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Effect of Water Deficit-Induced at Vegetative and Reproductive Stages on Protein and Oil Content in Soybean Grains

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Abstract: Soybean is one of the most common grain crops worldwide, representing an important protein and oil source. Although genetic variability in the chemical composition of grains is seen in soybean, the mean levels of proteins have remained stagnant or, in some cases, have decreased over time, arousing concern in the agricultural industry. Furthermore, environmental conditions influence the chemical composition of grains. Thus, the present study evaluated the effect of water deficit (WD) induced at the vegetative period (vegetative stress (VS)) and reproductive period (reproductive stress (RS)) on the protein and oil contents of grains in different soybean genotypes. Yield and its components were evaluated to evaluate the interrelation of these traits. The experiment was completed over three crop seasons under field conditions in Londrina, Paraná (PR), Brazil. WD was induced using rainout shelters and then stress treatments with irrigated and non-irrigated conditions were compared. WD negatively affected yield and its components. All evaluated genotypes showed similar responses for oil and protein contents under different water conditions. Higher protein content and lower oil content were observed in grains under RS. Such a relationship was not equally established under VS. Additionally, negative relationships between protein and oil content and between protein content and yield were confirmed.

Keywords: *Glycine max* L. Merrill; drought; grain quality

1. Introduction

Soybean is the most-grown oilseed in the world, with a planted area of around 120 million hectares and an annual production of around 352 million tons [1]. Brazil is the second largest producer of soybeans worldwide, with more than 33 million hectares planted and an estimated production of more than 102 million tons [2]. In addition to supplying the internal market, the surplus volume of this crop has made it the main Brazilian agricultural export product.

Soybean grains on average contain 40% protein, 20% oil, 35% carbohydrates, and 5% minerals on a dry basis [3]. Protein and oil content determine the commercial value of soybeans since soybeans are the main raw material in the oil and bran industry [4]. In soybean grains, protein and oil content may range from 31.7 to 57.9% and from 6.5 to 25.6%, respectively [5]. Although the genetic variability

of soybean is expressed in the chemical composition of grains, their protein and oil content has not increased over time. Thus, the soybean industry, both in Brazil and worldwide, has expressed overwhelming concern about a reduction in grain protein content. The focus of soybean breeding programs on yield improvement and resistance to diseases is partly responsible for this problem. Furthermore, the negative correlations between protein content and yield, and between protein and oil content, require more time and effort in genetic breeding [6].

In addition to the genetic factor, environmental conditions also influence the chemical composition of soybean grains [7]. Factors including geographical distribution, climate, soil fertility, and soil and crop management have been reported to interfere with protein and oil content in grains [8–10]. Dornbos and Mullen [11] described the effect of an increase in temperature and the availability of water on the protein and oil contents in grains. At higher temperatures and lower water availability, an increase and a reduction in the protein and oil contents were observed, respectively. Furthermore, Pípolo et al. [9], in an in vitro assay, studied the effect of nitrogen (N) supply on the protein and oil contents in soybean and observed that higher N levels favored protein synthesis. Despite these studies, the mechanisms by which climate conditions affect the chemical composition of soybean grains still require more clarification.

Since soybean crops have a wide geographic distribution in Brazil and across the globe, an understanding of the impact of environmental factors on yield and grain quality under different climate conditions is of great significance. The increased global average temperature and frequency of extreme climate phenomena, such as drought, have directly affected the yield and production stability of several crops, including soybean [12]. Thus, drought has been considered one of the main factors responsible for crop failure in global agriculture, leading to drastic reductions in yield and in the quality of seeds and grains [13].

This study aimed to evaluate the effect of WD induced at the vegetative and reproductive stages on the protein and oil contents in grains of different soybean genotypes. Yield and its components were evaluated to determine how these traits are interrelated.

2. Material and Methods

2.1. Experimental Design and Assay

Experiments were completed in the 2010–2011, 2011–2012, and 2013–2014 crop seasons at an experimental station belonging to the Brazilian Agricultural Research Corporation (Embrapa Soja), located in Londrina, Paraná (PR), Brazil (23°11'37" S, 51°11'03" W; 630 m altitude). The soil was classified as dystrophic Red Latosol (Oxisol), with 71% clay. The local annual average temperature was 21 °C, with higher averages in February (28.5 °C) and lower averages in July (13.3 °C). The annual rainfall was around 1600 mm over 123 days, concentrated mainly between January and March. This region has a Cfa climate according to the Köppen climate classification, which is described as a humid subtropical climate with hot summers. Temperature, relative air humidity, and rainfall were monitored through a weather station located in the experimental area. Rainfall and temperature data were used to calculate the water balance, according to Thornthwaite and Mather [14]. Rainfall data were those corresponding to the entrance of rain into the system; the entrance of water by irrigation was also included. Air temperature data were used to calculate the potential evapotranspiration, which depends on climate factors.

The experimental design was a completely randomized block design, according to a split-plot scheme, with four replicates. The whole plots included four water conditions and the subplots of four soybean genotypes. Water conditions were as follows: irrigated (I), rainfed (RF), WD induced at the vegetative period (vegetative stress) (VS), and WD induced at the reproductive period (reproductive stress) (RS). Four soybean genotypes were evaluated, including two conventional cultivars (BR 16 and Embrapa 48) and two non-commercial transgenic lines developed by Embrapa Soja, named P58 and

P2193. BR 16 and Embrapa 48 were used based on previous studies that indicated Embrapa 48 was more tolerant and BR 16 more sensitive to water deficit [15].

In all crop seasons, manual sowing was performed with 0.5 m spacing between rows and 16 plants/m with a density of 32 plants/m². Seeds were previously treated with fungicide and insecticide (200 g carboxin and 250 g fipronil/100 kg) to prevent attack by soil pests. At sowing, in-furrow inoculation with *Bradyrhizobium japonicum* strains SEMIA 5079 and SEMIA 5080 was performed. Fertilization was performed based on the results of soil analysis. Cultural practices followed the technical recommendations for soybean crops [16].

2.2. Control of Water Conditions

At vegetative and reproductive stages, WD was induced by using rainout shelters that moved on rails to cover plots at the beginning of the rainfall, and then to uncover at rainfall completion (Figure 1).



Figure 1. Rainout shelters used to induce water deficit in soybean in Londrina, Brazil.

For the 2010–2011 crop season, sowing was performed on 4 November 2010. The VS lasted from 1 December 2010 to 26 January 2011. The RS lasted from 26 January 2011 until 17 February 2011, which was the harvest time. For the 2011–2012 crop season, sowing was completed on 3 November 2011. The VS lasted from 1 December 2011 to 27 January 2012, and the RS occurred from 27 January 2012 to 8 February 2012 when the crop was harvested. In the 2013–2014 crop season, sowing was completed on 5 November 2013; the VS lasted from 2 December 2013 to 30 January 2014, and the RS occurred from 30 January 2014 to 19 February 2014 when the crop was harvested.

In treatments with no WD induction, plants were either rainfed (RF treatment) or rainfed with added irrigation (I treatment). The need for irrigation was monitored using tensiometers installed in the field at a depth of 0.30 m so that irrigation was triggered to maintain soil matrix potential between -0.03 and -0.05 MPa.

2.3. Traits

At the harvesting time (R8 stage), five plants from each plot were randomly collected and the following traits were evaluated per plant: total pod number (NP), total seed number (NS), total seed dry weight (SDM), 100-seed dry weight (g) (HSW), and apparent harvest index (AHI = (Grain dry matter/shoot dry matter) \times 100)). For yield determination, plants of three rows with a length of 3 m were manually harvested and the moisture content was immediately determined. Grain yield (kg/ha) was adjusted to 13% moisture content.

Protein and oil contents (%) were quantified in the Chemical Analysis Laboratory at Embrapa Soybean, by Fourier transform near-infrared spectroscopy (FT-NIR, model Antaris II, ThermoFisher Scientific, Waltham, MA, USA) using 30-g samples of grain according to Heil [17] and using an integrating sphere with readings ranging from 1100 to 2500 nm. Mathematical models developed

by Embrapa Soja in 2011–2012 were used to predict the protein content including 180 standards, correlation coefficient (r) = 0.97, and root mean square error of calibration (RMSEC) = 0.64; and for the oil content: 170 standards, r = 0.98, and RMSEC = 0.45.

2.4. Statistical Analysis

Data were submitted to the analysis of variance (ANOVA). When significant differences were detected by the F-test ($p \leq 0.05$), means were compared using Tukey's test ($p \leq 0.05$).

3. Results and Discussion

Based on ANOVA, a significant triple interaction between water condition (WC) \times genotype (G) \times agricultural year (Y) was detected for yield, 100-seed dry weight (HSW), and protein content in grains (Protein). Total pod number (NP), total seed number (NS), and total seed dry matter (SDM) presented a significant interaction for WC \times Y. The significant interactions (WC \times Y) and (WC \times G) were observed for oil content (Oil) and apparent harvest index (AHI).

Considering the yield results, a more pronounced WD was noted in the 2011–2012 and 2013–2014 crop seasons compared to the first season (Table 1). These results are due to the climate conditions involving low rainfall combined with high temperatures, as shown in Figure 2A–F. When yield was compared among the different WCs in each crop season, WD had a more negative impact on reproductive stress (RS) than on vegetative stress (VS) for all genotypes (Table 1). In the 2013–2014 crop season, under less favorable climate conditions (Figure 2E,F), yield decreased 70% on average in plants under RS (Table 1). Although differences among these genotypes were previously observed under water deficit [14], all genotypes evaluated in the present study had a severe reduction in yield.

Table 1. Grain yield (kg/ha) in soybean genotypes grown under different water conditions (WC), during three crop seasons in Londrina, Paraná (PR), Brazil.

Yield (kg/ha)					
2010–2011					
WC	Embrapa 48	BR 16	P58	P2193	Mean
I	3527.18 ^{Ab}	3300.33 ^{Ab}	3116.53 ^{Ab}	4197.91 ^{Aa}	3535.5 ^A
RF	3187.79 ^{Ab}	3376.41 ^{Ab}	2994.32 ^{Ab}	4178.77 ^{Aa}	3434.3 ^A
VS	3492.06 ^{Aa}	2853.34 ^{Ab}	2715.51 ^{Ab}	3048.44 ^{Bab}	3027.3 ^B
RS	1843.69 ^{Bab}	1679.42 ^{Bab}	1359.06 ^{Bb}	1982.47 ^{Ca}	1716.2 ^C
2011–2012					
WC	Embrapa 48	BR 16	P58	P2193	Mean
I	3612.06 ^{Aa}	3185.5 ^{Aab}	3006.43 ^{Ab}	3282.58 ^{Aab}	3271.6 ^A
RF	3449.23 ^{Aa}	3059.63 ^{Aab}	2992.97 ^{Aab}	2835.72 ^{Ab}	3084.4 ^A
VS	1937.67 ^{Bab}	1490.25 ^{Bb}	1825 ^{Bab}	1978.98 ^{Ba}	1807.0 ^B
RS	887.02 ^{Cns}	568.3 ^C	504.12 ^C	537.81 ^C	624.3 ^C
2013–2014					
WC	Embrapa 48	BR 16	P58	P2193	Mean
I	3278.13 ^{Aa}	2929.31 ^{Aab}	2568.13 ^{Ab}	2515.45 ^{Ab}	2822.8 ^A
RF	2169.68 ^{Bns}	1938.75 ^B	1868.04 ^B	1902.01 ^B	1969.6 ^B
VS	2126.33 ^{Ba}	1439.66 ^{Bb}	1635.96 ^{Bb}	1534.2 ^{Bb}	1684.0 ^C
RS	1252.61 ^{Ca}	766.21 ^{Cab}	724.38 ^{Cb}	819.61 ^{Cab}	890.7 ^D

Numbers followed by the same uppercase letter in the column and lowercase letter in the line do not differ by Tukey's test ($p \leq 0.05$). Ns: non-significant. Irrigated (I), rainfed (RF), water deficit (WD) induced in the vegetative period (vegetative stress (VS)), and reproductive period (reproductive stress (RS)).

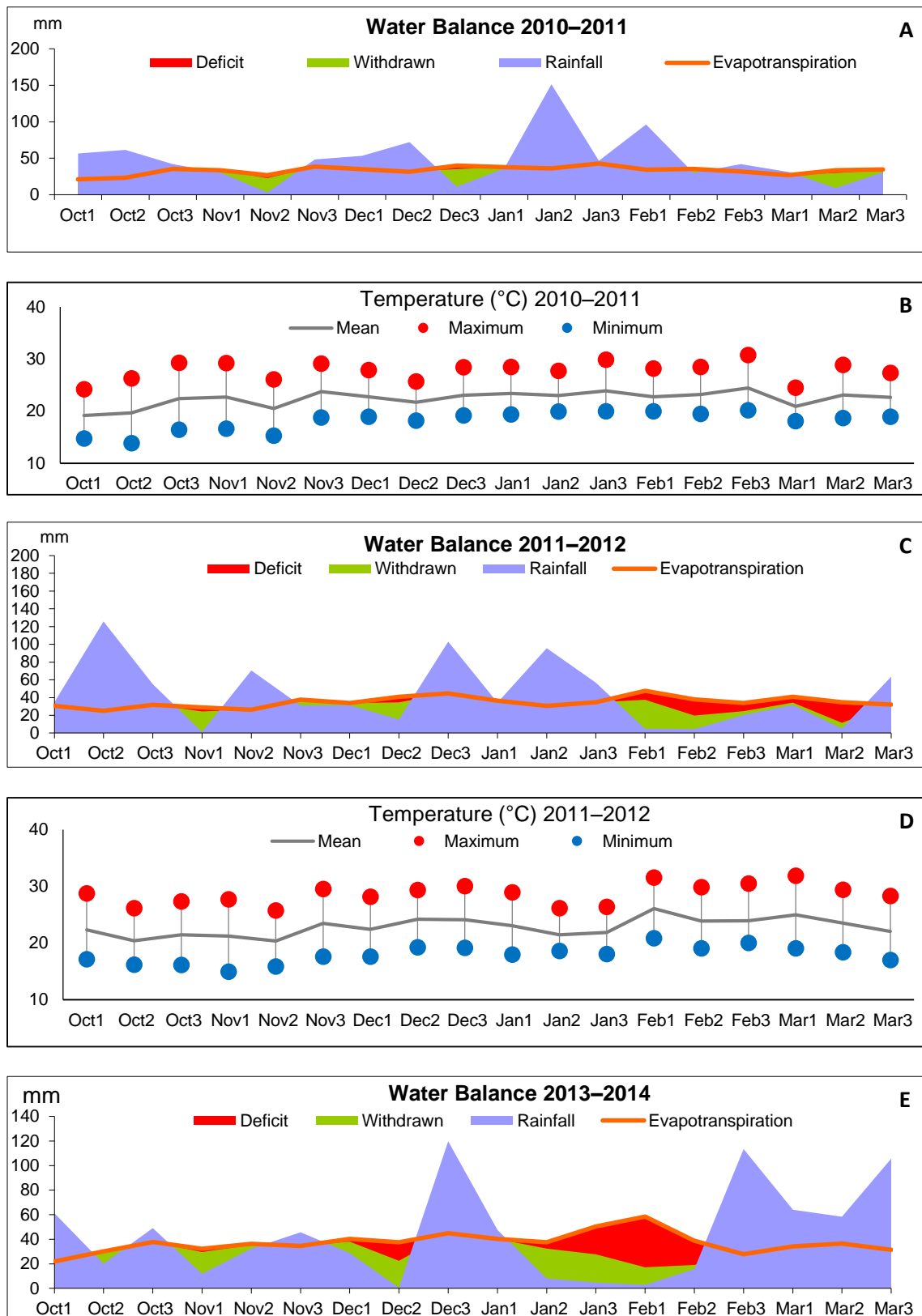


Figure 2. Cont.

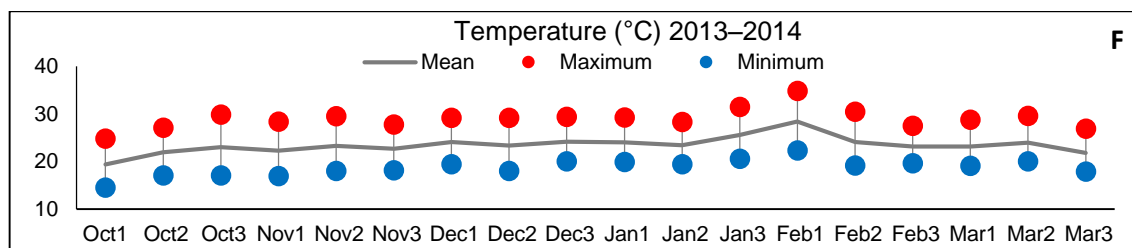


Figure 2. Water balance (WB) calculated according to Thornthwaite and Mather [15] and mean temperature (T°) for 10-day periods (1, 2, 3) over three crop seasons in Londrina, PR, Brazil. (A). WB 2010/2011; (B). T° 2010/2011; (C). WB 2011/2012; (D). T° 2011/2012; (E). WB.2013/2014; (F). T° 2013/2014.

Although water is important throughout the soybean crop cycle, the reproductive stage is the most critical period [18]. When WD is induced during the vegetative stage, its effect on yield can be reversed with subsequent rainfall. Conversely, WD induced during the reproductive stage tends to have a direct impact on yield, so that grain filling is the most critical period for water [19,20].

HSW presented a similar pattern to that of yield. WD had a more negative effect on the RS than on the VS (Table 2). The variation among WC was lower in the first crop season when climate conditions were favorable throughout the crop cycle (Figure 2).

Table 2. Dry weight (g) of 100 seeds (HSW) in soybean genotypes grown under different water conditions (WC) over three crop seasons in Londrina, PR, Brazil.

HSW (g)					
2010–2011					
WC	Embrapa 48	BR 16	P58	P2193	Mean
I	12.41 ^{Ab}	13.97 ^{Ab}	13.48 ^{Ab}	15.82 ^{Aa}	13.92 ^A
RF	12.33 ^{Ac}	14.55 ^{Ab}	13.07 ^{Abc}	16.72 ^{Aa}	14.17 ^A
VS	12.08 ^{ABC}	15.49 ^{Aab}	14.02 ^{Ac}	15.8 ^{Aa}	14.35 ^A
RS	10.36 ^{Bbc}	12.03 ^{Bab}	9.66 ^{Bc}	12.48 ^{Ba}	11.13 ^B
2011–2012					
WC	Embrapa 48	BR 16	P58	P2193	Mean
I	13.37 ^{Ac}	15.24 ^{Ab}	15.8 ^{Aab}	17.08 ^{Aa}	15.37 ^A
RF	11.53 ^{Abb}	14.09 ^{Aa}	15.45 ^{Aa}	14.76 ^{Ba}	13.96 ^B
VS	10.56 ^{Bb}	10.24 ^{Bb}	10.44 ^{Bb}	12.52 ^{Ca}	10.94 ^C
RS	10.12 ^{Bab}	9.91 ^{Bb}	11.64 ^{Ba}	10.67 ^{Cab}	10.58 ^C
2013–2014					
WC	Embrapa 48	BR 16	P58	P2193	Mean
I	10.96 ^{Ans}	12.2 ^A	11.37 ^A	11.38 ^A	11.48 ^A
RF	8.92 ^{Bns}	9.61 ^{BC}	9.87 ^A	9.55 ^A	9.49 ^B
VS	9.58 ^{Abns}	9.98 ^B	9.69 ^A	10.07 ^A	9.83 ^B
RS	8.16 ^{Bns}	7.78 ^C	6.99 ^B	7.64 ^B	7.64 ^C

Numbers followed by the same uppercase letter in the column and lowercase letter in the line do not differ by Tukey's test ($p \leq 0.05$). Non-significant (ns), irrigated (I), rainfed (RF), and water deficit (WD) induced at the vegetative period (vegetative stress (VS)) and reproductive period (reproductive stress (RS)).

WD led to a reduction in NP, NS, and SDM (Table 3) regardless of the genotype, with a higher intensity in the RS than the VS. In drier crop seasons, such as 2011–2012 and 2013–2014, differences in NS and SDM were detected even in the treatment RF compared to condition I.

Table 3. Decomposition of the interaction water condition (WC) × agricultural year (Y) for total pod number (NP), total seed number (NS), and total seed dry weight (SDM) in soybean. Data shown represent the mean of four genotypes in Londrina, PR, Brazil.

WC	NP			NS			SDM (g)		
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2	Y3
I	41.1 Aab	36.3 Ab	41.5 Aa	76.4 Aab	70.5 Ab	84.4 Aa	10.5 Aa	10.3 Aa	9.9 Aa
RF	44 Aa	32.7 Ab	36.6 Ab	79 Aa	63.5 Ab	70.3 Bab	10.8 Aa	8.2 Bb	6.7 Bc
VS	34.7 Ba	25.8 Bb	27.4 Bb	69.6 Aa	48.4 Bb	53.9 Cb	9.8 Aa	4.9 Cb	5.5 Bb
RS	21 Ca	11.5 Cb	22.1 Ba	36.8 Ba	21.6 Cb	35.7 Da	4.3 Ba	2 Db	2.7 Cb

Numbers followed by the same uppercase letter in the column and lowercase letter in the line do not differ by Tukey's test ($p \leq 0.05$). Ns: non-significant. Irrigated (I), rainfed (RF), and water deficit (WD) induced at the vegetative period (vegetative stress (VS)) and reproductive period (reproductive stress (RS)). Y1 (2010–2011), Y2 (2011–2012), and Y3 (2013–2014).

Protein content ranged from 34.3% to 40.6% (Table 4), which is below the average values reported in the literature, with around 40% protein on a dry basis [3]. Thakur and Hurburgh [10] stated that the decrease in protein content of soybean grains has aroused concern in the main soybean producing countries. These authors compared the quality of samples from different locations including Brazil, the U.S., and Argentina and observed that Brazilian soybeans had the highest protein content, followed by those from the U.S. and Argentina.

Table 4. Protein content (%) in grains on a dry basis in soybean genotypes grown under different water conditions (WC) for three crop seasons in Londrina, PR, Brazil.

Protein (%)					
2010–2011					
WC	Embrapa 48	BR 16	P58	P2193	Mean
I	34.93 BCc	36.77 Abab	35.7 Bbc	37.4 Aa	36.62 B
RF	34.18 Cc	35.84 Bc	35.29 Bbc	37.59 Aa	36.24 B
VS	36.55 Abb	37.34 Aba	37.91 Aab	38.66 Aa	37.97 A
RS	37.55 Ans	37.55 A	37.41 A	37.26 A	37.41 A
2011–2012					
WC	Embrapa 48	BR 16	P58	P2193	Mean
I	34.33 Ac	37.32 Bab	36.29 Bb	37.98 Aa	36.48 BC
RF	35.36 Bb	37.69 Aba	36.46 Bab	37.21 Aba	36.68 B
VS	34.97 Bb	36.73 Ba	36.14 Bab	36.03 Bab	35.97 C
RS	39.18 Ans	39.08 A	38.6 A	38.53 A	38.85 A
2013–2014					
WC	Embrapa 48	BR 16	P58	P2193	Mean
I	36.34 Bns	37.55 B	37.41 A	37.07 B	37.09 C
RF	36.78 Bb	38.17 Ba	37.29 Aab	38.35 Aba	37.65 BC
VS	36.56 Bb	38.87 Ba	37.99 Aa	38.29 Aba	37.93 B
RS	39.78 Aab	40.63 Aa	38.59 Ab	39.77 Aab	39.69 A

Data followed by the same uppercase letter in the column and lowercase letter in the line do not differ by Tukey's test ($p \leq 0.05$). Ns: non-significant. Irrigated (I), rainfed (RF), and water deficit (WD) induced at the vegetative period (vegetative stress (VS)) and reproductive period (reproductive stress (RS)).

The main factor leading to a reduction in protein content has been the emphasis of breeding programs on traits such as productivity and resistance to diseases, instead of the chemical composition of grains. Wilson et al. [21] observed a reduction in protein content and a yield increase in soybean cultivars released in the U.S. over more than 80 years.

In Brazil, samples produced in the 2014–2015 crop season from several regions had on average 36% protein and 22% oil contents on a dry basis [22]. To produce soybean bran with a minimum protein content of 46%, the industry recommends a 36% minimum protein content in grains based on 14% moisture. When such levels are not met, an alternative found by industries is to remove the grain tegument in one of the steps during industrial processing, since this grain component is low in oil and protein contents but represents more than 7% total grain weight [6].

Regarding the effect of treatments, in general, protein content in grains tended to increase under RS (Table 4). Except for genotypes P2193 and P58 in the 2010–2011 and 2013–2014 crop seasons, respectively, all cultivars had higher protein accumulation in grains under RS for the three crop seasons (Table 4). Under more severe WD (2013–2014), a 2.6 percentage point (pp.) difference in protein content on a dry basis was detected between I and RS conditions, considering the average among all plant materials.

The effect of WD on soybean protein content was evaluated in several studies and different responses have been observed. Foroud et al. [23] detected an increase in protein content and yield under well-watered conditions and lower values of both traits under severe WD. Ghassemi-Golezani and Lotfi [24] reported an increase in protein content and a reduction in oil content in grains under WD induced at the reproductive stage, proving their inverse relationship. The authors also observed the effect of seed position in the plant: upper seeds had higher oil and protein contents than those from middle and lower regions. Angra et al. [25] evaluated soybean genotypes under WD in the grain-filling period and observed a higher soluble protein content in grains after the beginning of WD, followed by its reduction. According to these authors, at the beginning of WD, proteins related to the protection against drought, such as chaperones, are probably synthesized, whereas a reduction in protein content is due to their hydrolysis and degradation [26]. Moreover, the authors detected that the most tolerant cultivar had a higher soluble protein content, suggesting a more efficient protection mechanism. Based on data obtained in the present study, all evaluated genotypes showed similar responses in oil and protein contents under different water conditions.

Grain yield is another factor that might explain an increase in protein content under RS. Since WD leads to a reduction in yield by negatively affecting grain number and weight (Table 1), a reduced number of sinks was observed, leading to a higher protein content. Studies have reported that the genetic control of protein content in soybean is negatively correlated with yield [27] and oil content [28], which makes breeding such a trait difficult.

When WD was induced at the vegetative stage, its effect on protein content in grains was not as evident as RS (Table 4). Except for the 2010–2011 crop season, considering the average of all genotypes, the protein content under VS was similar or even inferior to those observed under the I and RF conditions (Table 4). This result may be due to lower vegetative growth under VS, with reduced leaf area expansion [29]. Since N translocated to grains is partly remobilized from leaves [30], a smaller leaf area decreases the availability of N to be remobilized.

Oil content ranged from 20.63 to 22.57% (Table 5), which is above the average values indicated in the literature and the minimum values required by the industry of around 20% [3]. Different to protein, the oil content tended to decrease under RS (Table 5), proving the negative correlation between protein and oil contents in grains. VS did not change oil content in any cultivar.

Lower AHI values were observed for RS (Table 5) due to NP and NS under such a treatment, resulting in lower sink strength.

Table 5. Decomposition of the interaction water condition (WC) \times genotype for oil content (%) in grains on a dry basis and apparent harvest index (AHI) in soybean genotypes grown for three crop seasons in Londrina, PR, Brazil.

WC	Oil (%)				AHI			
	Embrapa 48	BR 16	P58	P2193	Embrapa 48	BR 16	P58	P2193
I	22.57 ^{Aa}	21.58 ^{Ab}	21.96 ^{Ab}	21.81 ^{Ab}	0.44 ^{Bb}	0.47 ^{Bab}	0.46 ^{Bab}	0.48 ^{Aba}
RF	22.19 ^{Ans}	21.72 ^A	21.94 ^A	21.73 ^{AB}	0.45 ^{Bns}	0.46 ^B	0.47 ^B	0.46 ^B
VS	22.23 ^{Aa}	21.61 ^{Ab}	21.81 ^{Aab}	22.24 ^{Aa}	0.52 ^{Ans}	0.5 ^A	0.51 ^A	0.51 ^A
RS	20.87 ^{Bab}	20.63 ^{Bb}	21 ^{Bab}	21.23 ^{Ba}	0.37 ^{Ca}	0.33 ^{Cb}	0.32 ^{Cb}	0.33 ^{Cb}

Numbers followed by the same uppercase letter in the column and lowercase letter in the line do not differ by Tukey's test ($p \leq 0.05$). Ns-non-significant. Irrigated (I), rainfed (RF), and water deficit (WD) induced at the vegetative period (vegetative stress (VS)) and reproductive period (reproductive stress (RS)).

Although protein content in soybean grains increased under WD, the physicochemical quality of grains can be impaired under severe drought conditions. Under WD, a larger number of green grains were detected in soybean lots [31], increasing the acidity level of the grains. Crude oil obtained from such grains has a green color with a high free fatty acid content [32]. Further studies are needed to evaluate the effect of climate conditions on the quality and the stability of oil and proteins of soybean grains.

4. Conclusions

WD induced at the reproductive period led to a higher protein content and a lower oil content in soybean grains. This relationship was not equally established when WD was induced at the vegetative period. Additionally, a negative relationship between protein and oil content, and between protein content and yield, was confirmed.

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