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WATER STRESS AFFECTING NODULATION, OIL, PROTEIN AND GRAIN YIELD OF SOYBEAN CULTIVARS

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ABSTRACT: In the present study the accumulation of oil and protein, yield and nodulation of ten soybean cultivars (BR 16, Embrapa 48, BRS 133, BRS 134, BRS 245 RR, BRS 247 RR, BRS 183, BRS 184, BRS 214, and BRS 232) at three different water regimes in the field (natural rainfall-NR, irrigation-IR and water stress-WS) applied during the reproductive stage were evaluated. It was observed that the cultivar BRS184 had intermediate percentage of protein in the grain (36.32%), highest protein and oil content per ha, although no significant, due its highest yield under water stress. Nodule number and nodule dry mass did not differ significantly from the other cultivars and reduction index based on the nodule number was lower. Additionally the BRS184 was among the cultivars with highest grain yield (NR and IR), intermediate grain protein content (NR) and it did not significantly differ from the other cultivars regarding to grain oil content (NR and IR). Thus the cultivar BRS 184 was considered a promising choice under water stress.

Keywords: *Glycine max*, water stress, yield.

ESTRESSE HÍDRICO AFETANDO NODULAÇÃO, ÓLEO, PROTEÍNA E PRODUTIVIDADE DE DEZ CULTIVARES DE SOJA

RESUMO: No presente estudo avaliou-se o acúmulo de óleo e proteína, produtividade e nodulação de dez cultivares de soja (BR 16, Embrapa 48, BRS 133, BRS 134, BRS 245 RR, BRS 247 RR, BRS 183, BRS 184, BRS 214 e BRS 232) sob três regimes hídricos no campo [condições naturais, sem irrigação (NR), com irrigação (IR) e estresse hídrico aplicado no estágio reprodutivo (WS)]. Observou-se que a cultivar BRS 184 teve teor intermediário de proteína no grão (36,32%), teor de proteína e óleo por ha mais alto, embora não significativo, devido à sua maior produtividade sob estresse hídrico. Número de nódulos e matéria seca do nódulo não diferiu significativamente das outras cultivares e índice de redução baseado no número de nódulos, foi mais baixo. Adicionalmente a cultivar BRS 184 esteve entre as cultivares com maior produtividade (NR e IR), apresentou teor de proteína intermediário no grão (NR) e não diferiu significativamente das outras cultivares com relação ao teor de óleo no grão (NR e IR). Por essas razões a cultivar BRS 184 foi considerada uma escolha promissora em condições de déficit hídrico.

Palavras-chave: *Glycine max*, estresse hídrico, produtividade.

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INTRODUCTION

Soybean *Glycine max* (L.) Merrill is the world's leading source of oil and protein. It has the highest protein content (40%) of all food crops and is second only to groundnut in terms of oil content (20%) among food legumes (GURMU et al., 2009). It is a consolidated crop in Brazil, which is among the largest world producers. On average, protein content of commercial varieties is around 40%, but it can vary from 30% to 53% (MELLO-FILHO et al., 2004).

Protein and oil concentration is controlled by quantitative genetic factors but it is highly influenced by the cultivation conditions mainly during the grain filling stage (Ávila et al., 2007). Among the abiotic factors affecting the protein content are the temperature, water availability and nitrogen supply (SANTOS et al., 2010).

Soybean has high demand for nitrogen which is supplied in its virtual totality by the biological nitrogen fixation (BNF) (ZILLI et al., 2010). Biological nitrogen fixation is however extremely sensitive to water stress. Although the influence of water availability on plant growth and photosynthetic activity has been studied extensively (FELLOWS et al., 1987 and IRIGOYEN et al., 1992), less attention has been given to the role of nodule activity in plant performance under drought conditions (ARANJUELO et al., 2011).

Water stress impairs the *Bradyrhizobium* survival, the nodule formation and longevity and the leghemoglobin synthesis responsible for O_2 transportation. It also decreases nodule membrane integrity (MHADHBI et al., 2009), increases degradation of bacteroids (HERDER et al., 2008), increases proteinases activity in the nodules (GROTEN et al., 2006) and causes loss of N-fixation activity regardless of physiological and biochemical mechanisms of N_2 fixation inhibition (ASHRAF and IRAM, 2005; CHARLSON et., 2009). Severe stress can lead to nitrogen fixation inhibition (FAGAN et al., 2007) which in turn leads to diminished yield and grains with modified chemical composition. There is however, evidence that legume species have genetic variation in their ability to fix N_2 under water stress (ASHRAF and IRAM, 2005; CHARLSON et al., 2009).

In this context the present work aimed to evaluate the accumulation of oil and protein, yield and nodulation of ten soybean cultivars at three different water regimes in the field.

MATERIAL AND METHODS

The experiment was carried out at Embrapa Soja, Londrina (23°11'S, 51°10'W, 612m), Paraná state, Brazil during the 2006/07 growing season. Soil chemical corrections and cultivations were carried out according to recommendations for this crop (EMBRAPA, 2005). Daily precipitation and temperature (maximum, minimum and average) during the season (December 2006 to April 2007) was obtained from a meteorological station at Embrapa Soybean and is shown in Figure 1. Seeds were inoculated with *Bradyrhizobium spp.*, strains Semia 587+5019 just prior to sowing.

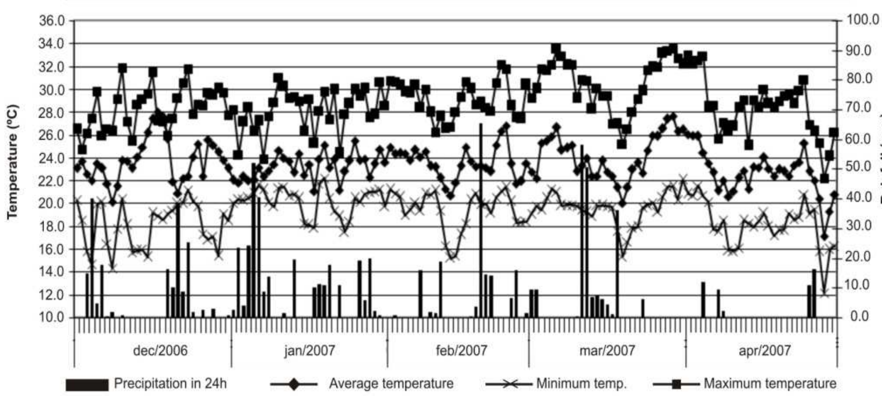

Figure 1 - Daily precipitation and temperature during the 2006/07 season.

The experimental design was the randomized complete block with treatments arranged in split plot and four replicates. The main plots received three different water regimes consisting of: **WS** –natural rainfall until the R_1 stage (early flowering), and then the plants were artificially drought stressed by sheltering them from rain (starting on 19 January, 2007); **NR** – natural rainfall as it occurred; and **IR** – manual irrigation to keep the matric soil-water potential between -0.03 and -0.05 MPa (five irrigations were made along the growing season). The sub-plots received ten soybean cultivars (BR 16, Embrapa 48, BRS 133, BRS 134, BRS 245 RR, BRS 247 RR, BRS 183, BRS 184, BRS 214, and BRS 232). Each WS plot of 4.5 m^2 contained three rows three meters long spaced 0.5 m apart and with a spacing of 0.6 m in the row. The NR and IR plots of 24 m^2 contained eight rows six meters long, also spaced 0.5m apart and with a spacing of 0.6 m in the row. Irrigation was performed manually on the IR plots using a hose with predetermined water flow rate/time. Soil humidity was monitored daily by tensiometers placed at 30 cm soil depth, and weekly by the gravimetric method and neutron probe.

At the R_8 stage, five plants were collected from each plot and analyzed for oil and protein percentage in the grain, yield (Kg ha^{-1}) and nodulation (nodule dry mass – NDM and number of nodules – NN). From these parameters, protein and oil per ha and the reduction index based on the nodule number, were calculated. For the calculation of the reduction index the following equation was used: RI=NNWS(NNWS-NNNRx100)/NNWS with the NNWS and NNNR meaning nodule number under water stress and natural rainfall respectively.

Total N accumulated in tissues was not examined since in previous studies with 152 soybean varieties, Bohrer and Hungria (1998) and Hungria and Bohrer (2000) found high correlation between the responses of shoot dry weight (SDW) and total N uptake by plants (r = 0,87** e r = 0,92**), eliminating the need for analysis of N content in tissues. Shoot dry weight was not significantly affected by the treatments and/or cultivars, except for BRS184 and BRS 232. Significance was found for these cultivars between the WS and NR treatments and these occurred mainly because its shoot dry weight values were the highest in the NR treatment (Table 3).

Oil accumulation in the grains was determined according to Pípolo et al. (2004) by the Soxhlet method (FEHR et al., 1968). N was analyzed in the remainings from the oil extraction by the Kjeldahl (1883) method. Grain N was determined by colorimeter semi-automated method and protein concentration was calculated by multiplying the N concentration by 6.25.

Grain yield (Kg ha^{-1}) in the NR and IR treatments was estimated by harvesting five meters of the three middle rows per plot which corresponds to 7.5 m^2. For the WS treatment yield was estimated harvesting two meters of the middle row of each plot which corresponds to 1 m^2. Plot grain yields (at 13% humidity) were calculated using the equation: Yield (Kg/ha) = (((100 –grain humidity at harvest, %) x ((harvested grain weight, Kg x 10000) / plot harvested area, m^2))) / 87. Oil and protein yields were obtained multiplying yield/ha by the percentage of oil and protein in the grain.

The methods of statistical analysis applied to all response variables consisted of an exploratory diagnostic, checking assumptions of normality and independence of the residue, the additivity of the model, and the homogeneity of treatment variances, followed by analysis of variance (ANOVA). After these analyses and when the F test showed statistical significance, the Tukey test for multiple comparisons among treatment means, at the level of significance of $\alpha = 0.05$, was applied.

RESULTS AND DISCUSSION

Biological nitrogen fixation (BNF) of soybean cultivars under water stress

Biological nitrogen fixation is highly affected by water stress. I our studies it was shown that water stress significantly reduced the nodule number (NN) (Table 1) and the nodule dry mass (NDM) (Table 2) of all cultivars except for BRS 183 (NN) and BR 16, BRS 183 and BRS 184 (NDM). Differences among cultivars inside each treatment were mainly non significant except for the BRS 134 that had highest NN when compared to the cultivar BRS 232 in the NR and when compared to all other cultivars (except BRS 245 RR and BRS 247 RR) in the IR treatments.

Table 1 - Means for *Bradyrhizobium* nodule number (NN) of ten cultivars of soybean. WS, NR and IR represents water stress applied in the reproductive stage, natural rainfall and irrigated treatments respectively. Means followed by the same lowercase letter in the columns and uppercase in the rows do not differ by Tukey test at 5% probability

Cultivars	water availability			Average
	WS	NR	IR	
BR 16	126 a B	334 ab A	303 b A	254
Embrapa 48	220 a B	443 ab A	380 b AB	348
BRS 133	105 a B	401 ab A	293 b A	266
BRS 134	142 a B	461 a A	617 a A	407
BRS 183	97 a A	261 ab A	259 b A	205
BRS 184	168 a B	336 ab A	266 b AB	256
BRS 214	90 a B	415 ab A	320 b A	275
BRS 232	79 a B	256 b A	319 b A	218
BRS 245 RR	91 a B	376 ab A	440 ab A	302
BRS 247 RR	105 a B	448 ab A	460 ab A	338
Average	122	373	366	
CV Pot (%) = 17.16		CV Subplot (%) = 30.78		

Table 2 - Means of *Bradyrhizobium* nodule dry mass (NDM) of ten cultivars of soybean. WS, NR and IR represents water stress applied in the reproductive stage, natural rainfall and irrigated treatments respectively. Means followed by the same lowercase letter in the columns and uppercase in the rows do not differ by Tukey test at 5% probability

Cultivars	water availability									Average
	WS			NR			IR			
BR 16	0.57	ab	A	1.14	bc	A	1.21	bc	A	0.97
Embrapa 48	1.25	a	B	2.18	a	A	1.55	abc	AB	1.66
BRS 133	0.70	ab	B	1.79	abc	A	1.69	abc	A	1.39
BRS 134	0.69	ab	B	1.93	ab	A	2.33	a	A	1.65
BRS 183	0.39	ab	A	0.94	c	A	1.01	c	A	0.78
BRS 184	0.67	ab	A	1.21	bc	A	1.14	c	A	1.01
BRS 214	0.29	b	B	1.04	bc	A	0.87	c	AB	0.73
BRS 232	0.39	ab	B	1.39	abc	A	1.74	abc	A	1.17
BRS 245 RR	0.38	ab	B	1.35	abc	A	1.64	abc	A	1.12
BRS 247 RR	0.50	ab	B	1.81	abc	A	2.07	ab	A	1.46
Average	0.58			1.48			1.53			
CV Pot (%) = 17.83	CV Subpot (%) = 32.41									

It was also observed that there was great variability among cultivars regarding to their reduction index (RI) (Figure 2) based on the number of nodules. The cultivars Embrapa 48 and BRS 184 had the lowest RI under water stress. According to Aguiar et al. (2008), low RI is a good criterion to identify promising genotypes.

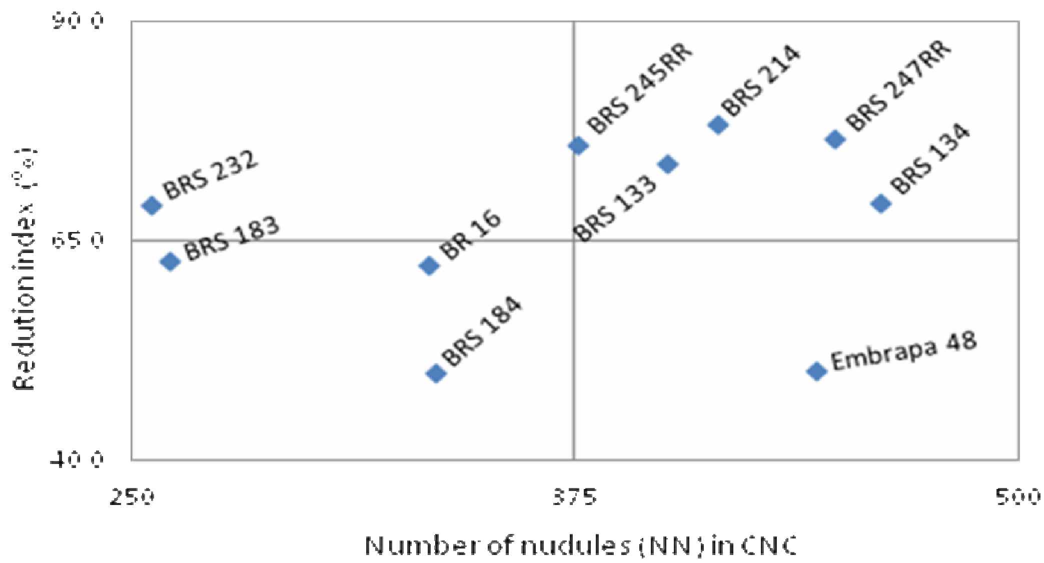

Figure 2 - Reduction Index of ten soybean cultivars. RI=NNWS(NNWS-NNNRx100)/NNWS with the NNWS and NNNR meaning nodule number under water stress and natural rainfall, respectively.

The extent of the reduction of the NDM under water stress (2.5 times, Table 2) was similar to that found by Calvache and Reichardt (1996) who observed that water deficit during flowering and pod filling reduced BNF in 2.2 times.

Nodule number (Table 1) mean values of non stressed plants (NR and IR) were consistent with those reported in the literature at similar conditions (VIEIRA-NETO et al., 2008). Nodule dry mass mean values (Table 2) obtained for Embrapa 48 and BRS 134 in all treatments (WS, NR and IR) were also similar to that found by Hungria et al. (2006).

Although no significant when compared to some of other cultivars, irrigation promoted highest NN and NDM in BRS 134, however under drought it had an intermediate RI (Figure 2).

Formation and growth of nodules can be altered by factors affecting plant development. A decrease in water potential can markedly affect root hair (WORRAL and ROUGHLEY, 1976), retard nodule growth (GALLACHER and SPRENT, 1978) and N2 fixation (RAMOS et al., 1999). Although root hair and N2 fixation were not evaluated, these traits may have limited nodule number (NN) (Table 1) and nodule dry mass (NDM) (Table 2) in some cultivars in the present study.

Nodule functioning can be altered by limitation on nitrogenase (N_{ase}) activity. Deleterious effects of drought on N_{ase} activity have been confirmed by several authors (LADRERA et al., 2007; LARRAINZAR et al., 2009 and ARANJUELO et al., 2011). Consequences of limitation in the N_{ase} activity are low production of ammonia and decrease in ammonia assimilating enzymes (KAUR et al., 1985). Reduced content of asparagine, which is the major N-transporting amino acid, in the nodules has also been reported (ARANJUELO et al., 2011).

Accumulation of oil and protein, shoot dry weight and yield of soybean cultivars under water stress

Shoot dry weight was not significantly affected by the treatments and/or cultivars, except for BRS184 and BRS 232. Significance was found for these cultivars between the WS and NR treatments and these occurred mainly because its shoot dry weight values were the highest in the NR treatment (Table 3).

Table 3 - Means of soybean shoot dry weights. WS, NR and IR represents water stress applied in the reproductive stage, natural rainfall and irrigated treatments respectively. Means followed by the same lowercase letter in the columns and uppercase in the rows do not differ by Tukey test at 5% probability

Cultivars	water availability									Average
	WS			NR			IR			
BR 16	89.58	a	A	74.89	a	A	93.74	a	A	86.07
Embrapa 48	94.59	a	A	78.29	a	A	63.52	a	A	78.80
BRS 133	85.53	a	A	84.22	a	A	110.69	a	A	93.48
BRS 134	82.97	a	A	128.81	a	A	144.15	a	A	118.64
BRS 183	71.41	a	A	114.11	a	A	99.69	a	A	95.07
BRS 184	63.37	a	B	147.21	a	A	107.16	a	AB	105.91
BRS 214	133.47	a	A	123.57	a	A	124.48	a	A	127.17
BRS 232	84.03	a	B	143.40	a	A	74.17	a	B	100.53
BRS 245 RR	80.18	a	A	97.75	a	A	127.44	a	A	101.79
BRS 247 RR	87.12	a	A	93.47	a	A	121.90	a	A	100.83
Average	87.23			108.57			106.69			
CV Pot (%) = 11.77	CV Subpot (%) = 34.50									

Grain protein content (%) was positively affected by water stress and inter-cultivar differences were observed within each treatment (Table 4).

Table 4 - Means of grain protein content (%) of ten soybean cultivars. WS, NR and IR represents water stress applied in the reproductive stage, natural rainfall and irrigated treatments respectively. Means followed by the same lowercase letter in the columns and uppercase in the rows do not differ by Tukey test at 5% probability

Cultivars	**water availability**									**Average**
	WS			**NR**			**IR**			
BR 16	37.82	abc	A	34.64	c	B	34.16	bc	B	35.54
Embrapa 48	38.62	ab	A	35.17	bc	B	32.91	c	C	35.57
BRS 133	36.93	bcd	A	35.12	bc	B	34.26	bc	B	35.44
BRS 134	35.80	d	A	35.96	abc	A	35.07	b	A	35.61
BRS 183	39.23	a	A	36.49	ab	B	37.07	a	B	37.60
BRS 184	36.32	cd	AB	37.42	a	A	35.17	b	B	36.30
BRS 214	39.23	a	A	36.41	abc	B	34.13	bc	C	36.59
BRS 232	37.76	abc	A	35.78	abc	B	35.62	ab	B	36.39
BRS 245 RR	37.68	abc	A	35.17	bc	B	35.15	b	B	36.00
BRS 247 RR	37.45	abcd	A	35.20	bc	B	34.50	bc	B	35.72
Average	37.68			35.74			34.80			
CV Plots (%) = 0.9	CV Subplots (%) = 2.19									

Although not significant when compared with some of the other cultivars, highest grain protein content (39.23%) was found under WS for the cultivars BRS 183 and BRS 214. The cultivars BRS 184 and BRS 183 were the ones with the highest protein contents (~37%) under NR and IR, respectively. According to Ávila et al. (2007), the protein content in the grain is controlled by quantitative genetic factors and it correlates positively with water stress (Albrecht et al., 2008).

Commercial value of soybean is determined by the protein content in the grain and to be classified as normal or HyPro, the grain must have 41.5 and 43% of protein respectively (MORAES et al., 2006). Using such criterion, and under the conditions studied here, all cultivars are classified as LowPro, not reaching the limits necessary to the production of an enriched soy meal for animal nutrition and exports (MELLO-FILHO et al., 2004). Protein values reported for BRS 183 and BRS 214 are 40.62% and 39% respectively (ALMEIDA et al., 2001 and EMBRAPA, 2008).

Maehler et al. (2003) explained that under water stress high protein accumulation in the grain is related to a phenomenon named “dilution factor”. According to it, when water is available, proteins are distributed to a large number of grains which causes its dilution. Contrarily, under water stress, proteins are distributed to fewer grains leading to its accumulation. Considerations about protein contents must then take into account rain occurrence and distribution during the grain filling stage (ALBRECHT et al., 2008).

Contrarily to the effect on protein, water availability did not affect the percentage of oil in the grain (Table 5). However significant inter-cultivar differences were found by comparing cultivar mean values across treatments. The cultivars with the highest (20.91%) and the lowest (18.08%) oil content values (BRS 184 and BRS 232, respectively) were distinct from the others (Table 5). In order to see if this 2% difference in oil content is significant, one must consider the grain yield.

Table 5 - Means of grain oil content (%) of ten soybean cultivars. WS, NR and IR represents water stress applied in the reproductive stage, natural rainfall and irrigated treatments, respectively. Means followed by the same lowercase letter in the columns and uppercase in the rows do not differ by Tukey test at 5% probability

Cultivars	water availability			Average
	WS	NR	IR	
BR 16	19.70 a A	18.07 a A	19.14 a A	18.97
Embrapa 48	19.35 a A	19.26 a A	19.77 a A	19.46
BRS 133	19.68 a A	18.96 a A	18.88 a A	19.17
BRS 134	20.36 a A	18.47 a A	19.09 a A	19.31
BRS 183	19.45 a A	18.70 a A	18.54 a A	18.90
BRS 184	21.16 a A	20.32 a A	21.24 a A	20.91
BRS 214	19.86 a A	18.78 a A	19.20 a A	19.28
BRS 232	18.38 a A	18.20 a A	17.65 a A	18.08
BRS 245 RR	20.36 a A	18.98 a A	19.48 a A	19.51
BRS 247 RR	19.95 a A	18.79 a A	19.57 a A	19.44
Average	19.79	18.85	19.21	
CV Plots (%) = 2.18 CV Subplots (%) = 3.27				

The oil content in the conditions studied were below to that described in the literature, which for the cultivars BRS 184 and BRS 232 were 24,24% and 19,50% respectively (EMBRAPA, 2008). Pípolo et al. (2004) argues that seed oil and protein contents are genetically determined, however strongly influenced by the environment mainly during the grain filling. Negative correlation between oil and protein have been reported and according to Hanson (1991) and Pípolo (2002) it can be explained by the competition for C skeletons by these two biosynthetic processes.

Santos et al. (2010) studying the influence of soybean genotype on the contents of protein and oil at the R_5 stage showed that the N/C ratio determines the accumulation of these compounds in the grain. Therefore the cultivars BRS183 and BRS 214 which had highest protein content in the WS condition had a biochemical capability of synthesizing more protein when N was available.

Not surprisingly higher yields were obtained when water was available (NR and IR) for all genotypes (Table 6).

Considering the genotypes studied negative correlation between percentage of protein in the grain and yield was only encountered in the WS treatment for the BRS 184 cultivar.

Table 6 - Means of grain yield of ten soybean cultivars. WS, NR and IR represents water stress applied in the reproductive stage, natural rainfall and irrigated treatments, respectively. Means followed by the same lowercase letter in the columns and uppercase in the rows do not differ by Tukey test at 5% probability

Cultivares	**Disponibilidades hídricas**									**Médias**
	WS			**NR**			**IR**			
BR 16	1072	d	B	1946	c	A	1929	b	A	1649
Embrapa 48	1235	cd	B	1946	c	A	1953	b	A	1711
BRS 133	1130	cd	B	2525	ab	A	2534	a	A	2063
BRS 134	1274	cd	B	2297	b	A	2019	b	A	1863
BRS 183	1284	cd	B	1984	c	A	1986	b	A	1751
BRS 184	1647	a	B	2637	a	A	2552	a	A	2279
BRS 214	1309	bcd	B	1675	c	A	1543	c	A	1509
BRS 232	1380	bc	B	2369	b	A	2329	a	A	2026
BRS 245 RR	1116	d	B	2424	ab	A	2406	a	A	1982
BRS 247 RR	1539	ab	B	2376	b	A	2315	a	A	2077
Médias	1299			2218			2157			
CV Parcela (%) = 0,9	CV Subparcela (%) = 2,19									

According to Maehler et al. (2003) water deficiency severely reduces yield due to pod abortion and/or to the formation of small grains. Reduction of the grain size has being associated with the shortening of the grain filling period and acceleration of leaf senescence.

Under water stress, the highest and lowest yields were obtained with BRS 184 and BRS 247RR and BR 16 and BRS 245 RR respectively. Several reports regard BR 16 as sensitive to water stress. It is worthwhile to note that BRS 184 had the highest yield in all treatments. The highest yields in the NR and IR conditions were responsible for the highest values of protein and oil content per ha (data not shown), despite the highest accumulation of protein in the grains under water stress (Table 4).

According to Bonato et al. (2000) the relationship between oil content and grain yield can be elevated and positive depending on the genotype analyzed. In our studies such relationship was not observed as there were no differences among cultivars regarding to oil content in any of the treatments.

Means of all parameters analyzed were similar under NR and IR conditions. This can be explained by the constant rain distribution along the season under study (Figure 1) which did not allow major differences between these two water regimes.

CONCLUSIONS

Under water stress, cultivar BRS 184 shows highest protein and oil content per ha due to its highest yield, and did not differ from the other cultivars tested regarding to number of nodules and nodule dry mass;

When water is available, BRS 184 is among the cultivars with highest grain yield and grain protein content (%);

Among the cultivars tested under water stress, BRS 184 is considered a promising choice regarding to nitrogen biological fixation, grain yield and protein and oil per ha.

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