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# NDVI variation according to the time of measurement, sampling size, positioning of sensor and water regime in different soybean cultivars

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**Abstract** Although the information on the Normalized Difference Vegetation Index (NDVI) in plants under water deficit is often obtained from sensors attached to satellites, the increasing data acquisition with portable sensors has wide applicability in agricultural production because it is a fast, nondestructive method, and is less prone to interference problems. Thus, we carried out a set of experiments to investigate the influence of time, spatial plant arrangements, sampling size, height of the sensor and water regimes on NDVI readings in different soybean cultivars in greenhouse and field trials during the crop seasons 2011/12, 2012/13 and 2013/14. In experiments where plants were always evaluated under well-watered conditions, we observed that 9 a.m. was the most suitable time for NDVI readings regardless of the soybean cultivar, spatial arrangement or environment. Furthermore, there was no difference among NDVI readings in relation to the sampling size, regardless of the date or cultivar. We also observed that NDVI tended to decrease according to the higher height of the sensor in relation to the canopy top, with higher

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values tending to be at 0.8 m, but with no significant difference relative to 1.0 m—the height we adopted in our experiments. When different water regimes were induced under field conditions, NDVI readings measured at 9 a.m. by using a portable sensor were successful to differentiate soybean cultivars with contrasting responses to drought.

**Keywords** *Glycine max* L. Merrill · Canopy temperature · Remote sensing · Water regime

## Introduction

Brazil is among the world's top grain producers. The Brazilian agricultural production, in the 2012/13 season, was 187.09 million tons, representing an increase of 14 % compared to the previous season. Of the 187.09 million tons of grains produced in this season, soybean (*Glycine max* L. Merrill) accounted for 81.5 million tons, or more than 43 % of the Brazilian grain production (Conab 2013), a record production. In the 2013/14 crop season, Brazilian soybean production reached 86.12 million tons, representing an increase of 5.7 % compared to the previous season. The soybean complex has been the highlight of Brazilian agribusiness exports, and the export value reached US$33.8 billion between August 2013 and July 2014, representing an increase of 19.6 % in relation to the same period in the previous year (Conab 2014). In turn, the Brazilian soybean production was 96.2 million tons in the 2014/15 crop season, and it is estimated at 96.9 million tons in the 2015/16 crop season, representing an increase of 0.7 % relative to the previous season (Conab 2016). However, problems related to the poor rainfall distribution in the areas planted with soybean affect productivity and have a negative impact on the agribusiness, and, consequently, on the Brazilian economy.

Several studies have been conducted aimed to obtain a better adaptation of crops to different environmental conditions. The results of these studies may lead to both the expansion of the planted areas and the increase in the current farming areas. Therefore, remote sensing techniques are very useful, since they allow the monitoring of crops without the need for direct contact with the sample. One of the several remote sensing products is the vegetation indexes like the Normalized Difference Vegetation Index (NDVI) (Folhes et al. 2009; Esquerdo et al. 2011). NDVI is calculated through the difference between reflectance detected in red (0.58–0.68 μm) and near infrared (0.725–1.1 μm) bands shared by the sum of these quantities (Rouse et al. 1974), resulting in values ranging from $-1.0$ to $+1.0$ (Chen et al. 2002). Under normal conditions, vigorous vegetation absorbs the visible radiation for photosynthesis and reflects the near-infrared radiation due to the light scattering through mesophyllic tissues and the leaf water content (Gusso 2013). Several studies report the relationship between NDVI and physiological and biophysical characteristics of plants, such as biomass (Marti et al. 2007), soil cover (Mullan and Reynolds 2010), nitrogen content (Wright Jr et al. 2005), productivity (Royo et al. 2003) and water deficit (Yuhas and Scuderi 2009).

Within-field monitoring by satellite remote sensing has been successful, but limited mainly due to cloud cover, total cost, poor spatial resolution and lack of proper techniques and facilities to process imagery for agricultural applications (Steven 1993), in addition to the perceived risk of yield reduction (Kim and Chavas 2003; Koundauri et al. 2006; Samseemoung et al. 2012). Although the information on NDVI in plants under water deficit is often obtained from sensors attached to satellites (orbit sensors) including Moderate Resolution Imaging Spectroradiometer data (Zhang et al. 2013: Sruthi and

Mohammed Aslam 2015), advanced very high resolution radiometer images (Rulinda et al. 2012) or Landsat satellite images (Dorman et al. 2013), the increasing data acquisition with portable sensors (land surface) has wide applicability in agricultural production because it is a fast, nondestructive method, and is less prone to interference problems. However, reports involving portable sensors have described different procedures for NDVI evaluation with regard to sampling area, height of sensor positioning in relation to the canopy top, and time of measuring (Xavier et al. 2006; Hikishima et al. 2010; Monteiro et al. 2012).

Thus, we carried out a set of experiments aimed at investigating the influence of time, spatial plant arrangements, sampling size, positioning of sensor and water regimes on NDVI readings in different soybean cultivars in greenhouse and field trials during the crop seasons 2011/12, 2012/13 and 2013/14.

## Materials and methods

### The use of *GreenSeeker® 505 Handheld Sensor* for NDVI measurements

In all experiments, the GreenSeeker® 505 Handheld Sensor device manufactured by Ntech Industries, Inc. was used in the measurements of NDVI (supplementary figure). It calculates the referred index according to the following equation:

$$NDVI = \frac{\rho NIR - \rho R}{\rho NIR + \rho R} \tag{1}$$

where $\rho R$ is the red reflectance (0.58–0.68 μm, spectrum range used in the photosynthetic process) and $\rho NIR$ is the near infrared reflectance (0.725–1.1 μm, spectrum range with high reflectance of the internal structures of the leaves). This device provides both reflectance values separately and also the NDVI values directly, which we used in our experiments. It uses the software NTech capture, and the view angle of the sensor is around 53°.

This sensor performs scanning readings every 0.1 s. In greenhouse experiments, we used the highest NDVI value obtained, which corresponds to the reflectance of plants grown in the pot under evaluation, in order to minimize the interference of adjacent areas. The relief must be established in a fixed location and the albedo must not correspond to the green color in order to prevent overlap of reflectance between the plant and the surface. Under field conditions, we used the mean values of NDVI obtained in each row. The manufacturer recommends the positioning of the sensor at 0.8–1.2 m above the canopy top. In the current study, except when different times and heights of the sensor were investigated, all NDVI measurements were performed at 9 a.m. under sunny conditions, with the sensor positioned at 1.0 m above the canopy top, and walking along the full length of each row in the case of field trials. All times referred to in this article are Brazilian daylight saving time.

### Influence of time during NDVI measurements under greenhouse conditions

This experiment was carried out in a greenhouse belonging to Embrapa Soybean. We used the determinate cultivars BR 16 and Embrapa 48, both presenting a semi-early cycle. Seeds previously inoculated with *Bradyrhizobium japonicum* (SEMIA 5079, 1.5 × 106 cells) were sown in 11 pots filled with a mixture composed of soil–sand-organic compound

(1:3:1, 26 % water retention capacity). The experimental design was completely randomized, with ten replicates. Plants were kept in a greenhouse under well-watered conditions until reaching the V2 development stage, when the first NDVI readings were performed. In order to check the reproducibility of the method, NDVI was again measured in the same plants two days later. On both dates, NDVI readings were taken at 9 a.m., 10 a.m., 11 a.m., noon, 1 p.m., 2 p.m., 3 p.m. and 4 p.m.

## NDVI evaluation under field conditions

### *Experimental area*

The field experiments described below were carried out at the facilities of Embrapa Soybean (latitude 23°11′37″S, longitude 51°11′03″ and 630 m altitude, Londrina, PR, Brazil). Air temperature, relative air humidity and rainfall were monitored by the weather station installed in the experimental field. Based on rainfall and air temperature data, water balance was calculated according to Thornthwaite and Mather (1955). From relative air humidity (RH) and air temperature values, the vapor pressure deficit (VPD) was calculated according to the equation:

$$\mathrm{VPD} = (100 - \mathrm{RH})/100 \times \mathrm{es} \tag{2}$$

where saturated vapor pressure (es − kPa) was calculated through a psychrometric chart using the software available at http://physics.holsoft.nl/physics/ocmain.htm.

### *NDVI measurements in three times during the day considering different spatial plant arrangements*

Two experiments were carried out during the crop season 2011/12 in order to evaluate NDVI at three reading times—9 a.m., noon and 3 p.m.—considering different spatial plant arrangements. These experiments aimed to investigate a possible influence of spatial plant arrangements on the best time during the day for NDVI measurements. Both trials were set in a randomized complete block design, with three blocks, and followed the soybean production technologies of Embrapa Soybean (Embrapa Soja 2013).

The first experiment consisted of soybean cultivation in normal rows (0.4 m spaced parallel rows) and crossed rows (0.4 m spaced parallel and perpendicular rows), using the cultivars BRS 294RR (determinate) and BRS 359RR (indeterminate growth). Plot dimensions were 4.8 m width × 8.0 m length. Seeds were sown on Oct. 19, 2011 and NDVI readings were performed on 15 Dec. 2011, 1 Feb. 2012 and 7 Feb. 2012. In the second experiment, we evaluated NDVI in three different row spacings, 0.19 and 0.38 m between single rows, and 0.38 m between double rows (in each double row, 0.19 m between rows). In this experiment, we used the cultivars BRS 294RR (determinate) and BMX Turbo (indeterminate growth). Plot dimensions were 6.0 m × 6.0 m. Seeds were sown on Nov. 11, 2011 and NDVI readings were performed on 16 Dec. 2011, 31 Jan. 2012 and 17 Feb. 2012.

### *NDVI measurements with variation of the sampling size and the height of sensor*

During the 2013/14 crop season, we investigated changes in NDVI values according to differences in the sampling size and in the height of the sensor in relation to the canopy top.

To evaluate NDVI in different sampling sizes, we used the indeterminate early-cycle soybean cultivars BRS 359RR and BRS 284. The experimental design was in randomized blocks, with four blocks for each cultivar. Sowing was on 5 Nov. 2013 and all plots were kept under natural watering conditions. NDVI readings were made at 45 and 77 days after sowing (DAS), when both cultivars were in R2 and R5.4 development stage, respectively. The sampling sizes evaluated in each plot were: (a) one reading along the total row length; (b) two readings, from each extremity up to the middle of the row, thus splitting the row length into two equal parts (1/2A and 1/2B); (c) two readings, from each extremity up to ¼ of the total row length (1/4A and 1/4B).

To check the best height of sensor positioning in relation to the canopy top, NDVI readings were made at 42 DAS, when BRS 359RR and BRS 284 plants were in R1 and R2 stage, respectively. The sensor was positioned at 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 m height in relation to the canopy top. To certify the correct height of sensor positioning, the readings were always taken by the same operator and the sensor height values were established using a measuring tape.

*NDVI measurements under water deficit*

The following experiments were carried out during the 2012/13 and 2013/14 crop seasons. The plants of cultivars Embrapa 48 and BR 16, less sensitive and more sensitive to drought, respectively (Oya et al. 2004), were subjected to three water regimes: irrigated (IRR, soil matric potential between −0.03 and −0.05 MPa), and drought stress in vegetative (DSV) and reproductive (DSR) periods. Soil moisture was monitored by neutron probe, thermogravimetric analysis and tensiometry. Stress in the vegetative and reproductive periods was artificially simulated by the use of movable covers (rainout shelters) programmed to automatically close when rain begins, and to open again when rain stops. A randomized complete block split-plot design was used, with four blocks. The water regimes were distributed in the plots, while the cultivars were distributed in the subplots.

In each crop season, the planting dates for both cultivars and for the treatments were 5 Nov. 2012 and 5 Nov. 2013. The beginning of the water deficit induction in the vegetative period (DSV) was on 5 Dec. 2012 and 2 Dec. 2013. On 27 Dec. 2012 and 30 Dec. 2013, the plants changed from the vegetative to the reproductive stage and were then allowed to receive rainfall. On that date, another group of plots, which had previously been allowed to receive rainfall, was then subjected to water deficit (DSR).

During the 2012/13 crop season, the phenological development stage of plants was assessed three times a week from the date of germination, five DAS to maturity, according to Fehr and Caviness (1977).

Canopy temperature measurement was performed with an InfraPro® thermal infrared sensor (Oakton®*)*, always positioned in the central leaflet of the fully expanded third trifoliate leaf from the top. The distance and the sensor positioning angle in relation to the leaflet followed the manufacturer's recommendations.

Readings of NDVI and canopy temperature were made on 6 Dec. 13 and 19 2012 and 1 Mar. 2013, always at 9 a.m., 11 a.m., 1 p.m. and 3 p.m. In order to check whether the differences in NDVI were related to differences in the stage of development, plants of the two cultivars in the same development stage (R5.5) were assessed with the different water regimes on 25 Jan. 2013, with NDVI measurements made only at 9 a.m. The leaf area index (LAI) was calculated as the ratio between the leaf area and the soil area occupied by the plant. During the 2013/14 crop season, NDVI and canopy temperature were assessed (at 9 a.m.) on 5 Feb. 2014, which corresponds to the period presenting the most severe water deficit.

In both crop seasons, grain yield was also assessed at 13 % moisture using the following equation:

$$GY = \frac{(100 - HGM)}{(100 - DGM)} \times HGW \times \frac{10,000}{HPA} \tag{3}$$

where GY is the grain yield (Kg $ha^{-1}$), HGM the harvested grain moisture (%), DGM the desired grain moisture (%), HGW the harvested grain weight (kg) and HPA is the harvested plot area ($m^2$).

### Statistical analysis

Since the assumptions of the analysis of variance (ANOVA) were met, the data were submitted to ANOVA and the means compared by the Tukey's test ($p \leq 0.05$) using the software Sisvar 5.3 (Ferreira 2010).

## Results and discussion

### NDVI variation according to the time of measurement, sampling size and height of sensor

In order to check a possible effect of the time of measurement on NDVI values, we first evaluated well-watered plants of two determinate soybean cultivars (BR 16 and Embrapa 48) under greenhouse conditions at different times during the day and on two dates of sampling when plants of both cultivars reached the stage V2 (Fig. 1). We observed that there was no significant difference among times of measurement for the cultivar BR 16 on both dates, although NDVI values tended to be slightly higher at the first time of measurement (9 a.m.). Regarding the cultivar Embrapa 48, there was no significant difference among times of measurement on the first date of evaluation (10 Sept. 2012), but two days later, higher values were detected at 9 a.m., followed by decreasing values mainly at 1 and 2 p.m.

To evaluate differences in the time of NDVI measurement during the day under field conditions, we carried out two experiments considering different spatial plant arrangements under well-watered conditions (Fig. 2). The first experiment consisted of cultivation in normal rows (0.4 m-spaced parallel rows) and crossed rows (0.4 m-spaced parallel and perpendicular rows), using the soybean cultivars BRS 294RR (determinate) and BRS 359RR (indeterminate). In the second experiment, we evaluated NDVI in three different row spacing, 0.19, 0.38 m between single rows and 0.38 m between double rows (in each double row, 0.19 m between rows), using the cultivars BRS 294RR (determinate) and BMX Turbo (indeterminate). In both experiments, except for the first date of sampling, when plants were younger, the highest NDVI values were always obtained at 9 a.m. in almost all evaluations, regardless of spatial arrangement, date or cultivar. This fact might be related to the leaf movement in soybean plants, in which leaves' extremities are downwards at dawn and remain almost horizontal (diaheliotropism) in the first hours of the day. During the morning, leaf extremities continue their upward movement and remain almost vertical (paraheliotropism) just before midday until around 4 p.m. Afterwards, a

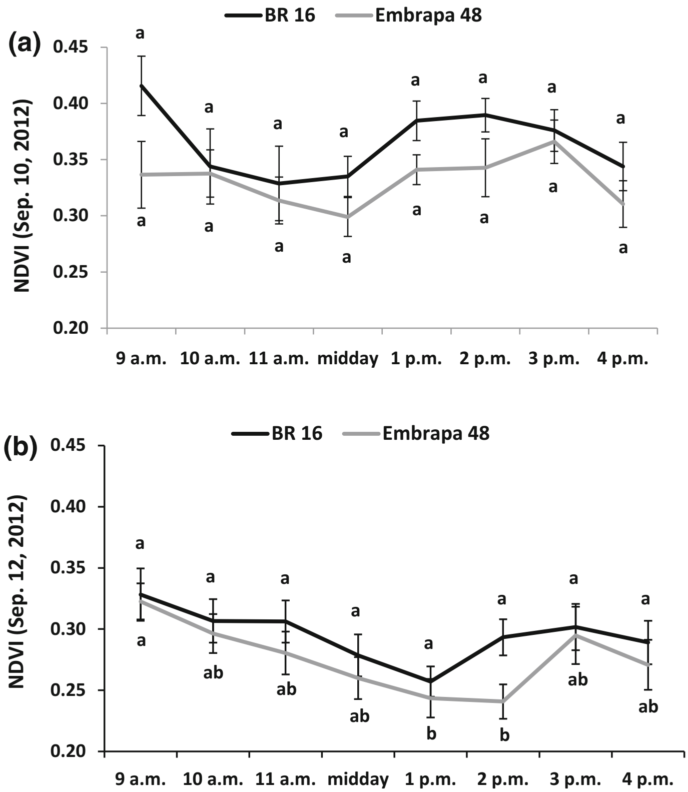



**Fig. 1** Values of NDVI for cultivars BR 16 (*black lines*) and Embrapa 48 (*gray lines*) in well-watered plants under greenhouse conditions. **a** 10 Sept. 2012; **b** 12 Sept. 2012. On each date of assessment and for each cultivar, mean ± standard error followed by the *same letter* do not differ by Tukey's test ($p \leq 0.05$). n = 10

reverse leaf movement is observed, so that most leaves remain almost horizontal around 5:30–6 p.m. and keep their extremities downwards at the end of the day just before dark (Boller et al. 2011). Thus, canopy reflectance is directly impacted by diurnal paraheliotropic movements, and NDVI can significantly vary during the day due to leaf movement as solar irradiation changes (Chávez et al. 2014). Consequently, under well-watered conditions, higher NDVI is expected in the early hours of the day due to the greater exposure of leaves in the sun.

We also investigated a possible influence of the sampling size on NDVI readings in a field experiment carried out under well-watered conditions. Thus, we evaluated the indeterminate soybean cultivars BRS 359RR and BRS 284 and NDVI readings were made at 45 and 79 DAS, when both cultivars were in R2 and R5.4 development stage, respectively. The sampling sizes evaluated in each plot were: (a) one reading along the full row, i.e. along the total row length; (b) two readings, from each extremity up to the middle of the row, thus splitting the row length into two equal parts (1/2A and 1/2B); (c) two readings, from each extremity up to ¼ of the total row length (1/4A and 1/4B). There was no difference among NDVI readings regardless of the sampling size, date or cultivar (Fig. 3a–d). This information is important mainly when NDVI needs to be evaluated in rows in

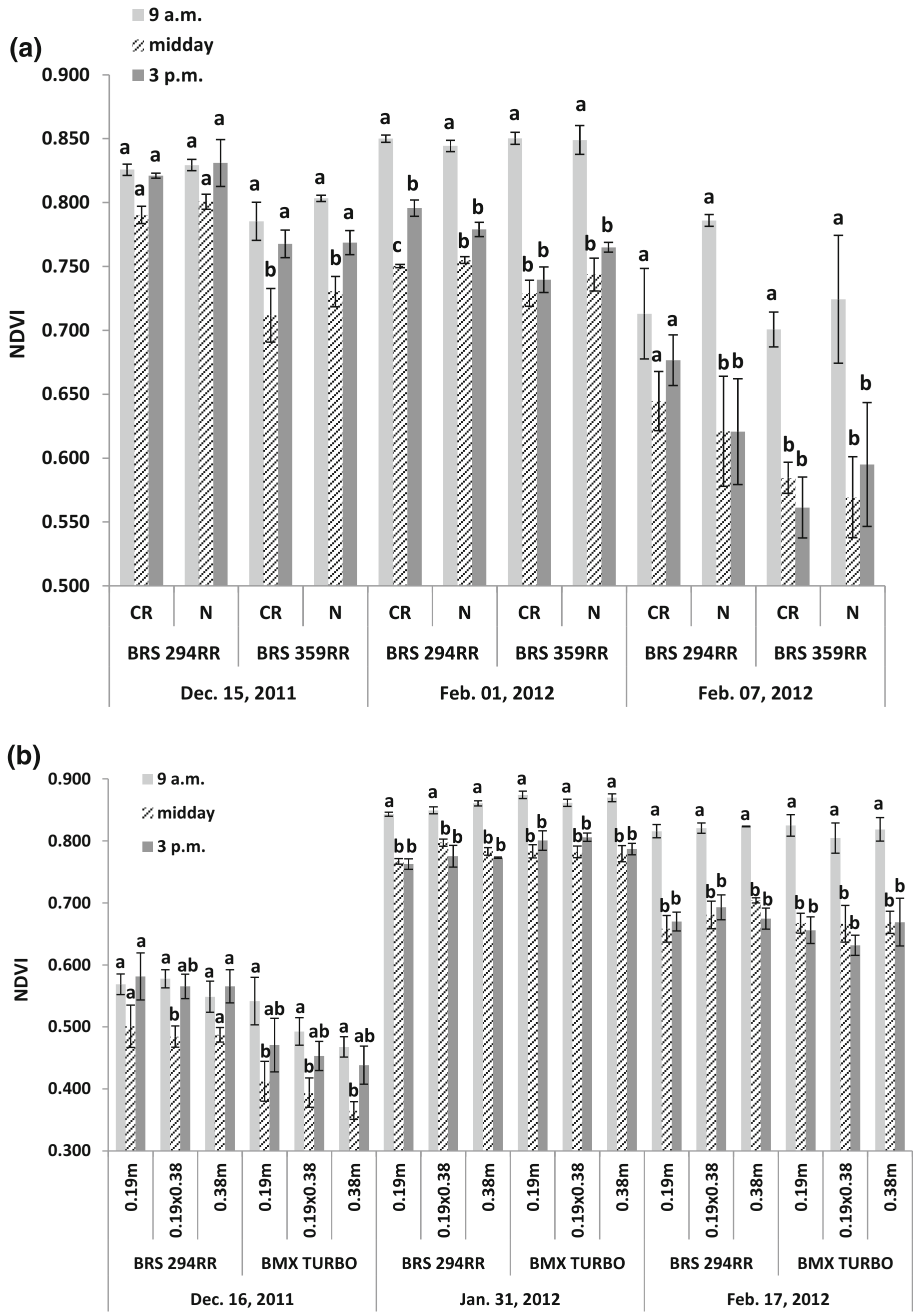



**Fig. 2** Values of NDVI at three times during the day in different spatial plant arrangements. **a** Normal (*N*) and crossed (*CR*) rows in the cultivars BRS 294RR and BRS 359RR. **b** Three row spacing measurements (0.19, 0.38 m, 0.19 × 0.38 m) in the cultivars BRS 294RR and BMX Turbo. On each date, cultivar and arrangement, mean ± standard error followed by the *same letter* among different times of measurement do not differ by Tukey's test ($p \leq 0.05$). n = 3

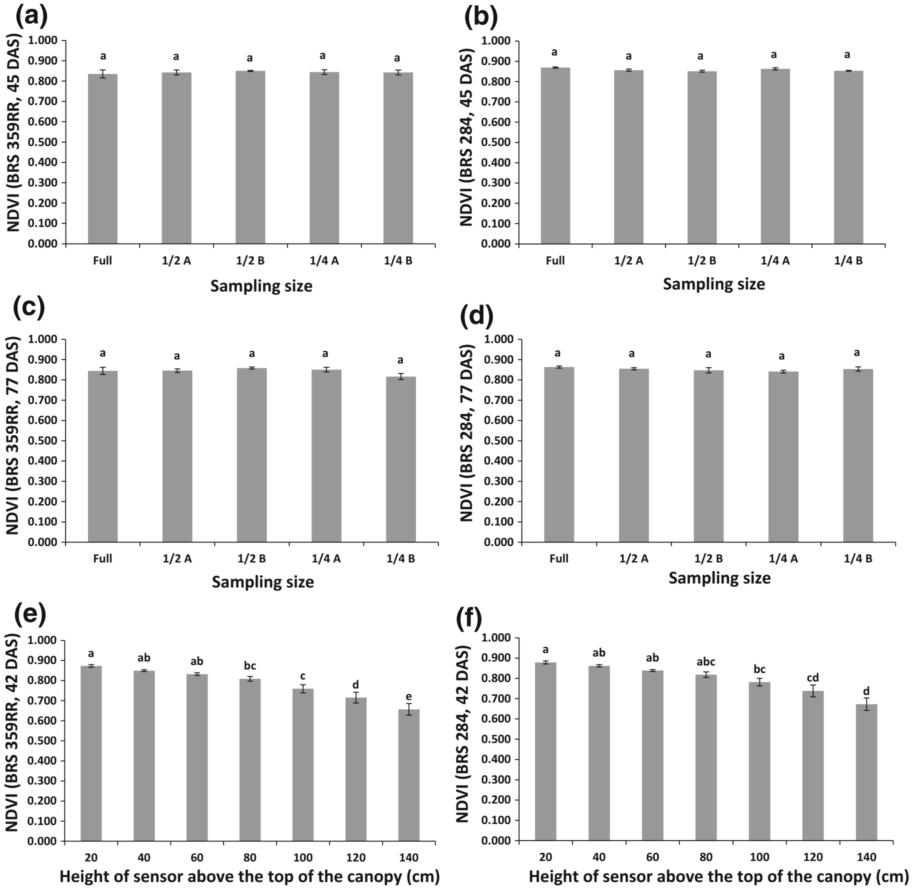


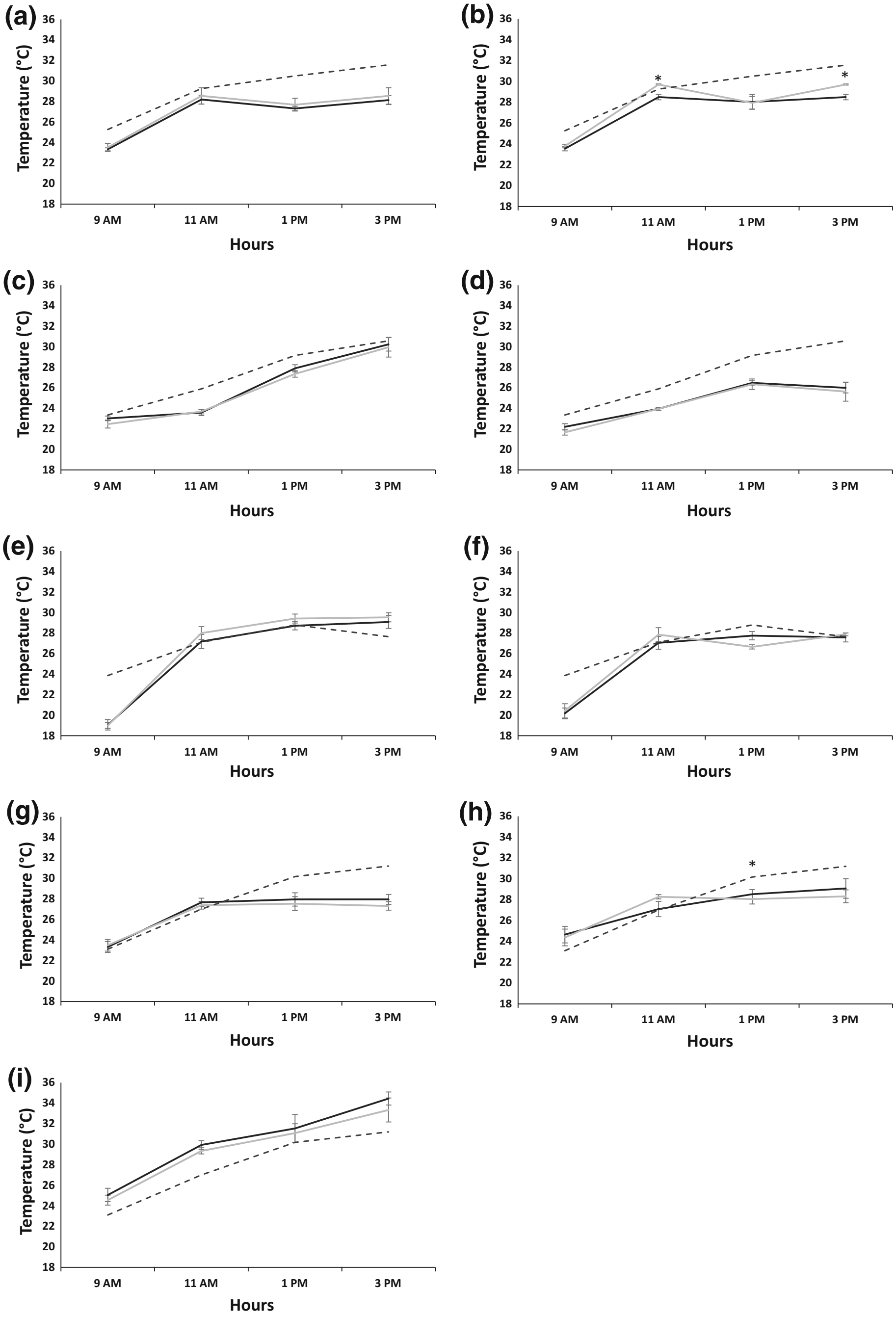

**Fig. 3** Values of NDVI in the soybean cultivars BRS 359RR and BRS 284 measured in different sampling sizes (**a**–**d**), and with the sensor positioned at several heights above the canopy top (**e**, **f**). Mean ± standard error followed by the *same letter* do not differ by Tukey's test ($p \leq 0.05$). n = 4

which plants are very tall or branched, so that readings could be taken with no need of walking along the row, thus preventing plant injuries.

Concerning the choice of the best height for positioning the sensor in relation to the canopy top, NDVI readings were made at 42 DAS, when BRS 359RR and BRS 284 plants were in R1 and R2 development stage, respectively. We observed that NDVI values tended to decrease with the increase in height of the sensor (Fig. 3e, f). Considering the range of height recommended by the manufacturer of GreenSeeker® 505 Handheld Sensor (0.8–1.2 m), NDVI values tended to be higher at 0.8 m, but with no significant difference from 1.0 m, the height that we adopted in our experiments.

## NDVI measurements under water deficit

In the current study, the possible difference in the NDVI of two soybean cultivars with contrasting responses to drought—BR 16 and Embrapa 48, more sensitive and less sensitive to drought, respectively—were investigated under continuous irrigation (IRR) and


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
Temperature (°C)
36
34
32
30
28
26
24
22
20
18
9 AM
11 AM
1 PM
3 PM
Hours
*

◀**Fig. 4** Air temperature values (*dashed lines*) and canopy temperature for cultivars BR 16 (*black lines*) and Embrapa 48 (*gray lines*). *Bars* represent the standard error of the mean. **a** 6 Dec. 2012, DSV, **b** 6 Dec. 2012, IRR, **c** 13 Dec. 2012, DSV, **d** 13 Dec. 2012, IRR, **e** 19 Dec. 2012, DSV, **f** 19 Dec. 2012, IRR, **g** 01 Mar. 2013, DSV, **h** Mar. 01, 2013, IRR, **i** 01 Mar. 2013, DSR. n = 6 (DSV and DSR); n = 4 (IRR). *DSV* water stress in the vegetative period, *IRR* continuous irrigation, *DSR* water stress in the reproductive period

under water deficit applied during the vegetative period (DSV), and reproductive period (DSR) under field conditions during the 2012/13 and 2013/14 crop seasons. Infrared thermometry was used with the purpose of verifying the occurrence of water stress in the plants, detected when canopy temperature is higher than air temperature (Carvalho et al. 2015). Although our preliminary results allowed us to infer that 9 a.m. represents the best time for NDVI readings under greenhouse conditions and in the field regardless of spatial arrangement in well-watered plants, we decided to investigate a possible effect of water deficit on NDVI readings measured four times under field conditions (9 a.m., 11 a.m., 1 p.m. and 3 p.m.) during the 2012/13 crop season.

On the first two assessment dates (6 Dec. and 13, 2012), the plants subjected to water deficit (Fig. 4a, c) or continuous irrigation (Fig. 4b, d) during the vegetative period were not under water stress since their canopy temperatures were lower than air temperature. The similarity of canopy temperature values between the plants subjected to the two water regimes on the first collection date (Fig. 4a, b) can be explained by the fact that the plants remained under movable covers for a short period of time, which is corroborated by the soil moisture in the 0.0–0.2 m layers, under the two water regimes (Fig. 5b), measured on 4 Dec. 2012. On 13 Dec. 2012, at 3 p.m., the plants under continuous irrigation (Fig. 4d) had lower canopy temperatures than the plants exposed to water deficit during the vegetative period (Fig. 4c). At this time, the temperature difference was 6 °C, the same value as obtained on 1 Mar. 2013, when comparing plants under stress in the reproductive period (Fig. 4i) and under continuous irrigation (Fig. 4h).

According to Mengistu et al. (1987), soybean plants subjected to water stress had higher canopy temperatures compared to non-stressed plants, considering the reproductive stages. Besides, Rao (1985) detected an increase of up to 8 °C in the canopy temperature of soybean plants subjected to water stress compared to well-irrigated plants.

On 19 Dec. 2012, the canopy temperature of the two cultivars tended to be equal under the two water regimes, because there was a decrease in the average air temperature (Fig. 4e, f). Since low VPD values were observed in this day (Fig. 5a), it is possible that higher stomatal conductance and increased rates of leaf transpiration had occurred, cooling the leaves and equaling the air and canopy temperatures. In a study that assessed the effects of $CO_2$ in the growth of soybean plants under environmental conditions, the authors suggested that stomatal conductance decreases with increase in VPD and response to sensitivity decreases at high temperatures (Wilson and Bunce 1997). Regulation of transpiration depending on VPD was also observed by Seversike et al. (2013) for some soybean genotypes.

Despite the high VPD values observed on the first date assessed (6 Dec. 2012), no water stress was detected in the plants under continuous irrigation or under water stress in the vegetative period. Therefore, it is possible that the soil moisture at the early stages of crop development was sufficient to meet the water needs of the vegetation, as well as the high deficit of vapor in the atmosphere.

The rise in canopy temperature was proportional to the rise of VPD throughout the following days: 13 Dec. 2012 (DSV) and Mar. 1, 2013 (DSR), when the maximum values

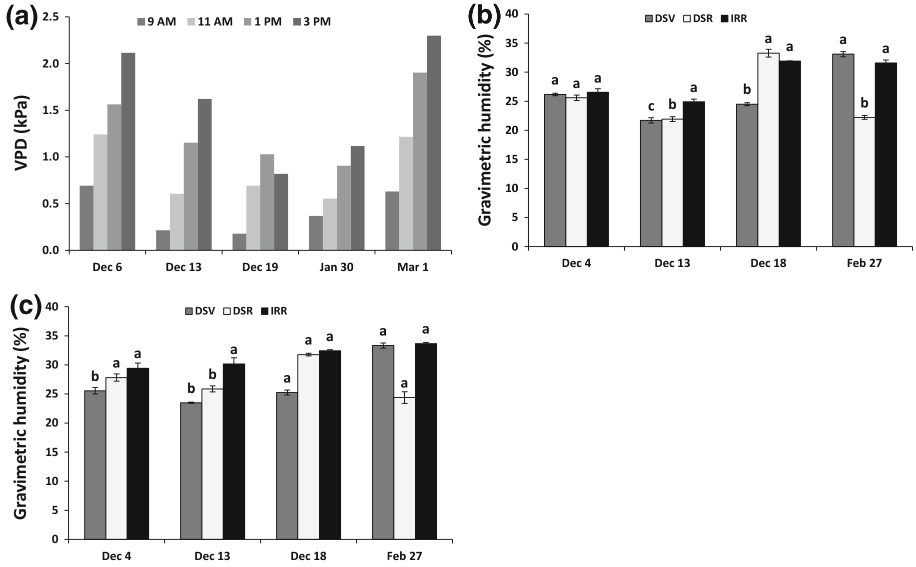



**Fig. 5** VPD (**a**) and gravimetric humidity (GH) of the soil in 0.0–0.2 m (**b**) and 0.2–0.4 m **c** layers in the 2012/2013 crop season. On each date of assessment of GH, mean ± standard error followed by the same letter do not differ by the Tukey's test ($p \leq 0.05$). n = 4 (GH)

of canopy temperature (around 35 °C) were observed at 3 p.m. in VPD values of 2.0–2.5 kPa.

Although equalization of air and canopy temperatures was observed for plants under water stress and continuous irrigation, for the plants subjected to water stress in the vegetative period (DSV) the rise in canopy temperature compared to air temperature was observed at 1 p.m. in both cultivars (Fig. 4e). On the previous assessment date (18 Dec. 2012), the soil moisture value was close to the one observed on days 4 Dec. and 13, 2012 under water deficit (DSV) or tending to increase in the days of continuous irrigation (IRR) (Fig. 5b, c).

In the current study, regarding the four analyzed dates, only on 1 Mar. 2013 there was water stress at the four times of measurement in the plants subjected to water deficit in the reproductive period (Fig. 4i). At 3 p.m. the canopy temperature of the plants under movable covers reached 35 °C, most likely due to stomatal closure and limited transpiration. Analysis of soil moisture on 27 Feb. 2013, the closest date to 1 Mar. 2013, showed that on the 0.0–0.2 and 0.2–0.4 m layers the values of plots under DSR were around 22 %, that is, far below the value obtained in the DSV and IRR treatments (around 32 %) (Fig. 5b, c). It is possible that such values were maintained or reduced on 1 Mar. 2013, based on the sequential water balance of the crop (Fig. 6a).

Similarities in canopy temperatures of plants under the DSV and IRR regimes (Fig. 4g, h) on Mar. 1, 2013 are explained by the fact that the plants initially subjected to water deficit during the vegetative period (Fig. 4g) were given rain water between 27 Dec. 2012 and 1 Mar. 2013. The total rainfall accumulated between these dates was 446.3 mm, and the plots of the irrigated treatment (IRR) totaled an additional 22.09 mm in the same period.

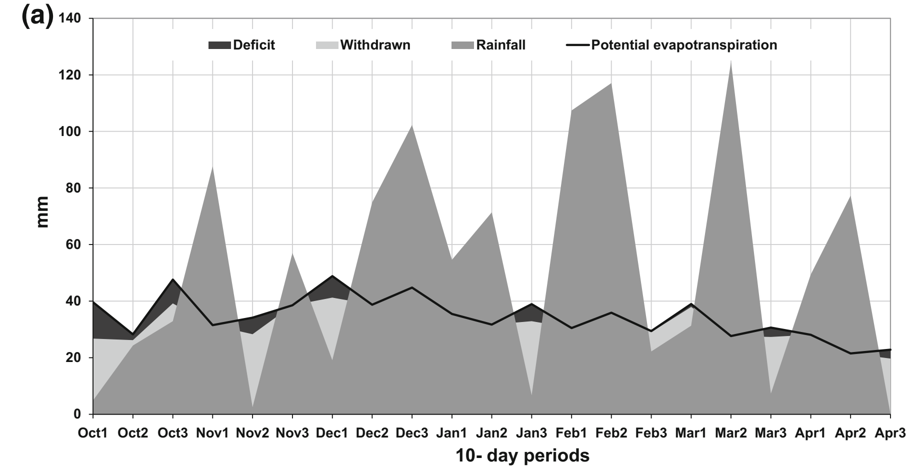
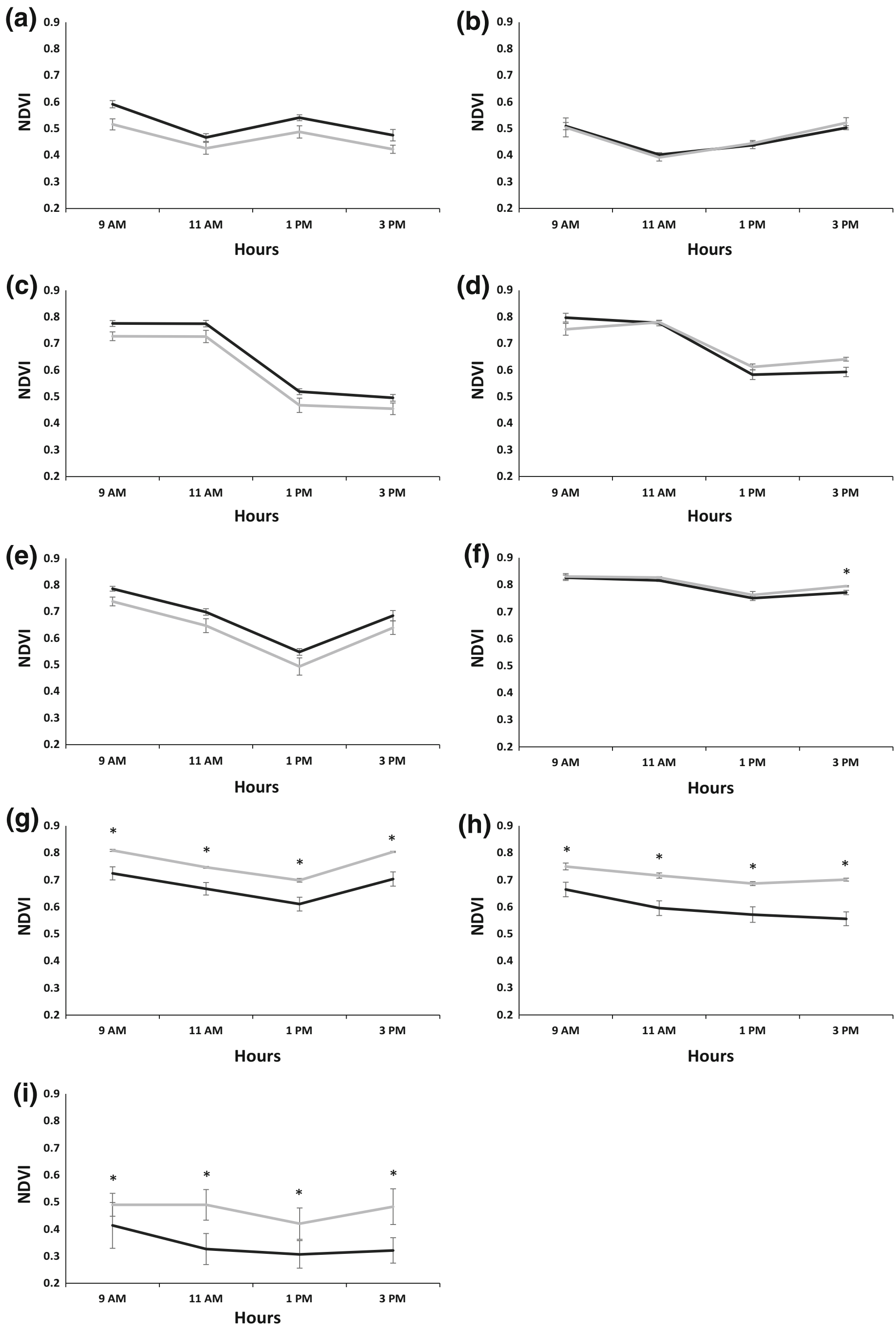



**Fig. 6** Sequential water balance of the 2012/13 (**a**) and 2013/14 (**b**) crop seasons

Differences in canopy temperatures between the two cultivars were observed under continuous irrigation on 6 Dec. 2012, at 11 a.m. and 3 p.m. (higher for Embrapa 48) and on Mar. 1, 2013, at 1 p.m. (higher for BR 16) (Fig. 4b, h).

The NDVI values of both cultivars analyzed in the three dates (6 Dec. 13 and 19, 2012) were similar (Fig. 7a–f), with slight differences observed in the plants under water deficit during the vegetative period (DSV), when soil moisture in the 0.0–0.2 and/or 0.2–0.4 m layers was lower than in the plots under continuous irrigation [4 Dec. (0.2–0.4 m), 13 (0.0–0.2 and 0.2–0.4 m), 18 (0.0–0.2 and 0.2–0.4 m), 2012] (Fig. 5b, c). Although not statistically significant, higher NDVI values during the vegetative period were obtained for cultivar BR 16 on the first three dates of analysis—6 Dec. 13 and 19, 2012 (Fig. 7a, c) in the DSV treatment and most probably reflected the small differences in the development stages of the two cultivars (Table 1).

In more advanced development stages (1 Mar. 2013) with plants subjected to atmospheric VPD tending to higher values, in combination or not with low soil moisture




(Fig. 5a, c) it was possible to distinguish the two cultivars with respect to their NDVI values. In this case, higher NDVI values were obtained for the cultivar less sensitive to drought, Embrapa 48 (Fig. 7g–i).

◀ **Fig. 7** Values of NDVI for cultivars BR 16 (*black lines*) and Embrapa 48 (*gray lines*). **a** 6 Dec. 2012, DSV, **b** 6 Dec. 2012, IRR, **c** 13 Dec. 2012, DSV, **d** 13 Dec. 2012, IRR, **e** 19 Dec. 2012, DSV, **f** 19 Dec. 2012, IRR, **g** 1 Mar. 2013, DSV, **h** 1 Mar. 2013, IRR, **i** 1 Mar. 2013, DSR. At each time of measurement, mean ± Standard Error followed by the same letter do not differ by the Tukey's test ($p \leq 0.05$) n = 6 (DSV and DSR); n = 4 (IRR). *DSV* water stress in the vegetative period, *IRR* continuous irrigation, *DSR* water stress in the reproductive period

From analysis of the development stages of the two cultivars on 1 Mar. 2013 (Table 1), it was found that the differences in NDVI values observed in plants under the DSR regime may have been caused by differences in the development stage of these cultivars. Also, while cultivar Embrapa 48 was changing from the full seed stage (R6) to the beginning of maturity stage (R7), cultivar BR 16 was at harvest maturity stage (R8). According to Heatherly and Elmore (2004), acceleration of the cycle may be related to a drought escape strategy. In this context, according to Lawlor (2013), the vegetative growth in annual crops takes advantage of the rainy season, while grain maturation occurs during dry seasons, which allows the drought-escaping mechanism.

Equalization of the stages of development in the different water regimes was observed on 25 Jan. 2013 with the plants in the beginning stage R5, or else, at the beginning of grain formation (Table 1).

Only on 18 Mar. 2013, except for treatment DSR, the two cultivars had equal development stages, being both in the R 7.8 and R8 stages in the DSV and IRR treatments, respectively (Table 1). Therefore, it was assumed that in cultivar BR 16, yellowing of leaves (chlorophyll degradation), as well as leaf fall, had started earlier than in Embrapa 48, resulting in lower NDVI values for cultivar BR 16.

In order to check whether the differences in NDVI were related to differences in the stage of development, plants of the two cultivars in the same development stage (R5.5) were assessed with the different water regimes on 25 Jan. 2013, with NDVI measurements made only at 9 a.m. It was found that when both cultivars were subjected to water deficit in the reproductive stage (DSR), the NDVI of cultivar Embrapa 48 was higher than that of cultivar BR 16 (Fig. 8a), indicating a different response of this cultivar to water deficit, regardless of the LAI. Significant differences in LAI were observed only in the DSV water regime, where the LAI of cultivar BR 16 was higher than that of cultivar Embrapa 48. On that date, the soil moisture values in the 0.0–0.2 and 0.2–0.4 m layers were around 22 % (Fig. 5b, c) and VDP had low values (0–1 kPa, Fig. 5a).

In the DSV and IRR, similar values of soil moisture were detected in the 0.2–0.4 m layer (Fig. 5b, c) because on 25 Jan. 2013 the plants on the DSV regime were receiving rainwater. Thus, similar NDVI values for both cultivars were found in the two treatments (Fig. 8a).

Throughout the period studied, changes in NDVI were observed during the times of assessment, which can be associated with heliotropic leaf movements and leaf water content. Some differences in daily heliotropic movements were observed between soybean cultivars by Rosa and Forseth (1996). Furthermore, the mechanism of heliotropic control is associated with turgor variations (Rakocevic et al. 2010).

Since there was an acceleration in the growth cycle of cultivar BR 16 compared to cultivar Embrapa 48 (Table 1) in both the vegetative and reproductive periods, it is suggested that cultivar BR 16 has drought escape mechanisms associated with acceleration of its life cycle. Such a mechanism allows vegetative growth to occur in the rainy season, while grain maturation occurs in the dry season. The drought-escaping ability by acceleration of the life cycle or the evolution of mechanisms to prevent or tolerate drought have been considered important strategies of adaptation to drought (Levitt 1972; Chaves et al.

**Table 1** Developmental stages of cultivars BR 16 and Embrapa 48 (E48) in vegetative (05–18 Dec. 2012) and reproductive (25 Jan.–18 Mar. 2013) periods under three water regimes (DSV, DSR and IRR) during the 2012/13 crop season

| | Evaluation dates and cultivars | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 05 Dec. | | 13 Dec. | | 18 Dec. | | 25 Jan. | | 25 Feb. | | 27 Feb. | | 01 Mar. | | 18 Mar. | |
| | BR16 | E48 | BR16 | E48 | BR16 | E48 | BR16 | E48 | BR16 | E48 | BR16 | E48 | BR16 | E48 | BR16 | E48 |
| A | 4 | 3.8 | 6.2 | 6 | 7.6 | 6.6 | 5.5 | 5.3 | 6.2 | 6 | 6.6 | 6.4 | 6.6 | 6.4 | 7.8 | 7.8 |
| B | 3.8 | 3.8 | 6.2 | 6.4 | 8.4 | 8 | 5.3 | 5.4 | 6.2 | 6 | 6.6 | 6.2 | 6.8 | 6.2 | 8 | 8 |
| C | | | | | | | 5.4 | 5.3 | 6.4 | 6 | 6.6 | 6.4 | 7.2 | 6.4 | 8 | 7.8 |

Data are average of readings in each block

*A* drought stress in the vegetative period, *B* irrigated, *C* drought stress in the reproductive period

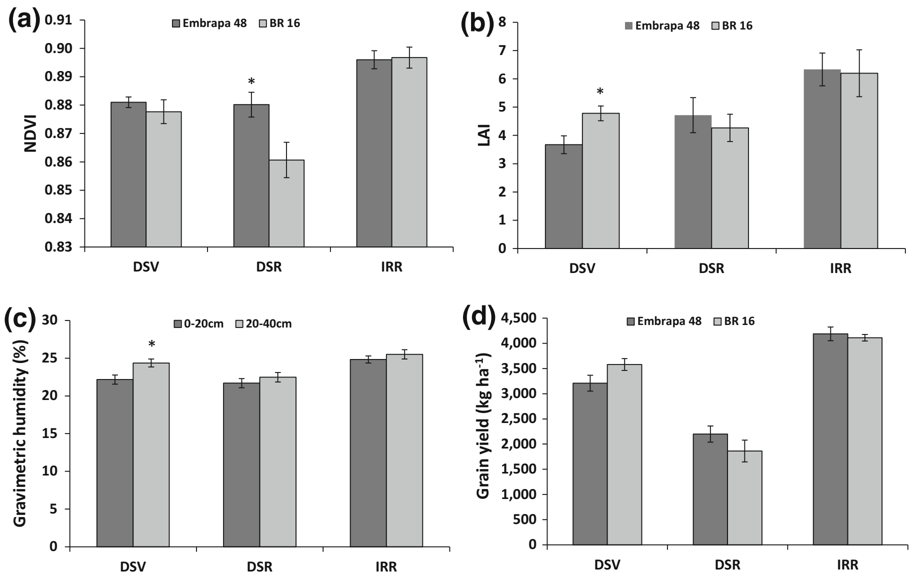



**Fig. 8** NDVI, LAI of cultivars BR 16 and Embrapa 48 at stage R5.5, soil gravimetric humidity and grain yield, during the 2012/13 crop season under water stress in the vegetative period (DSV), water stress in the reproductive period (DSR) and under continuous irrigation (IRR). In each water condition, mean ± standard error indicated by *asterisks* are significantly higher by the Tukey's test ($p \leq 0.05$). n = 6 (DSV and DSR); n = 4 (IRR)

2003). Escape strategies often depend on successful reproduction before the beginning of severe stress (Campos et al. 2004), through a short life cycle with high growth rates and efficient storage and use of reserves for seed production.

Considering the productivity of the two cultivars in the three water regimes during the 2012/13 crop season (Fig. 8d), it was found to be similar for both cultivars under DSV and IRR. However, under DSR, lower productivity levels were observed. Thus, the lowest productivity levels were obtained for plants under a higher water stress level detected by infrared thermometry, as well as lower NDVI values. The absence of differences in the productivity levels in both cultivars can be explained by the regular rainfall distribution throughout the crop cycle, so that the atmospheric conditions of low relative humidity and high temperatures, typical of dry seasons, were sporadic, making it difficult to simulate water stress even under movable covers. Although not statistically significant, increments of 15 % were observed for cultivar Embrapa 48 compared to cultivar BR 16 in the DSR water regime (Fig. 8d).

In order to check the behavior of both cultivars in the following crop season (2013/14) in the same water regimes under field conditions, we evaluated NDVI and canopy temperature (at 9 a.m.) on 5 Feb. 2014, a period corresponding to the most severe water deficit (Fig. 6b). The less drought-sensitive cultivar (Embrapa 48) had higher NDVI values relative to BR 16 plants under DSR (Fig. 9a)—a condition in which both cultivars were under water stress according to the canopy temperature (Fig. 9b). Although a small difference related to the phenological stage was detected under such condition (R5.2 for Embrapa 48 and R5.4 for BR 16), both cultivars had statistically similar LAI values during NDVI measurements (Fig. 9c) under DSR. Interestingly, under DSR the cultivar Embrapa 48 presented a higher yield than BR 16 plants (Fig. 9d). Since the crop season 2013/14 was

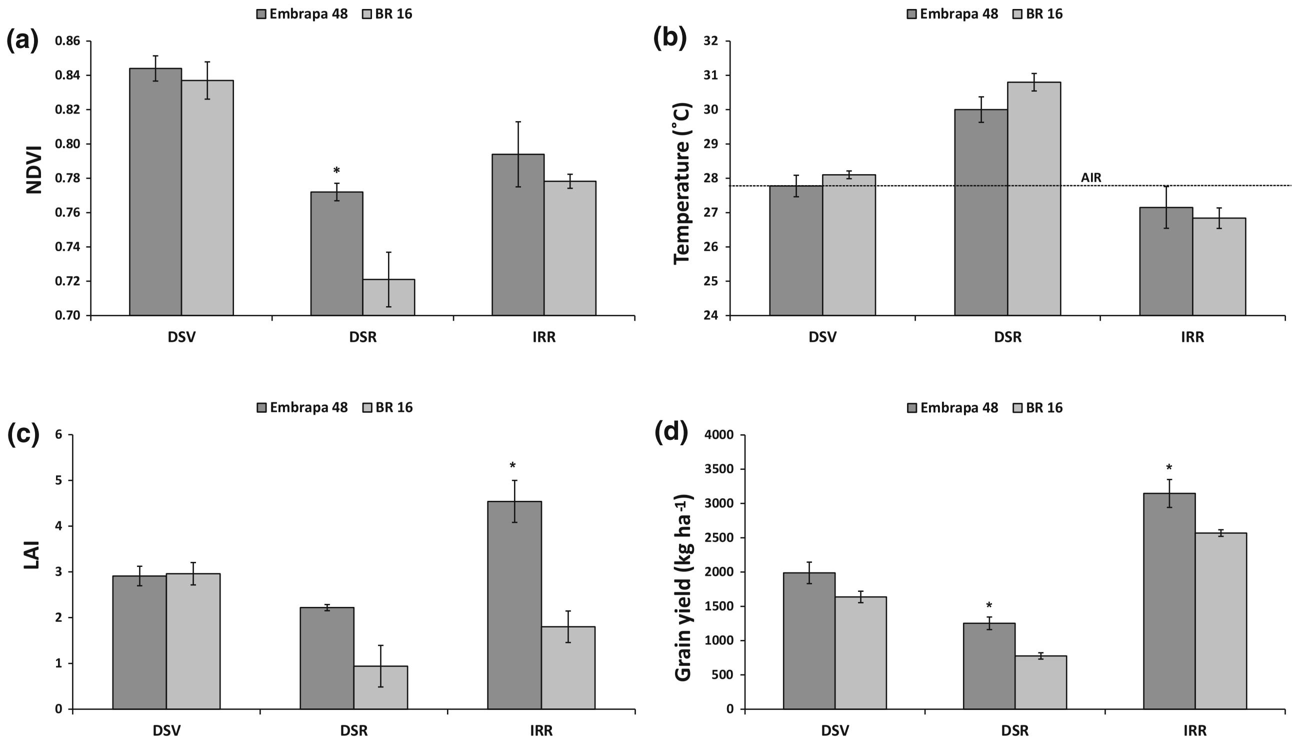



**Fig. 9** NDVI, canopy temperature and LAI of cultivars BR 16 and Embrapa 48 in the stages R5.4 and R5.2, respectively, and grain yield during the 2013/14 crop season under water stress in the vegetative period (DSV), water stress in the reproductive period (DSR) and under continuous irrigation (IRR). In each water condition, mean ± standard error indicated by *asterisks* are significantly higher by Tukey's test ($p \leq 0.05$). n = 4

characterized by a severe water deficit that occurred during the reproductive period, the best performance of the cultivar Embrapa 48 related to NDVI readings and yield under DSR compared to those showed by the cultivar BR 16 could be related to the differential response of both genotypes to water deficit.

## Conclusions

As we have mentioned, there is no consensus in reports involving the use of portable sensors for NDVI evaluation with regard to sampling area, height of sensor positioning in relation to the canopy top, and time of measuring. In this sense, papers have studied different crops and, not necessarily, the same evaluations have been made. Thus, the purpose of this manuscript was to present a set of NDVI measurements under different experimental conditions in order to standardize the methodology for NDVI evaluation in soybean cultivars submitted to water deficit. Our team has been making these evaluations over the last few years. Thus, our experiments involving different soybean cultivars in greenhouse and field trials during three consecutive crop seasons indicated that, regarding time of measurement, 9 a.m. showed to be the most suitable time for NDVI readings in well-watered plants regardless of soybean cultivar, spatial arrangement or environment (greenhouse or field). Moreover, 9 a.m. also showed to be a suitable time for NDVI readings by using a portable sensor to differentiate soybean cultivars with contrasting responses to drought induced under field conditions. With respect to sampling size, there was no difference among NDVI readings regardless of cultivar, which is important mainly when NDVI needs to be evaluated in rows in which plants are very tall or branched, so that readings could be taken with no need of walking along the row, thus preventing plant injuries. With respect to the positioning of the sensor, NDVI values tended to decrease with the increase in height of the sensor. Considering the range of height recommended by the manufacturer of GreenSeeker® 505 Handheld Sensor (0.8–1.2 m), NDVI values tended to be higher at 0.8 m, but with no significant difference from 1.0 m, the height that we adopted in our experiments. Thus, our procedures are within the manufacturer's recommendation. Regarding water regimes, when cultivars showing differential response to drought were subjected to water deficit in the reproductive stage under field conditions, the NDVI of cultivar Embrapa 48 (less drought-sensitive) was higher than that of cultivar BR 16 (more drought-sensitive), thus indicating that NDVI can be used to differentiate soybean cultivars with contrasting responses to drought. We recommend further studies in other soybean cultivars and other important crops under well-watered conditions and submitted to water deficit in the field in different sites in order to improve the precision regarding NDVI evaluation using portable sensors.

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