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ORIGINAL PAPER

Water Stress Response of Conventional and Transgenic Soybean Plants Monitored by Chlorophyll *a* Fluorescence

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Abstract Two soybean cultivars, one conventional and a glyphosate-tolerant (transgenic), were submitted to the water stress and the chlorophyll *a* fluorescence induced by UV light was monitored daily during 16 days. In this work, 40 pots in total, 20 per cultivar were used in the investigation. Each cultivar was divided in two groups, the control group and the group submitted to the water stress. The stress response of the cultivars was monitored by red to far-red fluorescence ratio. The data indicate that the water stress induced the earliest changes on the fluorescence ratio and chlorophyll content for the conventional cultivar. In addition, a comparative analysis of the fluorescence ratios of the cultivars reveals that conventional plants have higher chlorophyll content than transgenic ones. This result might be useful in the development of methodologies able to distinguish conventional to transgenic apart.

Keywords Fluorescence Spectroscopy · *Glycine max* · Water stress

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Introduction

Photosynthetic organisms such as plants make use of light energy to produce carbohydrate (glucose). Equation (1) shows that in a photosynthetic process the carbon dioxide and water are used to produce glucose and oxygen. In this process of interaction between light and plant, chlorophylls and carotenoids are responsible for light absorption, and then for providing chemical energy to the plant. Chlorophyll *a* (Chl*a*) plays a main role in this process, while chlorophyll *b* and carotenoids do not participate directly in photosynthetic reactions since they are only able to transfer their absorbed energy from sunlight to Chl*a* in vivo. The upper left inset in Fig. 1 shows that the basic structure of the Chl*a* molecule is a porphyrin ring, with magnesium atom at its center, attached to a long saturated hydrophobic carbohydrate chain.

$$6CO_2 + 6H_2O \xrightarrow[\text{Chlorophyll}]{\text{Sunlight}} C_6H_{12}O_6 + 6O_2 \qquad (1)$$

The main features of the absorption spectrum of Chl*a* are the $Q_{x,y}$ and Soret bands which are placed in the spectral range of 600–720 nm and 400–450 nm, respectively [1]. The absorption of ultraviolet-light by green leaves can originate blue/green fluorescence (400–600 nm range) as well as red/far-red fluorescence (600–800 nm). The blue/green fluorescence is associated to several leaf fluorophores such as hydroxycinnamic acids, flavonols, isoflavones, flavanones and phenolic acids, while, in vivo, the red/far-red fluorescence is produced solely by Chl*a* [2].

Several studies report that fluorescence induced by ultraviolet (UV) light possesses a large potential to assess accurately the physiological state of plants and to detect

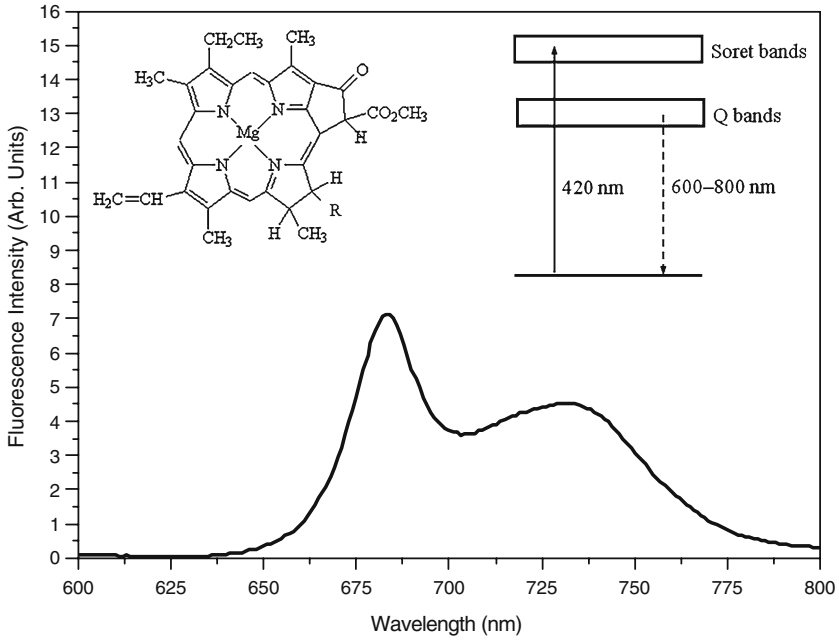

Fig. 1 Typical fluorescence spectrum of Soybean plants in the 600–800 nm range induced by UV-light. The insets show the molecular structure of Chlorophyll *a* with R = $CH_2CH_2CO_2CH_2CH{=}C(CH_2CH_2CH_2CH)_3CH_3$ and its energy-level diagram

precociously the impacts of environmental stress on them [2–4]. Furthermore, methods based on optical spectroscopy are very valuable for the study of plants because it provides a noncontact and nondestructive method as well as the possibility of a remote investigation in real-time [5]. In this context, Chl*a* fluorescence has been used as an accurate and non-destructive probe of photosynthetic efficiency that can directly or indirectly reflect the impacts of environmental factors and changes on the physiological state of plants. The photosynthetic efficiency of many plants decreases when they are subjected to stress conditions [6, 7]. Therefore, the Chl*a* fluorescence has been used as a standard method to investigate the chlorophyll content in plants, identify plant class, and detect plant stresses caused by nutrient deficiency, polluting agents, etc. [5]. In a recent paper Ohashi et al. have reported changes in stem diameter, photosynthetic gas exchange, and chlorophyll fluorescence in soybean plants submitted to water stress [8]. In this work, the Chl*a* fluorescence was used as a probe to evaluate the chlorophyll content as well as the water stress response in two cultivars of soybean plants, one conventional and other transgenic.

Materials and methods

Two soybean cultivars were used in this study, a conventional cultivar, BRS 133, and an herbicide tolerant, namely BRS 245RR. The soybean cultivar BRS 245RR was originated through the insertion of the cp4-epsps gene which confers tolerance to the non-selective herbicide glyphosate [9]. BRS 245RR was developed by EMBRAPA from six backcrosses with the BRS 133 cultivar. Under this breeding scheme the two cultivars have a 99,21% of genetic similarity [10].

Seeds of both soybean [(Glycine max L.) Merr.] cultivars were sown in 20 cm-diameter-plastic pots containing 2,500 cc of a Rhodic Eutrudox soil. The soil from each pot was previously fertilized with 25 g of a 0-20-20 NPK commercial fertilizer formulation. Soybean seeds were treated with the fungicide Derosal Plus® (carbendazim and thiram—200 mL commercial formulation 100 kg^{-1} seeds). Six soybean seeds were sowed per pot. Each cultivar was sowed in 20 pots and separated in two groups of 10 pots. Two weeks after germination, three plants were left in each pot. Pots were watered with a volume of tap water to maintain the soil at 100% of field capacity. This procedure was adopted until soybeans reached the V2 phenological stage [11]. At that stage ten pots of each soybean cultivar were not watered and the remaining two groups still received water as cited above. Hereafter, they will be called watered and non-watered group, respectively. It is worth to point out that both cultivars remained in the same environment during all experiments. Plants of the two groups, watered and non-watered, from both cultivars were evaluated daily during 16 days because after day 16 most of plants started to lose their leaves. Evaluations consisted of recording the

fluorescence emission spectra using a fluorescence spectrophotometer (Cary Eclipe—Varian). The excitation light at 420 nm was used to promote the Chl*a* molecules to Soret band in the 400–450 nm range and the fluorescence was collected in the 600–800 nm range. The energy levels of the Chl*a* are shown in the upper right inset in Fig. 1. The fluorescence spectra of the in vivo leaves were collected using an optical fiber linked to the spectrophotometer. All measurements were carried out at room temperature.

Results and discussion

Figure 1 shows a typical fluorescence spectrum collected for soybean plants when UV-light at 420 nm was used to excite the leaves. Two emission bands were observed between 600 and 800 nm, the red and far-red bands with the maximum at 683 and 733 nm, respectively. These fluorescence bands are associated to the Chl*a* molecule because it is the unique molecule present in the green leaves of plants able to emit in that spectral range during in vivo analyses when excited by UV-light [2].

Similar Chl*a* fluorescence spectrum profiles were obtained from both watered soybean cultivars. Nevertheless, the herbicide-tolerant cultivar presented a higher fluorescence signal than the conventional one throughout the experiment (data not shown). Several papers have reported that the red to far-red fluorescence ratio is a useful probe to evaluate the chlorophyll content of the leaf in many plant species because as higher this ratio the lower the concentration of chlorophyll [2]. In fact, the red to far-red ratio depends on the chlorophyll content of the leaf owing to the selective re-absorption of red relative to far-red fluorescence by Chl molecules [2, 12]. In those reports the ratio is calculated on the basis of the relationship between the peak fluorescence intensity at 683 to 733 nm, namely F683/F733 ratio. Figure 2 shows the F683/F733 ratio for the two watered cultivars. Based on that, it is plausible to state that the transgenic cultivar has lower chlorophyll content in the leaves than the conventional soybean cultivar.

The influence of the water stress on fluorescence spectrum profile and fluorescence intensity of the conventional cultivar is shown in Fig. 3. In that figure each spectrum was obtained from the average over all plants of the non-watered group and its respective watered group. There was no alteration in the fluorescence band of the watered and non-watered groups in the day immediately after irrigation was suspended. On the other hand, 8 days later there was a slight difference in fluorescence intensity of the watered and non-watered conventional cultivar. In the sixteenth day a remarkable divergence was observed in the fluorescence spectrum profiles and intensities of the non-and watered groups. Besides, it is possible to observe an increase of the fluorescence intensity around 600 nm which has been associated to the decrease of the concentration of chlorophyll in the leaves [13–16]. Analogous behaviors were also observed for the non-watered and watered groups of the herbicide-tolerant cultivar, despite of the difference in the spectrum profiles has become noticeable only after 12 days.

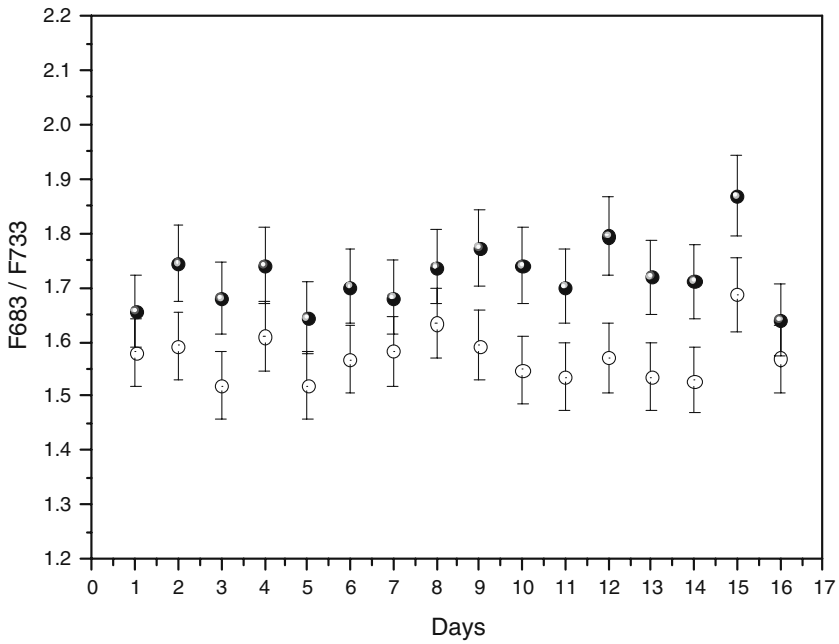

Fig. 2 Fluorescence ratio of watered plants: (○) conventional and (●) herbicide—tolerant

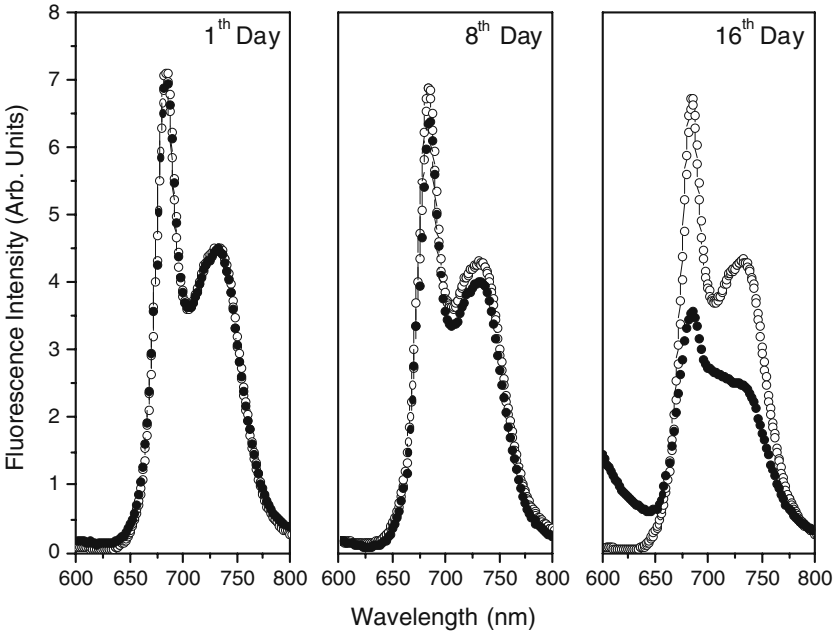

Fig. 3 Fluorescence spectra of the conventional cultivar as a function of time: (○) watered group and (●) non-watered group. The "1th day" means the first day that the plants were submitted to the water stress

The influence of the water stress on the conventional and herbicide-tolerant cultivars can be evaluated by means of the F683/F733 ratio as shown in Fig. 4. While the ratios associated to the watered groups remained almost constant, the ratio related to the two soybean cultivars exhibit a different response to the water stress. Initially the ratio related to the plants submitted to the water stress remained constant and then started to increase gradually. Eight days after the water stress was imposed to the conventional soybean plants an increase of the F683/F733 ratio was detected, whereas for the herbicide-tolerant cultivar this trend was observed only 5 days later to the appearance of this increase in the conventional cultivar.

The contrast in time response of the chlorophyll content change might be associated to a different dynamic of stomatal closure of the cultivars because the dynamics of opening and closing of stomata could be diverse between cultivars of the same species [17]. Stomatal apertures regulate the water content in plants in a way, for instance, to minimize the water loss when the plant is submitted to a

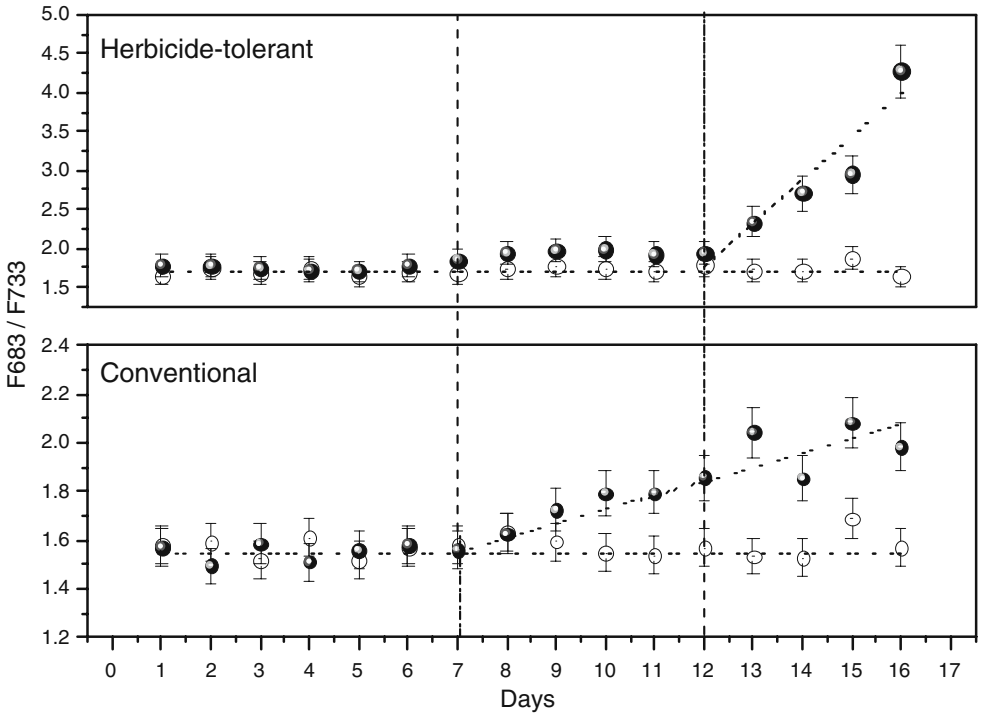

Fig. 4 Fluorescence ratio of the cultivars as a function of the time: (○) watered group and (●) non-watered group. The day 1 means the first day that the plants were submitted to the water stress

water stress condition [17]. In the present case, the longer time period the chlorophyll content of the transgenic cultivar remained unchanged as compared with conventional plants may be explained by the higher rate of stomatal closing of transgenic plants than conventional plants.

Despite of the fact that the herbicide-tolerant cultivar presented a later response to water deficiency as compared to the conventional plant, it showed a steeper increase in the F683/F733 ratio than one verified in the conventional cultivar after the deterioration process started. In other words, a faster reduction of the Chl content in the leaves of the glyphosate-tolerant plants was observed in the time period after initiated the degradation of the leaves when compared to the conventional cultivar. This different response could be associated with the chlorophyll content that depends on the cultivar.

Conclusions

The results reveal the transgenic soybean cultivar BRS 245RR presented a lower chlorophyll content than conventional near-isogenic cultivar BRS 133 since it showed a higher F683/F733 ratio than conventional ones. Therefore, F683/F733 ratio might be useful in the development of a method to distinguish these two cultivars. The data also show that the water stress was responsible for changes in the F683/F733 ratio and chlorophyll content for both cultivars. Additionally, it was possible to detect a different response to the water stress by the conventional plants in relation to transgenic ones. In transgenic plants the chlorophyll content remained almost unchanged during the first 12 days. On the other hand, the chlorophyll content started to decrease for the conventional cultivar after 1 week. One possible explanation for such difference could be associated with a different dynamic of stomatal closure. Besides, the experiment showed that the transgenic plants presented more abrupt chlorophyll content changes after initiated the degradation process. In summary, this work reveals that genetic modification, which confers to the soybean plants resistance to glyphosate herbicide, gives to transgenic plants a different response to the water stress as well as lower chlorophyll content when compared to the conventional ones.

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