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Effect of Water Stress and Foliar Boron Application on Seed Protein, Oil, Fatty Acids, and Nitrogen Metabolism in Soybean[*]

Nacer Bellaloui

Crop Genetics and Production Research Unit, USDA-ARS, Stoneville, MS, USA.
Email: nacer.bellaloui@ars.usda.gov

ABSTRACT

Effects of water stress and foliar boron (FB) application on soybean (Glycine max (L) Merr.) seed composition and nitrogen metabolism have not been well investigated. Therefore, the objective of this study was to investigate the effects of water stress and FB on seed protein, oil, fatty acids, nitrate reductase activity (NRA), and nitrogenase activity (NA). A repeated greenhouse experiment was conducted where one set of soybean plants were subjected to water stress (WS), and the other set was watered (W). Foliar boron (B) was applied at rate of 0.45 kg·ha^{-1}. Treatments were watered-plants with no FB (W), watered-plants with FB (WB), water-stress plants with no FB (WS), and water-stress plants with FB (WSB). The results showed that seed protein and oil percentage were significantly (P < 0.05) higher in WB than other treatments. Oleic acid increased and linolenic acid decreased in WB and WSB. Significant (P < 0.05) increase in NRA in leaves and roots and NA occurred in WB compared to W. In WSB, NRA in leaves and roots or nitrogenase activities were higher than those in WS. Nitrate reductase activity in nodules was greater in WB than in W, and was higher in WSB than in WS. The concentration of B in leaves and seed were significantly (P < 0.05) higher in W than in WS. Seed $^{15}N/^{14}N$ and $^{13}C/^{12}C$ natural abundance were altered between watered- and watered-stressed plants. These results suggest that water stress and FB can influence seed composition, and nitrogen metabolism, and $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios, reflecting environmental and metabolic changes in carbon and nitrogen fixation pathways. Lack of B translocation from leaves to seed under water stress may suggest a possible mechanism of limited B translocation under water stress. These findings may be beneficial to breeders to select for B translocation efficiency under drought conditions. Altered $^{15}N/^{14}N$ and $^{13}C/^{12}C$ under water stress can be used as a tool to select for drought tolerance using N and C isotopes in the breeding programs.

***Keywords**: Boron Nutrition, Nitrate Reductase, Nitrogenase, Nitrogen Assimilation, Nitrogen Fixation, Nitrogen Metabolism, Seed Composition, Nitrogen and Carbon Isotopes*

1. Introduction

Soybean is a major crop in the world, and soybean seed is a major source of protein and oil. The quality of soybean seed is determined by the content and composition of protein, oil, and fatty acids. Soybean seed protein ranges from 34 to 57% of total seed weight, with a mean of 42%, and oil content ranges from 8.3 to 28%, with a mean of 19.5% [1]. The concentration of saturated fatty acids in soybean seed oil ranges from 10% to 12% palmitic acid (C16:0), and 2.2 to 7.2% stearic acid (C18:0) [2].

The mean concentration of unsaturated fatty acids in soybean seed oil is 24% oleic acid (C18:1), 54% linoleic acid (C18:2), and 8.0% linolenic acid (C18:3) [3]. Higher oleic acid and lower linolenic acid are desirable traits for oil stability and long-term shelf storage for industrial and processing purposes, but higher linolenic acids (essential polyunsaturated) are desirable for human nutrition. Hydrogenation of polyunsaturated fatty acids such as linolenic acid leads to trans-isomers, which are associated with increased incidence of heart disease [4]. However, monounsaturated fatty acids such as oleic acid are less susceptible to oxidative changes during refining, storage, and frying. Consequently, the food industry is becoming increasingly interested in producing soybean seed with a

[*]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

high content of oleic acid and low linoleic and linolenic acids [5]

Boron is an essential micronutrient for plant growth and development [6-8]. Boron was reported to have an important role in nodule development of *Vicia faba* [9], and was found to be an essential micronutrient for the development of nodules in pea (*Pisum sativum*) [10]. It was reported that Rhizobia inside nodules exhibited little or no ability to fix N2 in B-deficient plants, resulting in N deficiency and necrosis of nodulated pea plants [11]. Nitrogenase fixes atmospheric N2 in the bacteroids of nodules [12], and nitrate reduction (assimilation) is catalyzed by the enzyme nitrate reductase, a limiting step in nitrate assimilation. Both nitrate reductase and nitrogenase enzymes coexist in nodules and compete for a reducing agent (reductant) [13].

The role of B in flower set, fruit set, seed set, and seed quality was reported in other species, and it was found that floral and fruiting organs are sensitive to B deficiency [14,15]. Higher B requirements during flowering and seed set were shown even where B levels in leaves are in the adequate range [16]. Recently, it was found that foliar B application improved seed protein and seed oleic fatty acid [17], and seed yield and seed quality of alfalfa [16], and increased fruit set [18].

In spite of B role in structure, metabolism, and improving yield and seed quality in plants, its effect on seed composition and yield in soybean has not been yet established. Therefore, the objective of this research was to investigate the effects of foliar B application on seed composition (protein, oil, and fatty acids), nitrogen assimilation, and nitrogen fixation in soybean. Since changes in plant physiology and environment resulting from water stress or drought may alter natural abundances of carbon isotopes ($\delta^{13}C$) and nitrogen isotopes ($\delta^{15}N$) in higher plants [19-21], seed nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes were also investigated.

2. Materials and Methods

A greenhouse experiment was repeated twice. Seeds of soybean cultivar AG4903RR were germinated in flat trays in vermiculite. Uniform size seedlings at about V1 stage were transplanted into 9.45 L size pots filled with field soil. The soil was a Dundee silt loam (fine-silty, mixed, active, thermic Typic Endoqualfs) with pH 6.3, 1.1% organic matter, a cation exchange capacity of 15 cmol/kg, and soil textural fractions of 26% sand, 56% silt, and 18% clay, average B concentration was 0.72 mg·kg^{-1}, and it contained an abundant native population of *B. japonicum*. To induce water stress, soil in pots were weighed and then saturated with dionized water and left to drain and weighed again to obtain the water field

capacity as measured by soil water sensors inserted in pots. Soil water potential was measured daily using Soil Moisture Meter (WaterMark Company, Inc., Wisconsin, USA). Water stressed plants were kept between –90 and –100 kPa. This represented a moderate to severe water stress level for soybean under greenhouse conditions. Watered plants were kept between –15 to –20 kPa (this was considered field capacity for the control plants) [17]. Half of the plants from each B treatment was watered (W), and the other half was water stressed (WS). Treatments were watered plants with no foliar B (W), watered plants with foliar B (WB), water stress plants with no foliar B (WS), and water stress plants with foliar B (WSB). Boron, as boric acid, of a rate of 0.45 kg·ha^{-1} was foliar applied using hand sprayer, and measures to avoid boron drift to the control plants were taken [17]. Boron was applied at R1-R2 (flowering stage) and R5-R6 (seed-fill stage) [22], or not applied (control). Samples were taken five days after each foliar B application for NRA, nitrogenase, and leaf B. Mature seed were weighed at R8 (physiological maturity stage). Plants were considered fully matured when they reached R8 according to [22]. Treatments were arranged in a split plot design with irrigation as a main block and B treatment as sub-plot. Four replicates were used for each treatment and for each sampling time for each experiment. Each pot with four individual plants was considered one replicate Greenhouse conditions were about 34˚C ± 9˚C during the day and about 28˚C ± 7˚C at night with a photosynthetic photon flux density (PPFD) of about 800 - 2300 µmol·m^{-2}·s^{-1}, as measured by Quantum Meter (Spectrum Technology, Inc., Illinois, USA). The range of light intensity reflects a bright, sunny, or cloudy day. The source of lighting in the greenhouse was a mixture of natural light, bulb light (60 W), cool white (250 W). To be consistent with the normal photoperiod for soybean growth and to avoid differences in the day-length between the two experiments, the two experiments were conducted simultaneously at the same time and during the normal growing season (from April to September) for the Early Soybean Production System in the midsouth USA.

2.1. Nitrate Reductase Assay

Nitrate reductase activity (NRA) was determined according to [23,24]. Briefly, NRA was measured in leaves (fully expanded leaves), stems, roots, and nodules. Nodules were gently and carefully separated from roots and placed in NRA assay buffer solution. Approximately 0.3 g of plant tissue was placed in 10 mL of potassium phosphate buffer at a concentration of 100 mM, pH 7.5, containing 1% (v/v) 1-propanol, in the flask. The incu-

bation solution was vacuum filtered for 1 min, flashed with nitrogen gas for 30 s, and then incubated at 30˚C. Samples of 0.5 mL were taken at regular intervals (0, 60, 120, 180, and 300 min) for nitrite measurement. Samples were extracted with 5 mL of deionized water and reacted with 1.0 mL of 1% (w/v) sulfanilamide in 10% v/v HCl and 1.0 mL of *N*-naphthyl-(1)-ethylenediamine dihydro-chloride (0.1%). The samples were read at 540 nm after 30 minutes using a Beckman Coulter DU 800 spectro-photometer (Fullerton, CA). A standard curve made of nitrite concentrations was prepared and used to measure nitrite concentrations in the plant tissue (Bellaloui *et al*., 2006). Potential NRA (PNRA) was measured by adding exogenous nitrate to the incubation solution at a concen-tration of 10 mM as KNO_3.

2.2. Acetylene Reduction Assay

Nitrogenase activity (NA) was assayed using the acety-lene reduction assay as described elsewhere [25,26]. Briefly, roots with nodules intact were excised and incubated in 1 L Mason jars. Four roots were placed in the Mason jars and sealed. A 10% volume of air was then removed and replaced with an equal volume of acetylene. After 1 h of incubation at room temperature, duplicate 1.0 mL gas samples were removed and analyzed by gas chromato-graphy for ethylene formation. An Agilent HP6960 (Agi-lent Technologies, Wilmington, DE) gas chromatograph was equipped with manual injector, injector loop, sample splitter, flame ionization detector (FID), and thermal conductivity detector (TCD). A sample of 0.25 mL of gas was directed into a 30 m length × 0.53 mm i.d. alu-mina megabore column (115 - 3532) connected to the FID, and 0.25 mL of sample was injected into a HP-PLOT D column (30 m length × 0.53 mm i.d. megabore with 40 μm film; 1905D-Q04) connected to the TCD using helium as a carrier gas. Chromatographs were in-tegrated using Chem Station software. Standard curves for ethylene were developed for each day of analysis and used to determine ethylene. Nodules were obtained by carefully removing the nodules from the roots, and the dry weight was determined following oven-drying at 60˚C for 4 - 5 days.

2.3. Boron Determination

Boron in fully expanded leaves and seed was determined according to Azomethine—H method [17,27]. Briefly, 1 g of dry samples was placed in a porcelain crucible for ashing at 500˚C for 8 hr. Then, samples were extracted with 20 mL of 2 M HCl at 90˚C for 10 min. After filtra-tion, the samples were transferred to plastic vials and 2 mL of the solution was added to 4 mL of buffer solution and 4 mL of azomethine-H solution containing 0.45%

azomethine-H and 1% of ascorbic acid prepared right before the analysis [28]. The buffer solution contained 25% ammonium acetate, 1.5% EDTA, and 12.5% acetic acid. Boron concentration measurement was performed after 45 minutes of color development. Samples were read using a Beckman Coulter DU 800 spectrophoto-meter (Fullerton, California) at 420 nm. Boron analysis in soil was conducted at The University of Georgia, Soil, Plant, and Water Laboratory, Athens, GA. Briefly, a soil sample of 5.0 g: 20 mL Mehlich 1 solution was used. Boron concentration was analyzed using Inductively Coupled Plasma spectrometry (ICP) using Thermo Ele-mental, Thermo Jarrell-Ash model 61E ICP, USA.

2.4. Analysis of $\delta^{15}N$ ($^{15}N/^{14}N$ Ratio) and $\delta^{13}C$ ($^{13}C/^{12}C$ Ratio) Using Natural Abundance

Delta ^{15}N and ^{13}C natural abundance was determined from nitrogen isotope, $^{15}N/^{14}N$ ratio, and carbon isotope, $^{13}C/^{12}C$ ratio, using about 0.9 mg of ground seeds. Isotopic analysis was conducted using a Thermo FinniGlyn Delta Plus Advantage Mass Spectrometer with a FinniGlyn ConFlo III, and Isomass Elemetal Analyzer (Bremen, Ger-many). Isodat software version 2.38 was used to obtain Delta values [29,30]. The elemental combustion system was Costech ECS 4010 with an autosampler (Bremen, Germany).

2.5. Analysis of Seed Protein, Oil, and Fatty Acids

Seed protein, oil, and fatty acids were analyzed using near-infrared (NIR) reflectance [24,31], diode array feed analyzer, Perten. The calibration was developed by the University of Wisconsin, USA using Perten's Thermo Galactic Grams PLS IQ software. The calibration was developed for unique samples using AOAC methods [32, 33]. The analysis was performed on the basis of percent dry matter [31,34].

2.6. Experimental Design and Statistical Analysis

Treatments were arranged in a split plot design with irri-gation as a main block and B treatment as sub-plot. The data were subjected to analysis of variance using Proc GLM in SAS [35]. Means were separated by Fisher's least significant difference test at the 5% level of pro-bability. Since there were no interactions between the two experiments for the measured variables, the data were pulled and combined.

3. Results and Discussion

3.1. Seed and Nodule Weights

Seed (**Figure 1(a)**) and nodule (**Figure 1(b)**) weights

were higher in W and WB, with the highest weight observed in WB treatment. This was observed at both R1-R2 and R5-R6 stages (**Figures 1(b) and (c)**). This indicates the significance positive effects of foliar B on seed and nodule weights. The lowest seed and nodule weight were observed in WS and WSB. The increase of soybean seed weight by foliar B was previously reported by other researchers. For example, it was found that B application increased soybean seed yield [36,37]. In general, B application in the field had negative, positive, or no yield responses from direct applications of B fertilizer [36-39]. The increase of nodule weight by FB indicates the positive response of nodules to B. It was reported that B is an essential micronutrient for the development of nitrogenfixing root nodules in pea (*Pisum sativum*) [40]. This may indicate that B induces nodule formation and development, as suggested by [40], or lowers infection of the host plants with Rhizobium in plants grown in B-deficient medium compared with plants supplied with adequate B [40]. Recently, it was found that FB increased nodule weight under irrigated greenhouse conditions [17]. Since B concentrations in leaves of WS plants fall below 20 mg·B·kg^{-1}, critical level of B in leaves for normal plant growth [41], a positive response to FB was expected. However, the non-response of WS or WSB to FB may be due to water stress effects. The current results showed that, even though B concentration in leaves was above the critical level, FB had a positive effect on seed and nodule weights. Therefore, the current results agreed with previous research of those of [17,36,37] in that FB can increase seed and nodule weights in soybean.

3.2. Boron Concentrations in Leaves and Seed

Boron concentration in leaves was significantly higher in W and WB than in WS and WSB, with leaf B in WB being the highest (**Figure 1(d)**). This was noticed at R1-R2 and R5-R6 stages. Seed B concentration was the highest in WB (**Figure 1(d)**). The increase in B concentration in leaves of WSB did not result in higher B concentration in seed. The higher B concentrations in leaves of WSB than in WS indicate that FB leads to higher B concentrations in leaves under water stress. The lower level of B concentration in leaves of WS plants indicates that B was not taken up under water stress even when B concentration in soil was adequate. Although soil B in the used soil was considered adequate, B concentration in leaves of WS was significantly lower than those of WSB due to water stress. The lower B concentration in seed in spite of transferrable B from leaves to seed in in WS and WSB plants indicated that B translocation from leaves to seed was limited under water stress, suggesting that the positive or beneficial effect of FB on
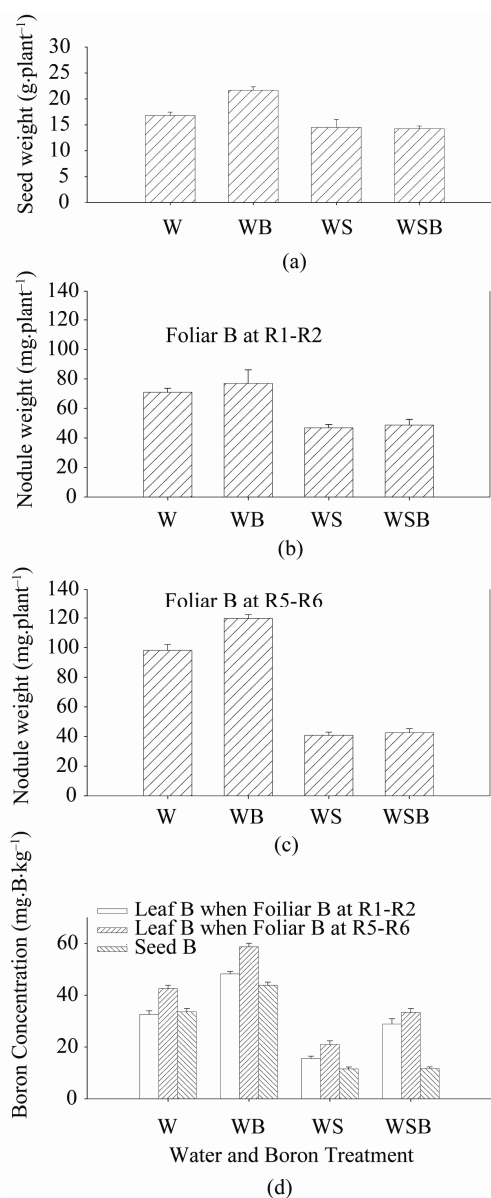

Figure 1. Effect of foliar boron (B) application on seed weight (a), nodule weight at R1-R2 stage (b), nodule weight at R5-R6 stage (c), boron in leaf and seed (d). Foliar B treatments were: W = plants were watered with no foliar B; WB = Plants were watered and received foliar B; WS = plants were water-stressed with no foliar B; WSB = plants were water-stressed and received foliar B. Foliar B was applied at R1-R2 (flowering stage and at R5-R6 (seed-fill stage). Bar Values are means ± SE.

soybean may depend on B translocation from leaves (source) to seed (sink). Limited translocation of B from leaves to seed under water stress condition could underline one of the mechanisms of how water stress affects B nutrition. If this is the case, then FB application under water stress or drought conditions in the field may in-

crease B concentrations in leaves and seed. This may explain the controversial literature on the effect of B on soybean yield. For example, during dry growing season, application of FB may increase seed yield, but under normal growing season with normal rainfall, application of FB may not increase seed yield. The higher cell wall B percentage in WS (91%) and WSB (86%) compared with that of W (71%) or WB (61%) plants (data not shown), emphasized the structural role of B in cell wall, as suggested by previous researchers [17,42].

3.3. *In Vivo* Nitrate Reductase Activity

Five days after FB, NRA at R1-R2 in leaves and roots of W and WB was significantly higher than in those of WS or WSB (**Figure 2(a)**). Leaves of WSB showed higher

NRA than those of WS. Same pattern of NRA was observed at R5-R6 growth stage (**Figure 2(b)**). Adding nitrate to the incubation solution (potential nitrate reductase activity, PNRA) to either leaves or roots resulted in a significant increase of PNRA in WS and WSB plants compared with W and WB (data not shown). The percentage increase of potential NRA in WS was 47% in leaves and 41% in roots, and in WSB was 35% in leaves and 31% in roots compared with their equivalent NRA without adding nitrate to the incubation solution (data not shown). Nitrate reductase activity per organ (leaves, stems, or roots) and per plant showed the same pattern as those of NRA per g fwt (**Figure 3(a)**). Similar pattern was observed five days after FB at R5-R5 (**Figure 3(b)**). Total NRA ($\mu mol \cdot nitrite \cdot plant^{-1} \cdot h^{-1}$) showed that W plants had the highest NRA followed by W, WSB, and finally WS, following similar pattern of those in NRA per g fwt or per organ (**Figure 4(a)**). The higher NRA in leaves and roots in W and WB indicates the nega-

Figure 2. Effect of foliar boron (B) application on nitrate reductase activity (NRA) in soybean leaves, roots, stems, and nodules. Foliar B treatments were: W = plants were watered with no foliar B; WB = Plants were watered and received foliar B; WS = plants were water-stressed with no foliar B; WSB = plants were water-stressed and received foliar B. Foliar B was applied at R1-R2 (flowering stage (a) and at R5-R6 (seed-fill stage) (b). Bar Values are means ± SE.
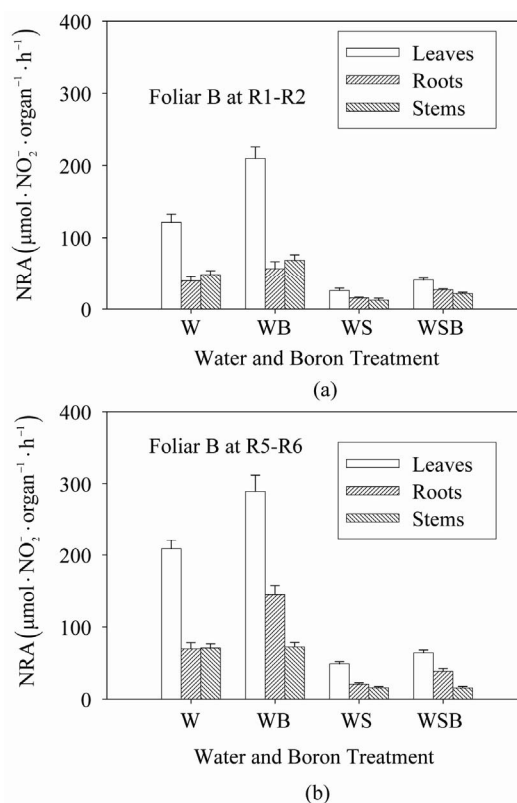

Figure 3. Effect of foliar boron (B) application on nitrate reductase activity (NRA) per soybean organ (leaves, roots, stems). Foliar B treatments were: W = plants were watered with no foliar B; WB = Plants were watered and received foliar B; WS = plants were water-stressed with no foliar B; WSB = plants were water-stressed and received foliar B. Foliar B was applied at R1-R2 (flowering stage (a) and at R5-R6 (seed-fill stage) (b). Bar Values are means ± SE.

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tive effect of water stress on nitrogen assimilation, leading to NRA inhibition. The increase of leaf NRA with FB indicates that B may stimulate NRA by, possibly, increasing nitrate uptake or translocation of nitrate from vacuoles (inaccessible nitrate for reduction) to cytoplasm (accessible nitrate for reduction). The higher increase in PNRA in roots in WS and WSB than those of W or WB indicates that water stress limited nitrate (substrate) availability to nitrate reductase, and this was reflected by the striking increase of PNRA when nitrate was added to the incubation solution. This observation also suggests that nitrate reductase was inactive under water stress conditions, and can be active when the substrate, nitrate, becomes available. Severe water stress (soil water potential between –150 to –195 kPa) led to complete irreversible NRA (data not shown).

The increase of NRA by FB may be due to an inducing, indirect effect [7] of B on nitrate assimilation [17]. The exact mechanism of B effects on NRA is not yet understood, but it was suggested that B may induce nitrate uptake and nitrate availability to nitrate reductase, enhance *de novo* synthesis of the enzyme and its effects on cell membrane. The influence of B on ion uptake was previously reported [7,43], and was suggested to be mediated by direct or indirect effects of B on the plasma membrane-bount H$^+$ ATPase [36,44,45], cell wall structure and membrane integrity [7,36]. The current results support those of [7] in that B has an indirect effects by inducing nitrate uptake and assimilation [17], increasing nitrate availability to NR, and contributes to membrane cell membrane cell wall integrity.

3.4. δ^{15}N (^{15}N/^{14}N ratio) and δ^{13}C (^{13}C/^{12}C Ratio) Natural Abundance

There was no difference in ^{15}N/^{14}N or ^{13}C/^{12}C between irrigated foliar applied and non-foliar applied soybean.

However, there were significant changes in ^{15}N/^{14}N and ^{13}C/^{12}C ratios between irrigated and non-irrigated soybean with or without FB (**Figures 4(b)** and **(c)**). Water stress altered ^{15}N/^{14}N by increasing ^{15}N (derived from soil nitrogen that is used for nitrate assimilation) and decreasing ^{14}N (derived from atmospheric nitrogen that is used for nitrogen fixation) (**Figure 4(a)**). This indicates that water stress inhibited nitrogen fixation, may be due to that nitrogenase is more sensitive than nitrate reductase. This shift in ^{15}N/^{14}N may reflect a possible mechanism to compensate for the inhibition of nitrogen fixation under water stress conditions. It has been reported that the δ^{15}N values in the xylem and plant tissues are associated with acquired N, and the value can be altered because of N metabolism [21]. There was also a change in ^{13}C/^{12}C ratio between irrigated and non-irrigated soybean with or without FB (**Figure 4(c)**). The
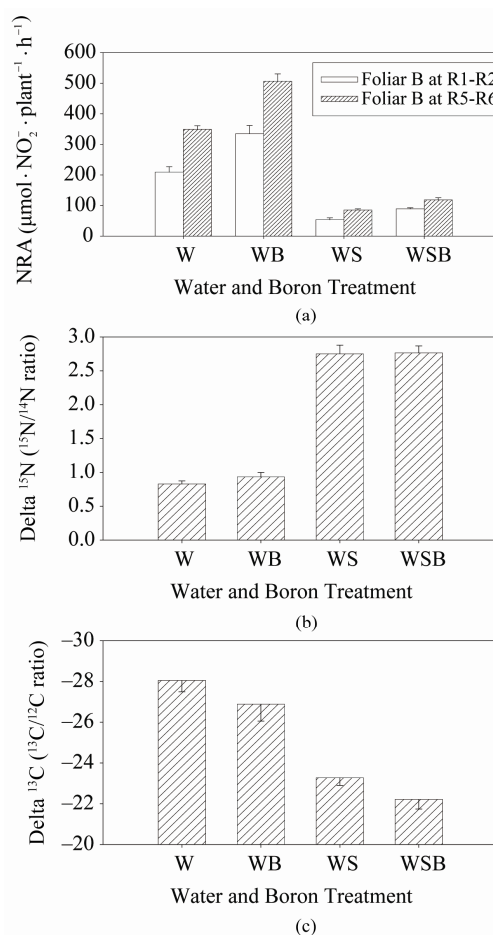

Figure 4. Effect of foliar boron (B) application on nitrate reductase activity (NRA) per whole plant (a) and on seed δ ^{15}N (^{15}N/^{14}N ratio) (b), and on seed δ ^{13}C (^{13}C/^{12}C ratio) (c). Foliar B treatments were: W = plants were watered with no foliar B; WB = Plants were watered and received foliar B; WS = plants were water-stressed with no foliar B; WSB = plants were water-stressed and received foliar B. Bar Values are means ± SE.

increase in δ^{13}C (higher ^{13}C/^{12}C ratio = less negative) in seed of water stressed soybean indicates that water stress altered the source of carbon fixation. It was reported that the δ^{13}C value in plant tissues can be influenced by water supply and temperature [46], plant physiology [47], and mycorrhizal infection [48]. There are two systems for carbon fixation in N$_2$-fixing plants, depending on where the carbon is fixed. In nodules carbon fixation is catalyzed by phosphoenolpyruvate (PEP) carboxylase; in leaves carbon is fixation is catalyzed by ribulose bisphosphate (RuBP) carboxylase. Therefore, the incorporation of fixed carbon by the nodules may change the δ^{13}C value in the nodulated plants. The δ^{13}C values of carbon fixed by PEP carboxylase are less negative than that of CO$_2$ fixed by RuBP carboxylase [46]. It was found that

for C_3 species, the variability of carbon isotope composition among and between genotypes correlated with water use efficiency, and during carbon fixation by photosynthesis, the naturally occurring stable isotope ^{13}C is discriminated against, and plants would have a smaller ^{13}C to ^{12}C ratio than ^{13}C to ^{12}C ratio in fixed CO_2 of the air [49]. The altered ^{13}C to ^{12}C ratio could be due closure of stomatal conductance under drought, resulting in an increase of ^{13}C fixation, leading to less ^{13}C discrimination [50,51]. The current results support previous research that environmental stresses, including drought, can alter $\delta^{13}C$ as a result of effects on the balance between stomatal conductance and carboxylation [50-52].

3.5. Acetylene Reduction Assay

Five days after FB at R1-R2, FB resulted in higher nitrogen fixation in WB and WSB plants, with nitrogen fixation being the highest in WB plants. This pattern was shown at R1-R2 and R5-R6 (**Figures 5(a)** and **(b)**). The relationship between B and nitrogen fixation is not yet clear.
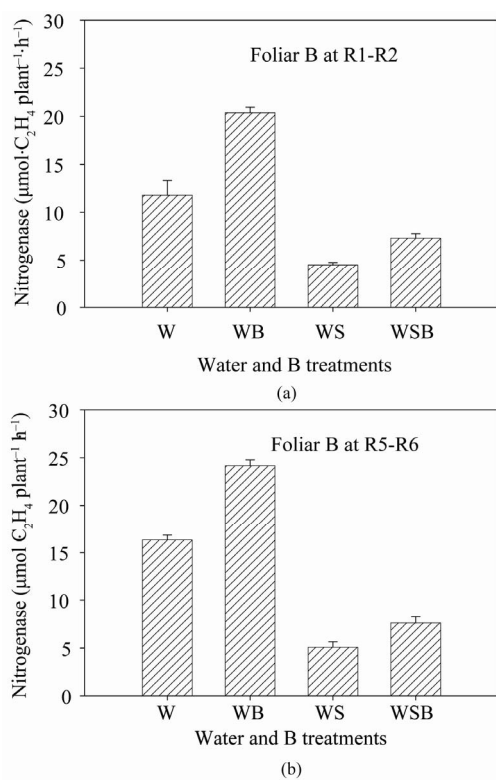

Figure 5. Effect of foliar boron (B) application on nitrogenase activity in soybean at R1-R2 stage (a) and at R5-R6 stage (b). Foliar B treatments were: W = plants were watered with no foliar B; WB = Plants were watered and received foliar B; WS = plants were water-stressed with no foliar B; WSB = plants were water-stressed and received foliar B. Foliar B was applied at R1-R2 (flowering stage (a) and at R5-R6 (seed-fill stage) (b). Bar Values are means ± SE.

However, previous research suggested that nodules under B deficiency had little or no ability to fix N_2, leading to N deficiency and necrosis of nodulated pea plants [11]. Recently, it was shown that FB increased nitrogen fixation under irrigated greenhouse conditions [17]. In spite of the possible explanation that limited B can reduce early nodullin protein (ENOD2) in nodule parenchyma cells and malfunction of oxygen diffusion barrier, especially under B deficiency [53]. There is no convincing evidence that there is a direct effect of B on nitrogen metabolism [7,17,53,54]. The current results showed that FB increased nitrogenase activity under both water stress and irrigated conditions, agreeing with previous studies [11,17,40]. It was suggested that B may protect nitrogenase against the oxygen damage to membrane integrity and function [53] or may maintain the proper conformation in nitrogen-fixing cells [14]. Further research is needed to elucidate the relationship between B and nitrogenase.

3.6. Seed Protein, Oil, and Fatty Acids

In seed of WB plants, protein and oil percentage were significantly ($P \leq 0.05$) higher than those of W, WS, or WSB (**Table 1**). Compared to seed protein in W plants, the increase in seed protein was 13.8% in WB, 3% in WS, and 8% in WSB. Seed oil increased 11% in WB, but decreased 9.8% in WS and 14% in WSB compared to those of W plants. The most noticeable change was in

Table 1. Effect of foliar boron (B) application on seed protein, oil, monounsaturated fatty acid (oleic), and polyunsaturated fatty acid (linolenic). Foliar B treatments were: W = plants were watered with no foliar B; WB = Plants were watered and received foliar B; WS = plants were water-stressed with no foliar B; WSB = plants were water-stressed and received foliar B[(*)].

Treatments	Concentration			
	Protein ($g \cdot kg^{-1}$)	Oil ($g \cdot kg^{-1}$)	Oleic ($g \cdot kg^{-1}$)	Linolenic ($g \cdot kg^{-1}$)
W	398 d	204 b	200 d	83 a
WB	453 a	226 a	299 a	59 b
WS	410 c	1844 c	220 c	85 a
WSB	431 b	175 b	274 b	58 b
Treatments	Content			
	Protein ($g \cdot plant^{-1}$)	Oil ($g \cdot plant^{-1}$)	Oleic ($g \cdot plant^{-1}$)	Linolenic ($g \cdot plant^{-1}$)
W	6.67 b	3.42 b	3.37 b	1.39 a
WB	9.81 a	4.90 a	6.51 a	1.28 b
WS	5.96 c	2.62 c	3.17 c	1.23 c
WSB	6.14 c	2.49 c	3.90 c	0.83 d

[*]Means within a column for each water treatment separately followed by the same letter are not significantly different at the 5% level.

oleic acid. Compared to those of W plants, oleic acid increased 49% in WB, 10% in WS, and 36.9% in WSB. On the other hand, linolenic acid was decreased by FB, opposing the pattern of oleic acid. Compared with those of W plants, the decrease in linolenic acid was 28.6% in WB and 29.8% in WSB. The increase of seed protein was not accompanied with a decrease in oil in WB compared with those of W plants. The inverse relationship between protein and oil in soybean is well established [55]. However, this inverse relationship was shown in W, WS, and WSB. The increase in oleic acid and decrease in linolenic acid with foliar B application in watered and water-stressed plants indicated that both FB and water stress can result in seed constituent altering. Expressing the seed constituents on seed weight per plant, water stressed plants with or without foliar B (WS or WSB) had lower protein, oil, and linolenic acid compared to watered plants (W), and this was expected based on previous research. Previous research showed that there was a positive relationship between soil B and seed protein and oleic acid, suggesting an indirect role of B in seed composition [56]. Other research showed that foliar B application increased soybean seed protein and oleic acid concentrations [17,57]. Recently, it was found that foliar B application under irrigated conditions increased seed protein and oleic acid, and decreased linolenic acid. Although the effect of fertilizers on seed composition is still inconsistent, the current results support previous research that foliar B alters seed composition.

4. Conclusions

Foliar B application can alter seed composition by increasing seed protein and oleic acid and decreasing linolenic acids. Foliar B application at both flowering and seed fill stages induced nitrogen assimilation and nitrogen fixation, suggesting a close relationship between B nutrition and nitrogen metabolism. The nature of this relationship is still unclear (Bellaloui *et al*., 2010). Water stress inhibited nitrogen assimilation and nitrogen fixation, and altered $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios. Boron translocation from leaves to seed was limited under water stress, suggesting a possible mechanism of B translocation within soybean plants. The current research suggests that soybean selection for B translocation efficiency is an important trait for soybean B nutrition under drought conditions such as in ESPS. Alteration in $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios under water stress indicates that nitrogen and carbon isotopes could be used as a tool in selecting for drought tolerant soybean lines.

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