

Elevated Atmospheric Carbon Dioxide and Temperature Affect Seed Composition, Mineral Nutrition, and ^{15}N and ^{13}C Dynamics in Soybean Genotypes under Controlled Environments

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Abstract

The seed nutrition of crops is affected by global climate changes due to elevated CO_2 and temperatures. Information on the effects of elevated CO_2 and temperature on seed nutrition is very limited in spite of its importance in seed quality and food security. Therefore, the objective of this study was to evaluate the effects of elevated atmospheric CO_2 and temperature on seed composition (protein, oil, fatty acids, and sugars) and mineral nutrition in two soybean cultivars under controlled environments. The treatments were ambient CO_2 concentrations ($360 \mu\text{mol mol}^{-1}$) and elevated CO_2 concentration ($700 \mu\text{mol mol}^{-1}$) as well as normal temperature ($26/16^\circ\text{C}$) and elevated temperature ($45/35^\circ\text{C}$). Plants were grown under greenhouse conditions until the R5 stage, and then, transferred to growth chambers until full maturity (R8). Elevated temperature or a combination of elevated temperature and elevated CO_2 resulted in a decrease in seed protein and linolenic acid concentrations and an increase in oil and oleic acid in cultivars Williams 82 (MGIII) and Hutcheson (MG V). Seed sucrose, glucose, and fructose decreased, whereas raffinose and stachyose remained relatively stable. Minerals also decreased under elevated CO_2 and temperature. Among those that decreased were N, P, K, Zn, Fe, and B. Natural abundance of ^{15}N and ^{13}C isotopes was altered only under high temperature, regardless of CO_2 concentration, indicating that changes in nitrogen and carbon metabolism occurred

at elevated temperature. The increase in oil and oleic acid and decrease in linolenic acid are desirable, as high oleic acid and low linolenic acid contribute to the stability and longer shelf-life of oil. The combination of low protein and high oil was due to the inverse relationship between them. This study showed that seed composition and seed mineral nutrients can be affected by elevated temperature alone or elevated CO_2 and temperature. This information is beneficial for selecting varieties with high seed nutritional qualities and efficient mineral nutrient use and uptake, traits that are related to seed production, seed quality, and food security. Also, it provides further knowledge on the effect of climate change on seed quality.

Key words: Soybean; seed composition; seed nutrition; seed protein; seed oil; seed sugars.

Introduction

Elevated carbon dioxide and temperatures due to global climate change affect crop yield and seed quality (Thomas et al., 2003; Prasad et al., 2005; Taub et al., 2008; Uprety et al., 2010), including chemical composition (Uprety et al., 2010). Global climate change, due to human activities, such as CO_2

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emission, or naturally caused threatens human life as it affects food security and human survival (Prasad et al., 2005; Upreti et al., 2010; Thomas et al., 2003; Taub et al., 2008). It also affects vegetation, including C_3 crops, such as soybean, barley, wheat, and rice, as well as C_4 crops such as corn and sugar beet (Prasad et al., 2005; DaMatta et al., 2010; Long et al., 2004; Easterling et al., 2007; Leakey et al., 2009) in spite of the difference in responses of C_3 and C_4 plants to elevated CO_2 and temperature. This is due to the process of carbon fixation pathway in C_3 versus C_4 plants. In C_3 plants, the CO_2 acceptor is ribulose biphosphate (RuBP), whereas the CO_2 acceptor in C_4 plants is phosphoenol pyruvate (PEP) (Pearcy and Ehleringer, 1984; Prasad et al. 2005; Upreti et al., 2010). It has been reported that Thomas et al. (2003) evaluated the impact of climate changes due to elevated CO_2 and temperature on seed composition. They used sinusoidal temperatures of 28/18, 32/22, 36/26, 40/30, and 44/34°C (day/night, maximum/minimum), and two levels of CO_2 , 350 and 700 $\mu\text{mol mol}^{-1}$. They found that temperature, but not CO_2 concentration, had significant effects on seed composition. They found that total oil concentration was the highest at 32/22°C, but decreased with the increasing temperature. Oleic acid concentration increased with the increasing temperature, but linolenic acid decreased. They also found that nonstructural carbohydrates decreased with the increasing temperature, but the proportion of soluble sugars to starch decreased. The concentrations of seed N and P increased with the increasing temperature until 40/30°C and then decreased. They concluded that high temperature alters soybean seed composition, whereas CO_2 had minimal effects. However, further research is needed to understand the chemical basis of climate change (Thomas et al., 2003).

In addition to crop growth and yield, crop quality is also affected by global climatic changes, including nutritional aspects (Hay and Porter, 2006). Micronutrients, such as zinc and iodine, and macromoles, such as proteins, in plant tissues are expected to change in response to future high CO_2 levels (Taub et al., 2008). The crops respond directly to elevated CO_2 through photosynthesis and stomatal conductance (Long et al., 2004), and this is due to the fact that current atmospheric CO_2 concentration is sub-optimal for photosynthesis of C_3 plant species, including soybean, dry bean, peanut, and cowpea (Prasad et al., 2005). The responses of crops to elevated CO_2 are highly dependent on temperature (Polley, 2002). Thus, understanding how crop species respond to global environmental changes is crucial for maximizing the potential benefits of elevated CO_2 and adjust agricultural practices with the increases in temperature and CO_2 (Challinor and Wheeler, 2008). Since previous studied indicated that elevated temperatures combined with high CO_2 are expected due to the greenhouse effect (Intergovernmental Panel on Climate Change, 1995), researchers predicted decreased soybean yields in the southeastern USA due to a 5°C increase in temperature (Curry et al., 1995). Additionally, grain quality will be impacted due to higher temperatures (Allen and Boote, 2000) and interactions of increased CO_2 and temperature (Pickering et al., 1994), resulting in physiological, growth, and seed yield changes (Baker and Allen, 1993; Allen and Boote, 2000).

The atmospheric CO_2 and temperature affect plant growth

and development and grain quality (Upreti et al., 2010). Carbon dioxide increased from 270 to 380 $\mu\text{mol mol}^{-1}$, since the industrial revolution (Upreti et al., 2010), and it is expected to be between 470 and 570 $\mu\text{mol mol}^{-1}$ by the year 2050 (IPCC 2007). It was expected to be increasing at the rate of 1.5 to 1.8 $\mu\text{mol mol}^{-1} \text{ yr}^{-1}$. Temperature is suggested to increase by 1.4 to 4.5°C in earth's temperature by the year 2100 according to model projections. Impact assessment on physiological and biochemical processes indicated that CO_2 and temperature are predicted to have significant changes in biochemical composition of grains and their nutritional quality (Stafford 2007; Upreti and Reddy, 2008; Upreti et al., 2009). Previous studies on elevated CO_2 and temperatures showed a decrease in the concentration of mineral nutrition, such Ca, S, Mg, Fe, and Zn, in plants, which has a potential negative impact on human nutrition sources. This information is useful for breeders as they select genotypes that maintain desirable grain nutritional qualities under future CO_2 scenarios. Elevated atmospheric CO_2 and temperatures as a consequence of global climate changes will present a challenge and provide opportunities to plant scientists, especially plant breeders to increase productivity and improve grain nutritional value.

Since soybean is one of the most valuable crops worldwide due to its content of oil (20%), protein (40%), carbohydrates (30%), crude fiber (5%), and ash (5%) (Hymowitz et al., 1972), it is essential to be evaluated under elevated CO_2 and increased temperature conditions. Soybean seed also contains minerals such as Fe, Cu, Mn, Ca, Mg, Zn, Co, P, and K (Augustin and Klein, 1985; Messina, 1997; Bellaloui et al., 2014) vitamins B1, B2, and B6, and isoflavones (Augustin and Klein, 1985; Messina, 1997). Only limited studies have been conducted to document changes in seed composition due to elevated CO_2 and high temperatures, resulting from global climate changes (Thomas et al., 2003). The objective of the current study was to evaluate the effects of elevated atmospheric carbon dioxide concentration and elevated temperature on seed composition (protein, oil, fatty acids, and sugars) and mineral nutrition (macro- and micro-nutrients) in soybean genotypes under controlled environment. Special attention was also given to ^{15}N and ^{13}C dynamics as nitrogen and carbon are sources of protein, oil, and sugars.

Materials and Methods

Growth Conditions

Two growth chamber experiments were conducted. Soybean seeds were planted in vermiculite and grown under greenhouse conditions. After germination, uniform size seedlings at about the V1 stage were transplanted into pots filled with field soil. The soil was 8% sand, 31.6% silt, and 60.4% clay and had an adequate concentration of macro- and micro-nutrients. Plants were grown in greenhouse until they reached R5 (beginning of seed-fill stage) (Fehr and Caviness, 1977), and then, transferred to the growth chambers until full maturity. CO_2 was supplied from a cylinder controlled by a regulator, monitoring CO_2 concentration. CO_2 flowed through a tube to the growth chamber. The plants were grown under growth chamber conditions supplied

with a light source of photon flux density of about 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, supplied with a combination of 10,400 W high pressure sodium and metal halide lights. Plants were watered as needed. Soil water potential was monitored daily using soil water potential sensors read by Soil Moisture Meter (WaterMark Company, Inc., Wisconsin, USA). Soil was kept near the water field capacity as read by Soil Moisture Meter (about -15 to -20 kPa). The treatments for carbon dioxide were ambient CO_2 concentrations (about 360 $\mu\text{mol mol}^{-1}$) and elevated CO_2 concentration (700 $\mu\text{mol mol}^{-1}$). For temperatures, the treatments were normal temperature (26/16°C) and elevated (45/35°C). There were four treatment combinations (T1 = 26/16°C and 360 $\mu\text{mol mol}^{-1}$; T2 = 26/16°C and 700 $\mu\text{mol mol}^{-1}$, T3 = 45/35°C and 360 $\mu\text{mol mol}^{-1}$; T4 = 45/35°C and 700 $\mu\text{mol mol}^{-1}$). Two soybean cultivars were used: Williams 82 (MGIII) and Hutcheson (MG V).

Soil and Seed Analyses for Minerals, N, S, and C

Mineral analysis of soil K, P, B, Fe, N, S, and C was conducted by the Soil, Plant, and Water Laboratory, University of Georgia, Athens, GA. For K concentration, a 5-g soil: 20-ml Mehlich-1 solution was used and analyzed by inductively coupled plasma spectrometry (Thermo Jarrell-Ash Model 61E ICP and Thermo Jarrell-Ash Autosampler 300). For N, S, and C percentages a 0.25-g sample of soil was used and the samples were combusted in an oxygen atmosphere at 1350°C to convert elemental N, S, and C into N_2 , SO_2 , and CO_2 , respectively. Then, the resulting gases were then passed through infrared cells and the N, S, and C and then were determined by an elemental analyzer using thermal conductivity cells (LECO CNS-2000 elemental analyzer, LECO Corporation, St. Joseph, MI, USA) (Bellaloui et al., 2014; Bellaloui et al., 2009).

Seed samples were ground using a Laboratory Mill 3600 (Perten, Springfield, IL, USA). The samples were analyzed for minerals, N, S, and C by digesting 0.6 g of dried, ground plant materials in HNO_3 in a microwave digestion system. The concentration of K was determined by inductively coupled plasma spectrometry (Bellaloui et al., 2014; Bellaloui et al., 2009). For N, C, and S measurements, a 0.25 g ground-dried sample was combusted in an atmospheric oxygen of 1350°C, and the combusted samples were the converted to gases as detailed above. The N, S, and C were determined by an elemental analyzer using thermal conductivity cells (LECO CNS-2000 elemental analyzer, LECO Corporation, St. Joseph, MI, USA) (Bellaloui et al., 2014; Bellaloui et al., 2009).

Analyses of Seed Protein, Oil, Fatty Acids, and Sugars

Seeds collected at harvest maturity were analyzed for protein, oil, and fatty acids. The seed samples of 25 g of seed were ground by the Laboratory Mill 3600 and analyzed by near infrared reflectance (Wilcox and Shibbles, 2001; Bellaloui et al., 2014; Bellaloui et al., 2009) 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 AOAC methods [AOAC, 1990a; AOAC, 1990b]. Protein and oil concen-

trations were determined based on a seed dry matter (Wilcox and Shibbles, 2001, Bellaloui et al., 2014) and the concentrations of palmitic, stearic, oleic, linoleic, and linolenic fatty acids were determined on a total oil basis (Bellaloui et al., 2014). Determination of seed sugars (sucrose, raffinose, and stachyose) were conducted by near infrared reflectance (Wilcox and Shibbles, 2001; Bellaloui et al., 2014) using the AD 7200 array feed analyzer. The analyses of sugars were based on a seed dry matter basis (Wilcox and Shibbles, 2001; Bellaloui et al., 2014; Bellaloui et al., 2009).

Determination of Seed Glucose and Fructose

The concentration of glucose in the 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 (Bellaloui et al., 2014). The concentration was determined by reading samples at absorbance of 340 nm using the Beckman Coulter DU 800 spectrophotometer, and the concentration was expressed as mg g^{-1} dry weight. The concentration of fructose in seeds 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 elsewhere (Bellaloui et al., 2014). The concentration of fructose was determined by the Beckman Coulter DU 800 spectrophotometer by reading the samples at absorbance of 340 nm, and the concentration of fructose in seeds was expressed as mg g^{-1} dry weight.

Boron Determination

The concentration of boron in seeds was determined using the Azomethine-H method (Bellaloui et al., 2014; Lohse, 1982; Doradas et al., 2007) as reported elsewhere (Bellaloui et al., 2014). Briefly, a ground sample of 1.0 g was ashed at 500°C and extracted with 20 ml of 2 M HCl at 90°C for 10 minutes. 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. Then, 4 ml of fresh azomethine-H solution (0.45% azomethine-H and 1% of ascorbic acid) (John et al., 1975) was added. The concentration of boron in seeds was determined by reading the samples at 420 nm using a Beckman Coulter DU 800 spectrophotometer (Beckman Coulter, Inc., Brea, CA, USA).

Iron Determination

The concentration of Fe in seeds was determined according to methods described elsewhere (Bandemer et al., 1944; Loeppert and Inskeep, 1996). Samples were acid wet digested, extracted, and reacted with reduced ferrous Fe with 1,10-phenanthroline and as described elsewhere (Bellaloui et al., 2014). Briefly, a sample of 2 g of dried ground seeds were acid digested, and the soluble constituents were dissolved in 2 M HCl. A phenanthroline solution of 0.25% (w/v) in 25% (v/v) ethanol and quinol solution (1% w/v) was used. Standard curves were prepared with fresh standard solutions of Fe ion concentrations in 0.4M HCl, ranging from 0.0 to 4 $\mu\text{g ml}^{-1}$ of Fe. The concen-

tration of Fe in the samples was determined by reading the samples at absorbance of 510 nm using the Beckman Coulter DU 800 spectrophotometer.

Phosphorus Determination

The concentration of P in seeds was determined according to Cavel (1955). The method was based on the yellow phosphor-vanado-molybdate complex as detailed elsewhere (Bellaloui et al., 2014). Leaf and seed samples of 2 g were dried, ground and ashed. A volume of 10 ml of 6M HCl was added. Phosphorus was extracted using 2 ml of 36% v/v HCl under heat and filtration and 5 ml of 5 M HCl. An amount of 5 ml of reagent (ammonium molybdate–ammonium metavanadate) were added to 5 ml of the filtrate. The reagent Ammonium molybdate–ammonium metavanadate was prepared in 500 ml of distilled water by dissolving 25 g of ammonium molybdate and 1.25 g of ammonium metavanadate. Standard curve solutions of P were prepared by preparing standard solutions of P concentrations ranging from 0–50 µg ml⁻¹ using dihydrogen orthophosphates. The concentrations of P were determined by reading the absorbance at 400 nm using the Beckman Coulter DU 800 spectrophotometer.

Experimental Design and Statistical Analysis

The experiments were a split plot arrangement of treatments in a randomized complete block design (RCBD) with four replicates. Main plot was temperature/CO₂ treatment and subplot was cultivar. Each experiment is a replicate for the main plot. One growth chamber was used for each temperature/CO₂ treatment. For each experiment, there were four treatments (T1 = 26/16°C (normal) and 360 µmol mol⁻¹; T2 = 26/16°C and 700 µmol mol⁻¹, T3 = 45/35°C and 360 µmol mol⁻¹; T4 = 45/35°C

and 700 µmol mol⁻¹. The four levels of temperature/CO₂ are each replicated twice. Two soybean cultivars were used. Two experiments were conducted (two experiments were repeated in time). The analysis of variance of data was conducted using PROC MIXED in SAS (SAS, 2002-2010). Experiment (E), temperature (T), CO₂ concentration (CO₂), and their interactions were considered fixed effects, and replication within E, and replication × T × CO₂ × CV within E were considered random effects. Level of significance was P ≤ 0.05.

Results and Discussion

Analysis of variance indicated that temperature (T), CO₂, and cultivar (CV) were the major factors affecting seed composition and mineral nutrition. Seed protein, oleic and linolenic acids, and sugars, especially glucose and fructose, were the major seed composition constituents affected by these major main factors (T, CO₂, CV) (Table 1). Seed N, P, K, Mg, S, Fe, B, and Zn were the major macro- and micro-nutrients affected by T, CO₂, and CV (Table 2). Since experiment (EXP) (two experiments were repeated in time) did not interact with other factors (T, CO₂, CV), the results were combined across the two experiments as the variables had the same pattern and ranking in the two experiments. Results were presented by cultivar as cultivars were of different maturity (genotype is confounded with maturity).

Mean Values for Seed Composition Constituents (Protein, Oil, Fatty Acids, and Sugars)

Seed protein and linolenic acid decreased under elevated temperature, and elevated temperature with elevated CO₂. For example in cultivar Williams 82, the percentage decrease was 8% for protein, 62% for linolenic acids (Table 3). Oil and oleic acid increased with high temperature, elevated CO₂, and ele-

Table 1. Analysis of variance for soybean (Williams 82 and Hutcheson cultivars) seed protein, oil, fatty acids (%) and sugars (glucose, fructose, sucrose, raffinose, and stachyose, mg g⁻¹) as affected by the main effect factors of experiment (EXP: two experiments were conducted), temperature (T), carbon dioxide (CO₂), cultivar (CV), and their interactions. Treatments were four: T1 = plants were grown at 26/16°C and 360 µmol mol⁻¹; T2 = plants were grown at 26/16°C and 700 µmol mol⁻¹, T3 = plants were grown at 45/35°C and 360 µmol mol⁻¹; T4 = plants were grown at 45/35°C and 700 µmol mol⁻¹. Plants were grown in the greenhouse, but they were transferred to growth chambers at the beginning of seed-fill stage until maturation (R8).

Treatments	Protein	Oil	Oleic	Linolenic	Glucose	Fructose	Sucrose	Raffinose	Stachyose
EXP	*	*	ns	ns	*	ns	ns	**	ns
T	**	*	***	***	*	**	*	**	*
CO ₂	*	*	*	*	**	***	*	**	*
CV	**	*	*	*	*	**	*	*	ns
EXP × T	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
T × CO ₂	**	*	**	*	*	*	**	*	*
T × CV	**	ns	*	*	**	*	**	*	*
CO ₂ × CV	*	ns	*	*	*	*	ns	*	ns
EXP × T × CO ₂ × CV	ns	ns	ns	ns	ns	ns	ns	ns	ns

*= significance at P ≤ 0.05; **= significance at P ≤ 0.01; ***= significance at P ≤ 0.001.

Table 2. Analysis of variance for soybean (Williams 82 and Hutcheson cultivars) seed macro- and micro-nutrients (N, P, K, Mg: %; Fe, B, Cu, Zn, Mn: mg kg⁻¹) as affected by the mains effect factors of experiment (EXP: two experiments were conducted), temperature (T), carbon dioxide (CO₂), cultivar (CV), and their interactions. Treatments were four: T1= plants were grown at 26/16°C and 360 µmol mol⁻¹; T2 = plants were grown at 26/16°C and 700 µmol mol⁻¹; T3= plants were grown at 45/35°C and 360 µmol mol⁻¹; T4 = plants were grown at 45/35°C and 700 µmol mol⁻¹. Plants were grown in the greenhouse, but they were transferred to growth chambers at the beginning of seed-fill stage until maturation (R8).

Treatments	N	P	K	Mg	Fe	B	Cu	Zn	Mn
EXP	*	*	ns	ns	*	ns	ns	ns	*
T	**	*	**	**	*	**	*	*	*
CO ₂	**	**	***	ns	*	*	ns	*	ns
CV	*	**	**	*	*	**	*	*	ns
EXP × T	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
T × CO ₂	**	*	**	ns	*	**	ns	*	ns
T × CV	**	**	***	*	*	**	*	**	*
CO ₂ × CV	*	ns	*	ns	ns	*	ns	ns	Ns
EXP × T × CO ₂ × CV	ns	ns	ns	ns	ns	ns	ns	ns	ns

*= significance at $P \leq 0.05$; **= significance at $P \leq 0.01$; ***= significance at $P \leq 0.001$.

Table 3. Effect of elevated temperature and elevated carbon dioxide on soybean (Williams 82 cultivar) seed protein, oil, fatty acids (%) and sugars (glucose, fructose, sucrose, raffinose, and stachyose, mg g⁻¹). Treatments were four: T1= plants were grown at 26/16°C and 360 µmol mol⁻¹; T2 = plants were grown at 26/16°C and 700 µmol mol⁻¹; T3 = plants were grown at 45/35°C and 360 µmol mol⁻¹; T4=plants were grown at 45/35°C and 700 µmol mol⁻¹. Plants were grown in the greenhouse, but they were transferred to growth chambers at the beginning of seed-fill stage until maturation (R8).

Treatments	Protein	Oil	Oleic	Linolenic	Glucose	Fructose	Sucrose	Raffinose	Stachyose
T1	39.3 a	20.3 d	23.5 c	8.7 a	1.5 b	0.91 a	54 a	12.2 a	43.3 a
T2	37.4 b	21.7 c	26.4 b	7.9 b	2.2 a	0.93 a	58 a	9.9 b	41.5 b
T3	36.4 c	22.4 b	28.6 a	5.4 b	0.7 c	0.53 b	28 c	7.7 d	34.1 c
T4	36.8 c	23.6 a	28.5 a	5.4 b	0.6 c	0.43 c	38 b	8.9 c	33.7 c

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.

vated temperature with elevated CO₂. The increase in oleic acid was 18% and oil 14%. Seed glucose, fructose, and raffinose decreased under elevated T (Table 3), and elevated temperature with elevated CO₂. No decrease was noticed in stachyose in both cultivars. It appeared that Hutcheson accumulated more seed composition constituents than Williams 82, which may be due to a longer period of maturity in Hutcheson.

Mean Values for Seed Macro- and Micro-Nutrients

Seed N, P, K, Fe, B, and Zn decreased with elevated temperature and elevated temperature with elevated CO₂ (Table 4). Seed Mn, and Cu did not show consistency in the two cultivars. Generally, both cultivars were affected by elevated CO₂ and elevated temperatures for protein, oil, oleic, linolenic, sugars, and minerals, although the magnitude of response of both cultivars differed due to genotype and maturity differences (Table 5 and Table 6). The higher levels of some nutrients in Hutcheson could be due to genotype and maturity differences as Hutcheson took

more time (MG V) to mature than Williams 82 (MG III), resulting in higher accumulation of nutrient levels.

Previous research showed that oil concentration increased with increasing temperature up to 32/22°C, then decreased (Gibson and Mullen, 1996; Dornbos and Mullen, 1992). Other researchers analyzed 20 soybean cultivars, representing 10 maturity groups in 60 locations throughout the USA, and found that oil increased with temperature up to a mean temperature of 28°C. A quadratic equation was the best fit for oil vs. temperature (Piper and Boote, 1999). They concluded that 32/22°C is the optimum temperature to give the highest oil concentration in soybean seed. They also reported that since temperatures during the soybean-growing season in the southern USA are at or higher than, 32/22°C, oil concentration at temperatures above 32/22°C could decrease, impacting the soybean oil industry in the southern USA, especially with the increase of 4°C due to global climate change conditions. Current research showed that oil increased at the higher temperature regardless of the concentration of CO₂, indicating that temperature is more important

Table 4. Effect of elevated temperature (T) and elevated carbon dioxide (CO₂) on soybean (Williams 82 cultivar) seed macro- and micro-nutrients (N, P, K, Mg: %; Fe, B, Cu, Zn, Mn: mg kg⁻¹). Treatments were four: T1= plants were grown at 26/16°C and 360 µmol mol⁻¹; T2 = plants were grown at 26/16°C and 700 µmol mol⁻¹; T3 = plants were grown at 45/35°C and 360 µmol mol⁻¹; T4 = plants were grown at 45/35°C and 700 µmol mol⁻¹. Plants were grown in the greenhouse, but they were transferred to growth chambers at the beginning of seed-fill stage until maturation (R8).

Treatments	N	P	K	Mg	Fe	B	Cu	Zn	Mn
T1	5.6 a	0.62 a	1.8 a	0.31 a	87 a	56 a	9.8 a	48 a	39a
T2	5.0 b	0.43 b	1.4 b	0.31 a	67 b	43 c	9.6 a	43 b	38 a
T3	4.0 c	0.34 c	1.2 c	0.32 a	57 d	42 c	9.8 a	35 c	39 a
T4	4.2 c	0.47 b	1.1 c	0.31 a	67 c	45 b	10.1 a	36 c	40 a

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.

Table 5. Effect of elevated temperature (T) and elevated carbon dioxide (CO₂) on soybean (Hutcheson cultivar) seed protein, oil, fatty acids (%) and sugars (glucose, fructose, sucrose, raffinose, and stachyose, mg g⁻¹). Treatments were four: T1= plants were grown at 26/16°C and 360 µmol mol⁻¹; T2= plants were grown at 26/16°C and 700 µmol mol⁻¹; T3 = plants were grown at 45/35°C and 360 µmol mol⁻¹; T4 = plants were grown at 45/35°C and 700 µmol mol⁻¹. Plants were grown in the greenhouse, but they were transferred to growth chambers at the beginning of seed-fill stage until maturation (R8).

Treatments	Protein	Oil	Oleic	Linolenic	Glucose	Fructose	Sucrose	Raffinose	Stachyose
T1	42.0 a	19.5 c	23.6 c	8.7 a	2.1 a	1.12 a	67 a	12.5 a	45 a
T2	40.1 b	20.5 b	28.7 a	7.6 b	2.0 a	0.87 b	69 a	10.5 c	46 a
T3	37.5 d	22.5 a	26.5 b	6.9 c	0.8 b	0.62 d	46 c	11.3 b	43 b
T4	38.8 c	22.6 a	28.7 a	7.4 b	1.1 b	0.78 c	53 b	10.5 c	42 b

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.

Table 6. Effect of elevated temperature (T) and elevated carbon dioxide (CO₂) on soybean (Hutcheson) seed macro- and micro-nutrients (N, P, K, Mg, %; Fe, B, Cu, Zn, Mn, mg kg⁻¹). Treatments were four: T1 = plants were grown at 26/16°C and 360 µmol mol⁻¹; T2 = plants were grown at 26/16°C and 700 µmol mol⁻¹; T3 = plants were grown at 45/35°C and 360 µmol mol⁻¹; T4 = plants were grown at 45/35°C and 700 µmol mol⁻¹. Plants were grown in the greenhouse, but they were transferred to growth chambers at the beginning of seed-fill stage until maturation (R8).

Treatments	N	P	K	Mg	Fe	B	Cu	Zn	Mn
T1	6.8 a	0.67 a	2.3 a	0.36a	98 a	61a	10.1 a	49 a	45a
T2	5.3 b	0.54 b	1.8 b	0.37 a	87 b	56 b	9.5 a	47 a	43 a
T3	4.3 c	0.32 c	1.1 c	0.24 b	67 c	54 c	8.6 b	38 b	36 b
T4	4.0 c	0.34 c	1.2 c	0.22 b	63 c	44 d	8.7 b	39 b	34 b

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.

than CO₂ and its effects is more significant than CO₂, and CO₂ had a minimal effects (Thomas et al., 2003). On the other hand, our results differ from those of Thomas in that CO₂ concentration alone significantly affected protein and oil.

The increase of oleic acid and the decrease of linolenic acid with the increase of temperature was previously reported. Thomas et al. (2003) found that oleic acid concentration increased and linolenic acid decreased with increasing temperatures from 28/18 to 44/34°C, and also oleic acid concentration increased and linoleic acid decreased with the increase of temperatures (Rennie and Tanner, 1989; Gibson and Mullen, 1996; Rebetzke et al., 1996). Our findings are supported by these researchers

as oleic acid decreased and linolenic decreased (Rennie and Tanner, 1989; Gibson and Mullen, 1996; Rebetzke et al., 1996; Thomas et al., 2003). The increase of oleic acid has a nutritional benefit as it is more stable and less susceptible to oxidation than linolenic acid, which is desirable nutritional aspect for storage longevity of soybean oil (O'Byrne, 1995).

Although the decrease of N and protein at higher temperature in our experiments was previously reported, others researchers also found that N and/or crude protein concentration increased with temperature to 40/30°C. However, above 40/30°C protein concentration decreased (Thomas et al., 2003). A similar trend was shown by others (Piper and Boote, 1999), who report-

ed that protein concentration was lowest at 22°C (mean), and then increased as temperature increased to 28°C (mean). Thomas et al. (2003) showed that protein increased. From 28/18°C (23°C mean) up to 40/30°C (35°C mean), but that protein concentration within a range of mean temperatures from 15.5 to 30.5°C was relatively stable, but increased significantly at the highest temperature. They suggested that the increase of protein concentration with the decrease of oil may be a mathematical side effect due to the decreased oil and the total non-structural carbohydrate concentration by temperature increases (Thomas et al., 2003). In addition, soybean protein concentration is inversely correlated with oil (Krober and Cartter, 1962; Burton, 1987; Dornbos and Mullen, 1992; Piper and Boote, 1999).

The decrease of monosaccharides (glucose and fructose) and disaccharides (sucrose) observed in our findings as the temperature increased may be due to their conversion to further assimilates such as lipid, protein, or structural components (Thomas et al., 2003). The other suggestion is that the movement of these assimilates to seed was limited (Thomas et al., 2003; Thorne, 1982) due to the inhibition of phloem unloading from the seed coat vascular bundles into the apoplast surrounding the cotyledon cells under anaerobic conditions (Thorne, 1982). This was also supported by Thomas et al. (2003) who hypothesized that O₂ availability to seed may be limited and respiratory demand is higher as temperature increases.

The results of the effects of elevated CO₂ on seed composition are still conflicting. For example, Thomas et al. (2003) found minimal effects on seed composition, while Heagle et al. (1998) found a significant effect of elevated CO₂ on soybean seed oil in cultivars Essex, Holladay, and NK6955. In that study oleic acid concentration was positively affected by CO₂, but no effect of CO₂ on protein concentration was observed. Jablonski et al. (2002) in their meta-analysis of 79 reports on plant reproduction under elevated CO₂ showed that legume seed N concentration was not affected. Allen et al. (1998) reported that CO₂ concentration had no significant effects on seed total non-structural carbohydrates when soybean (cv. Bragg) was grown at six levels of CO₂ from 160 to 990 μmol mol⁻¹. Our results showed that seed protein decreased and oil and oleic acid increased with elevated CO₂ at normal temperature, which is supported by other researchers (Heagle et al. (1998). Heagle et al. (1998) showed a positive, significant effect of CO₂ enrichment on soybean seed oil and oleic acid concentration in soybean cultivars Essex, Holladay, and NK6955, but elevated CO₂ had no effect on protein concentration. It appears that higher temperature severely affected seed composition compared with elevated CO₂. It is clear that the effects of elevated temperature and elevated CO₂ on seed composition is still complex, and further research is needed to understand the biochemical basis of this phenomenon (Upreti et al., 2010). It was also reported that the effects of CO₂ magnitude on grain quality will depend on the level of future CO₂ atmospheric concentration, its interactions with the biotic (genotype, species, diseases, and pathogens) and abiotic stresses such as elevated temperature, drought, and soil conditions), and agronomic practices such as irrigation and growth conditions. Our knowledge of these interactions is currently limited and further research needs to be devoted to this

aspect (Upreti et al., 2010).

It was reported that the effect of increased temperatures (2–4°C) was higher than the effect of elevated CO₂ on grain quality (Tester et al., 1995; Williams et al., 1995). Most of the nutrients in grains result from the mobility of nutrients from vegetative pools (source) to grains (sink) during grain filling. The decrease of macro- and micro-nutrients, shown by our findings, was also noticed in other species such as wheat. For example, CO₂ elevation resulted in a decrease in Na, Ca, Mg, S, Fe, Zn, and Mn (Manderscheid et al., 1995; Upreti et al., 2010). Hogg and Fangmeier (2008) reported that elevated CO₂ resulted in a significant decrease of macro-nutrients, ranging from 0.7–19.5%, except for K and P. In addition, the decrease was in Ca (9.7%), Mg (4.8%), Na (5.5%), Ca (14.5%), Mg (7.2%), S (12.3%), Fe (3.7%), and Zn (18.3) (Dong-Xiu et al., 2004; Upreti et al., 2010). The decrease of macro- and micro-nutrients was explained to be due to the dilution effect induced by the increase of carbohydrates in grains. The decrease of some or all essential nutrients as a result of CO₂ elevation will be a challenge for the scientific community, especially breeders, to select for higher seed mineral nutrition varieties with high nutrient efficiency uptake under harsh environment of high temperatures, drought, and elevated CO₂ concentrations resulting from global climate changes. Since the total quantity of mineral nutrients accumulated in grains per hectare is higher under high CO₂ due to increase in grain yield (Prasad et al., 2005; Upreti et al., 2010), the elevated CO₂ effects is minimal on grain production. However, we do not understand the interactions of elevated CO₂ with high temperature, drought, or flood. Therefore, research is needed to quantify the negative impact of elevated CO₂ and its interactions with biotic and abiotic stresses on grain quality to improve high quality grains and improve appropriate crop management systems (Prasad et al., 2005; Upreti et al., 2010).

Dynamics of $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$ Ratio) and $\delta^{13}\text{C}$ ($^{13}\text{C}/^{12}\text{C}$ Ratio) Natural Abundance Isotopes

High temperature and high temperature with elevated CO₂ resulted in alteration of $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios in the two cultivars (Figure 1 and Figure 2). The alteration in $^{15}\text{N}/^{14}\text{N}$ is indicated by the increase of ^{15}N derived from plant gas exchange through stomatal conductance and CO₂ fixation (Livingston et al., 1999; Matsushima and Chang, 2007). The alteration of $^{13}\text{C}/^{12}\text{C}$ ratio may have resulted from the increase of stomatal closure and ^{13}C fixation enrichment, leading to less discrimination against $\delta^{13}\text{C}$ and may be a shift in carbon fixation metabolism and ^{13}C enrichment (O'Leary, 1995). It is known that current atmospheric CO₂ concentration is sub-optimal for photosynthesis of C₃ plant, including soybean, dry bean, peanut, and cowpea. The fixation of CO₂ in C₃ plant goes through the primary CO₂ acceptor, which is ribulose biphosphate (RuBP). During this process the enzyme ribulose biphosphate carboxylase-oxygenase (Rubisco) catalyzes this reaction. Since Rubisco catalyzes both carboxylation and oxygenase reactions, CO₂ and O₂ compete for the same site on Rubisco. This competition leads the oxygenase activity of Rubisco (photorespiration) to about 25% (20 to 60%) loss of carbon in C₃ species (Bowes, 1996; Prasad et

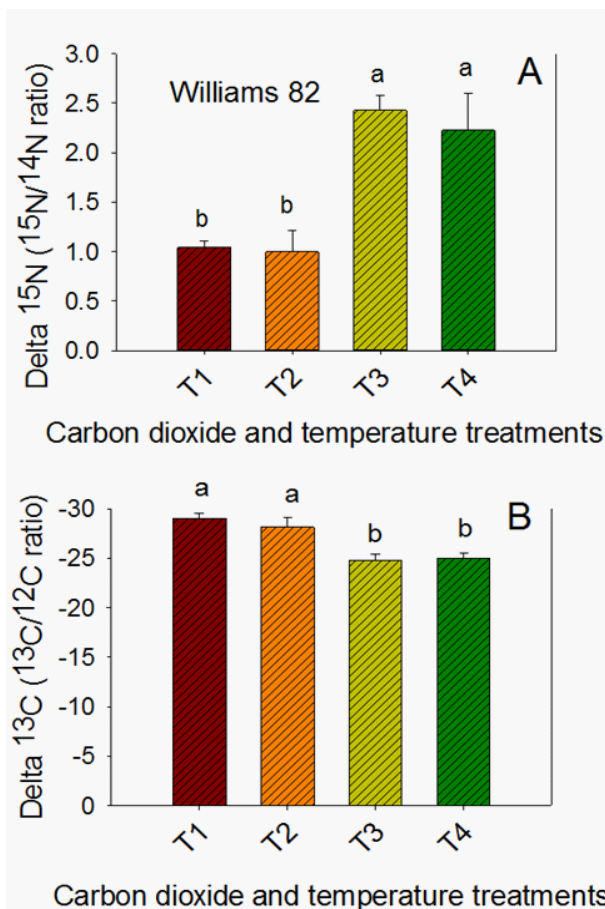


Figure 1. Cultivar Williams 82 (Maturity Group III). $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$ ratio) (A) and $\delta^{13}\text{C}$ ($^{13}\text{C}/^{12}\text{C}$ ratio) (B) natural abundance isotope in soybean seed as affected by elevated CO_2 and elevated temperature. Treatments were four: T1 = plants were grown at 26/16°C and 360 $\mu\text{mol mol}^{-1}$; T2 = plants were grown at 26/16°C and 700 $\mu\text{mol mol}^{-1}$; T3 = plants were grown at 45/35°C and 360 $\mu\text{mol mol}^{-1}$; T4 = plants were grown at 45/35°C and 700 $\mu\text{mol mol}^{-1}$. Plants were grown in the greenhouse, but they were transferred to growth chambers at the beginning of seed-fill stage until maturation (R8).

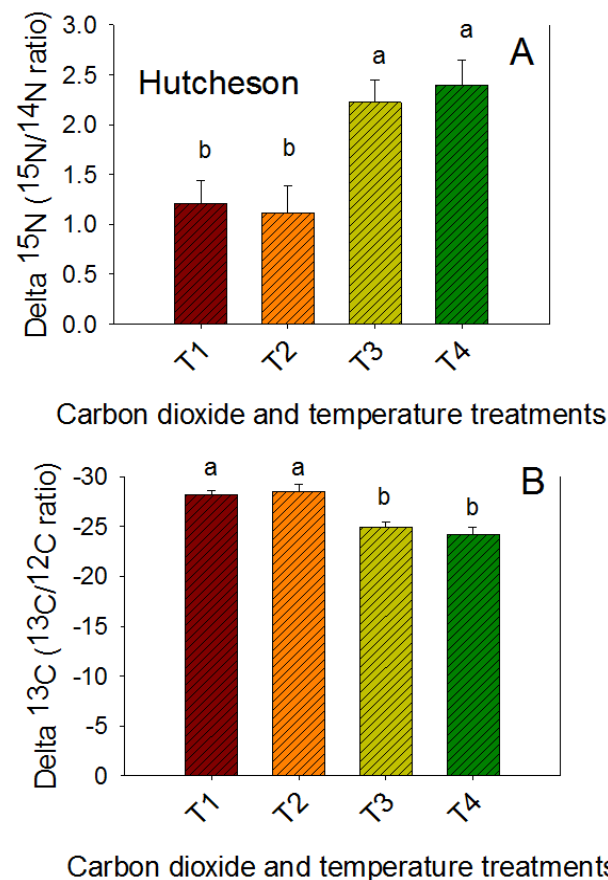


Figure 2. Cultivar Hutcheson (Maturity Group V). $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$ ratio) (A) and $\delta^{13}\text{C}$ ($^{13}\text{C}/^{12}\text{C}$ ratio) (B) natural abundance isotope in soybean seed as affected by elevated CO_2 and elevated temperature. Treatments were four: T1 = plants were grown at 26/16°C and 360 $\mu\text{mol mol}^{-1}$; T2 = plants were grown at 26/16°C and 700 $\mu\text{mol mol}^{-1}$; T3 = plants were grown at 45/35°C and 360 $\mu\text{mol mol}^{-1}$; T4 = plants were grown at 45/35°C and 700 $\mu\text{mol mol}^{-1}$. Plants were grown in the greenhouse, but they were transferred to growth chambers at the beginning of seed-fill stage until maturation (R8).

al., 2005). This was supported by previous research that photosynthetic rates of soybean plants were higher at higher levels of irradiance (ranging from 100 to 1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) compared to those at 330 $\mu\text{mol mol}^{-1}$ (Campbell et al., 1990). Increased photosynthetic rates of soybean leaves and/or canopies with elevated CO_2 have been reported in several other studies (Ferris et al., 1998; Ferris et al., 1999; Ziska et al., 2001). It appears that the alteration in $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios could be due changes in N and C metabolism brought about high by temperature and high CO_2 concentration. Further research is needed in this area to further understand the changes in $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios.

Conclusion

Our research showed that high temperature resulted in a decrease of protein and linolenic acid, but an increase of oil and

oleic acid. The increase of oil, oleic acid, and low linolenic acid is desirable as both high oleic acid and low linolenic acid contribute to oil stability and long shelf-life of the oil. Although the effect of CO_2 alone had significant effect on seed composition, the effect of temperature is more pronounced than the effect of CO_2 , which had relatively minimal effects (Tester et al. 1995; Williams et al. 1995; Thomas et al., 2003). Also, high temperature resulted in lower mono- (glucose and fructose) and di-saccharides (sucrose). Raffinose and stachyose are more stable than mono- and di-saccharides, which may be due to their possible role in heat stress response. High CO_2 elevation resulted in a decrease in some macro- and micro-nutrients. The change of $\delta^{15}\text{N}$ $\delta^{13}\text{C}$ natural abundance isotopes indicated possible alteration in nitrogen and carbon metabolism. The current research is beneficial to scientist as it provides further knowledge on the effects of increased temperature and elevated CO_2 on seed quality, especially seed nutrition. Also, the research is beneficial to breed-

ers, who will need to select varieties for higher seed nutritional qualities and efficient mineral nutrients use and uptake, as these traits are related to seed production, quality, and food security.

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