{14}N$ ratio) (A) and δ ^{13}C ($^{13}C/^{12}C$ ratio) (B) natural abundance isotope in soybean seed as influenced by drought and elevated CO_2. Treatments used were four: T1 = plants were grown irrigated and subjected to 360 μmol mol^{-1} CO_2 concentration; T2 = plants were grown irrigated and subjected to 700 μmol mol^{-1} CO_2 concentration; T3 = plants were grown under drought and subjected to 360 μmol mol^{-1} CO_2 concentration; and T4 = plants were grown under drought and subjected to 700 μmol mol^{-1} CO_2 concentration. Plants were grown in the greenhouse, and they were transferred to growth chambers at the beginning of seed-fill stage (R5) until full maturity (R8). The experiments were conducted under constant normal temperature of 26/16°C, day/night.

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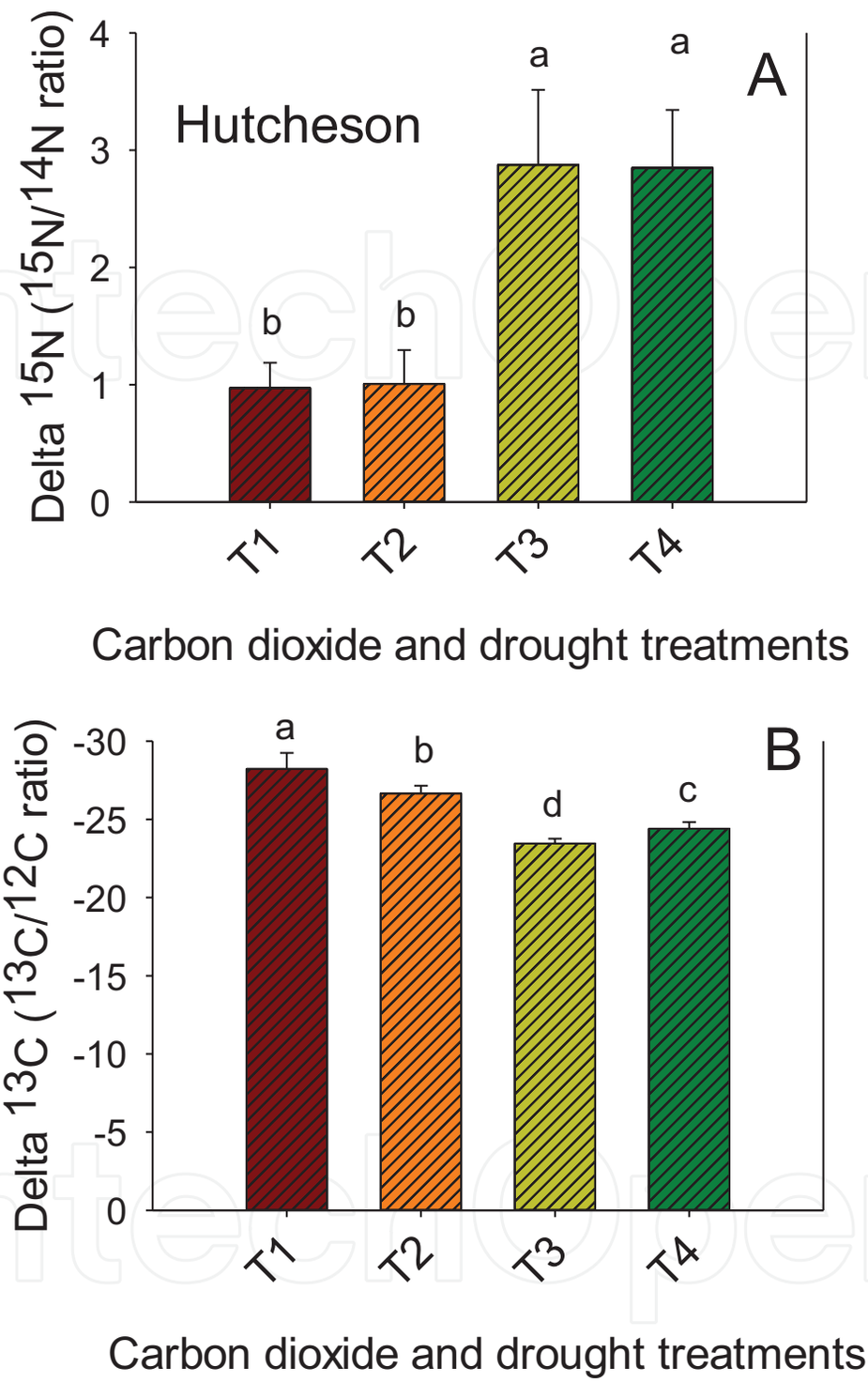

Figure 2. Cultivar Hutcheson (maturity group V). δ ^{15}N ($^{15}N/^{14}N$ ratio) (A) and δ ^{13}C ($^{13}C/^{12}C$ ratio) (B) natural abundance isotope in soybean seed as affected by drought and elevated CO_2. Treatments used were four: T1 = plants were grown irrigated and subjected to 360 μmol mol^{-1} CO_2 concentration; T2 = plants were grown irrigated and subjected to 700 μmol mol^{-1} CO_2 concentration; T3 = plants were grown under drought and subjected to 360 μmol mol^{-1} CO_2 concentration; and T4 = plants were grown under drought and subjected to 700 μmol mol^{-1} CO_2 concentration. Plants were grown in the greenhouse, and they were transferred to growth chambers at the beginning of seed-fill stage (R5) until full maturity (R8). The experiments were conducted under constant normal temperature of 26/16°C, day/night.

Our results showed that elevated CO_2 concentration enhanced oleic acid and carbohydrates (mainly sucrose and glucose) and reduced NPK and some micronutrients compared with ambient CO_2. The increase of carbohydrates due to elevated CO_2 could result from enhanced photosynthesis resulting in higher total carbohydrates [4]. The decrease of NPK and some micronutrients was due to the dilution of concentrations of the nutrients by higher levels of carbohydrates as supported by previous research [12, 39, 42–44]. Higher seed protein, oleic acid, raffinose and stachyose, and lower oil, linolenic acid, glucose, fructose, and sucrose under drought or drought with elevated CO_2 conditions can be used as biochemical indicators/markers to be used for breeding to select for drought tolerance.

Therefore, research is needed to quantify the negative impact of elevated CO_2 and its interactions with biotic and abiotic stresses, including drought, on seed quality to breed for higher seed nutritional qualities and develop appropriate crop management systems [43, 45]. It was reported that the physiological processes influenced by drought can be identified by the biochemical characterization of plant tissues under stress conditions [46]. Limited research was conducted to characterize the biochemical compounds such as protein, amino acids, carbohydrates, and phenolics in the breeding program as a tool for selection, but this tool has promise in selecting for abiotic stress tolerance [24, 47]. The changes of δ ^{15}N ($^{15}N/^{14}N$ Ratio) and δ ^{13}C ($^{13}C/^{12}C$ Ratio) natural abundance isotopes under drought and drought with elevated CO_2 indicated the increase of ^{15}N derived from plant gas exchange through stomatal conductance and CO_2 fixation [48, 49]. The alteration of $^{13}C/^{12}C$ ratio indicated a possible shift of carbon metabolism leading to less discrimination against δ ^{13}C [50].

4. Conclusions

The current research demonstrated that elevated CO_2, drought, and drought combined with elevated CO_2 altered seed biochemical compounds, including protein, carbohydrates, and minerals. This research increases our knowledge of the interaction between elevated CO_2 and drought resulting from climate changes for seed chemical composition and mineral nutrition.

The high level of oleic acid and low level of linolenic acid are desirable, and they contribute to oil stability and long shelf life of the oil. Sugars of both raffinose and stachyose may play a possible role in drought stress response. Alterations of δ ^{15}N and δ ^{13}C natural abundance isotopes indicated changes in nitrogen and carbon metabolism. The characterization of these seed biochemical compounds under elevated CO_2 or drought stress can be used as biomarkers in breeding program to select for crop drought tolerance and high seed nutritional qualities, as these traits are related to seed production, quality, and food security. Since limited research was conducted on the effects of climate change on seed quality and mineral nutrition, further research is needed.

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