Original Article

PEROXIDASE ACTIVITY AS AN INDICATOR OF WATER DEFICIT TOLERANCE IN SOYBEAN CULTIVARS

ATIVIDADE DE PEROXIDASE COMO INDICATIVO DE TOLERÂNCIA À DEFICIÊNCIA HÍDRICA EM CULTIVARES DE SOJA

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ABSTRACT: This study had as purpose to test the hypothesis that peroxidase enzyme activity could be used as an indicator of tolerance to water deficit in soybean cultivars. The experiment was carried out in pots containing 20-L of a Rhodic Hapludox soil in greenhouse conditions. Treatments were arranged in a randomized block design in a 2 × 4 factorial: two water regimes (with and without water deficit) and four soybean cultivars (CD 204, CD 215, CD 225RR and CD 226RR), with four replications. Soybean plants were grown until the beginning of flowering (R1-R2) with soil moisture content near at the field capacity, and then, it started the differentiation of treatments under water deficit by the suspension of water supply. Changes in relative water content (RWC) in leaves, protoplasmic tolerance, peroxidase activity, and plant growth were measured after four days of drought stress and 4-d recovery. The soybean cultivars used in this study have different responses under water deficit conditions. The CD 215 cultivar was the most sensitive to water deficit in the beginning of flowering stage. Soybean cultivars presented variable responses to the peroxidase enzyme activity, and this variable can be used in the selection of soybean genotypes with greater tolerance to drought.

KEYWORDS: *Glycine max*. Free radicals. Plasma membrane. Antioxidants.

INTRODUCTION

Soybean [*Glycine max* L. (Merrill)] is one of the most importance crops in Brazil, and has been cultivated in all national territory. However, the drought stress is a common environmental factor that constrains soybean plants to express their ecophysiological potential, causing short and long term effects in different hierarchical levels, from biochemical to morphological ones, and affecting crop yield (KRON et al., 2008; SOUZA; CARDOSO, 2003). Water deficit leads invariably to a decrease in photosynthetic rate, leaf area, transpiration and growth rate (KRON et al., 2008; CHAVES et al., 2002), as well as changes of key enzyme activities involved in carbon and nitrogen metabolism and changes of antioxidants levels (GUNES et al., 2008; FLEXAS et al., 2006; GONG et al., 2005). Some of these responses are part of strategies that aimed to reduce the deleterious effects of low water availability of soil, constituting, so, mechanisms of drought tolerance.

The water deficit can lead to the accumulation of chemically active molecules and free radicals in plant cells (i.e., oxidative stress), capable of changing the plant metabolic processes. According to Roy-Macauley et al. (1992), as the severity of water deficit increases, the metabolic processes at the cellular level are strongly affected. At this point, begins the enzyme activity of lysis that results in rupture and increased of membranes permeability and disruption of organelles and molecules present inside the cells. However, plants possess sufficient stability, thanks to their highly active antioxidant system, which inhibits free radical processes (CAVALCANTI et al., 2005). It is shown that under extreme conditions the protective mechanism of antioxidant system is activated. The higher is the antioxidant activity, the more resistant is the species toward the stressor (SMIRNOFF, 1998).

The protective antioxidant system consists of high and low-molecular substances. Enzymes peroxidases are high-molecular, which are capable of eliminating the hydrogen peroxide (H_2O_2) formed during nonenzymatic or enzymatic dismutation (CAVALCANTI et al., 2005). These enzymes are the most important in the elimination of H_2O_2 in the cytosol and chloroplast (INZÉ; MONTAGU, 1995). Changes in peroxidase activity have been frequently correlated to the response of resistance or susceptibility of plants to biotic stresses, such as disease and pest (CAVALCANTI et al., 2005; PEITER-BENINCA et al., 2008). Thus, based on

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this assumption, the peroxidase activity can be a useful tool in the selection cultivars resistant to environmental such as the water deficit; however, there is no study that proves this inference.

This study had as purpose to test the hypothesis that peroxidase enzyme activity could be used as an indicator of tolerance to water deficit in soybean cultivars.

MATERIAL AND METHODS

Pot experiments were carried out in a greenhouse in Marechal Cândido Rondon, Paraná, Brazil (24º31'S, 54º01'W, and 420 m a.s.l.), where the environmental conditions were: minimum and maximum mean air temperature of 16 and 34ºC, respectively; mean air relative humidity of 65%. The soil used in the experiment was collected from the plough layer of a clayey Rhodic Hapludox (Eutroferric Red Latosol in the Brazilian classification) with 830 g kg^{-1} of clay, 100 g kg^{-1} of silt, and 70 g kg^{-1} of sand. The soil had the following properties: pH (1:2.5 soil/$CaCl_2$ suspension 0.01M) 5.4, 25 g dm^{-3} organic matter, 20 mg dm^{-3} P (Mehlich-1), 49 $mmol_c$ dm^{-3} Ca, 6 $mmol_c$ dm^{-3} Mg, 3.7 $mmol_c$ dm^{-3} K, 65 $mmol_c$ dm^{-3} H+Al, 123 $mmol_c$ dm^{-3} CEC, 48% of base saturation. All the soil chemical properties were analyzed according to Lana et al. (2010). The field capacity of the soil sample under free draining was measured at –0.02 MPa using the Richards extractor (Embrapa, 1997) and the value obtained was 350 g kg^{-1}.

Lime (25% CaO, 12% MgO and ECC 90%) was applied before the experiment to raise base saturation up to 70%, according to Raij et al. (1997). The limed soil was kept in plastic bags for 20 days with water content at field capacity. Then, the soil was fertilized with applying 20 mg kg^{-1} of N (urea), 30 mg kg^{-1} of P (triple superphosphate), 20 mg kg^{-1} of K (potassium chloride), and transferred to 20-L plastic pots.

The experimental design was a 2 × 4 factorial in complete randomized blocks, with four replications. The treatments consisted of two water regimes (with and without water deficit) and four soybean cultivars (CD 204, CD 215, CD 225RR and CD 226RR).

Five soybean seeds were sown in each 20-L pot, and six days after emergence, seedlings were thinned down to two per pot. The soil surface was covered with a layer about 20 mm of straw, with the goal of reducing water loss through evaporation and avoids high temperatures. Until the beginning of flowering (R1-R2), the soil water content was monitored daily and maintained near at the field capacity (–0.02 MPa). Posteriorly, it started the differentiation of treatments under water deficit by the suspension of water supply. The treatments of water deficit remained without water replacement until the relative water content in leaves reached nearly 40%, that due the environmental conditions, occurred four days after imposition of water deficit. Then, was performed the rehydration of treatments in water deficit, being then, the whole experiment conducted without water deficiency (well watered) until physiological maturity of the crop.

During the period of exposure to water deficit and recovery of the plants was determined the leaf relative water content (RWC) and soil moisture content (SMC). The RWC indicates the water content (i.e., water status of plant) of a given amount of leaf relative to its fully hydrated or fully turgid state. Leaf discs (diameter 10 mm) were collected at 5 am from two plants per treatment and processed according to Barrs (1968). The RWC was determined using the following equation: RWC = [(FW – DW)/(TW –DW)], where FW, DW and TW are fresh, dry and turgid weight, respectively. Soil moisture content (SMC, g $kg^{–1}$) was determined by gravimetric method as described by Embrapa (1997).

Protoplasmic tolerance was assessed after four days of drought stress and 4-d recovery as described by Leopold al. (1981), with modifications. Twenty leaf discs (diameter 5 mm) were placed in closed tubes containing 30 mL of deionized water; subsequently the electrical conductivity of solution in the 1st and 24th hour was measured. Samples were then boiled at 100 °C for 1 h, and the last electrical conductivity was obtained after equilibration at 25 °C. Measuring in the first hour and after boiled was considered the free conductivity (FC) and total conductivity (TC), respectively. From these data were calculated the percentage absolute integrity of cell membrane [PAI (%) = 1 – FC/TC], the percentage relative integrity of cell membrane [PRI (%) = PAI without water deficit/PAI with water deficit], and percentage of membrane damage (MD (%) = 100 – PRI) (VASQUEZ-TELLO et al., 1990).

Peroxidase activity was also determined after 7-d of drought stress and 3-d recovery. Were collected the central leaves of the trifoliate from the middle third of plants. The collected material (about 2.0 g) was weighed and macerated in 4 mL of phosphate buffer 0.01 M (pH 6.0) at 4 °C. The homogenate was centrifuged at 6,000 rpm for 20 minutes and the supernatant collected. In glass bucket with capacity of 3.0 mL, were added 2.9 mL of reaction buffer (153 µL H_2O_2 + 6.25 mL of guaiacol (2%) in 50 mL of extraction buffer) and 0.1 mL of supernatant. The reaction was followed by spectrophotometer at 470 nm for 2 minutes (HAMMER-SCHMIDT et al., 1982). The unit of peroxidase activity was defined with the increase of one unit of absorbance per min^{-1} mg^{-1} of fresh matter.

After 7-d of exposure to water deficit, in one pot of each treatment, the crop yield was evaluated in terms of dry matter production of shoots (SDM, g $plant^{-1}$) and roots (RDM, g $plant^{-1}$). Plants of all treatments were harvested separately, dried for three days at 60 ± 2 ºC, and then weighed. The leaf area (LA, cm^2 $plant^{-1}$) was determined using the following equation proposed by Benincasa (2003): LA = [(LAs × TLDM)/DMs], where LAs is the leaf area of the sample collected, TLDM is the total leaf dry matter and DMs is the dry matter of the sample collected. At physiological maturity of soybean (R8), in the other pot of each treatment, the number of pods per plant (PP), grains per pod (GP), thousand grains weight (TGW, g) and grain yield (GY, g $plant^{-1}$) was measured.

Data were subjected to analysis of variance (ANOVA) and the means of water regimes and soybean cultivars were compared by F test and Tukey test, respectively. For all statistical tests, a P value of 0.05 or less was considered significant. All analyses were performed using Sisvar software for Windows (version 5.1, Statistical Analysis Software, UFLA, Lavras, MG).

RESULTS AND DISCUSSION

The soil moisture content (SMC) remained constant in the treatments under normal irrigation management, varying from 330 to 360 g kg^{-1} (Figure 1A). In treatments with water deficit, the desiccation of the soil occurred rapidly, being that the SMC passed from 330 g kg^{-1} on the first day to 200 g kg^{-1} on the second day, due to conditions of high temperature and low relative humidity of the air increasing the soil water withdrawal by plant. After the second day of water deficit the SMC remained constant until the fourth day when the rehydration occurred. As the soil dries, it becomes more difficult for plants to absorb water, because retention force increases and the water availability in soil to plants decreases (SANTOS; CARLESSO, 1998).

Water supply (rehydration) in the fourth day after the imposition of water deficit provided increase of SMC to values higher than the treatments under normal irrigation management (Figure 1A). This difference in SMC after rehydration occurred as a result of the plants with normal irrigation are more efficient in water uptake due to greater leaf area, and consequently resulted in higher evapotranspiration demand. In turn, the plants under water deficit absorb water with less efficiently, due to lower leaf area, which is one of the changes suffered by plants under water deficit conditions.

The relative water content (RWC) in leaves of soybean decreased with the passing of the days of water deficit (Figure 1B), reaching the lowest value on the fourth day after the beginning of water deficit. On the third day after rehydration, the RWC of treatments with deficit was similar to maintained with normal irrigation. The maintenance of cells turgor in water deficit conditions enables a rapid recovery of the plant in the case of rehydration and can give continuity to the processes affected in a short period (CHAVES et al., 2002).

The soybean cultivar presents RWC different on the second day of water deficit (Figure 1B). The cultivar CD 215 presented CRA lowers than the other cultivars, indicating that this cultivar has a greater susceptibility to water deficit. This difference between the cultivars may be related with some intrinsic characteristics in tolerate to water deficit. Among the characteristics, can be cited the stomatal density, the efficiency in controlling water loss through the stomata and cuticle thickness in the leaf. The control of water loss through the stomata when the plant suffers water deficit is essential for maintenance of cell metabolism. The rapid drying of the soil, as this study, even that does not affect plant water relations, causes an increase of abscisic acid (ABA) concentration in xylem, leading to stomatal closure and to reduction in cell expansion (CHAVES et al., 2002). Besides, the low amount of epicuticular wax, as occurs in plants grown in a vegetation house is one of the main factors for reducing the RWC in plants. After the third day of exposure to water deficit, was not possible to see a difference in RWC among the cultivars possibly due to the major severity of water deficit imposed to plants.

The free conductivity (FC) increased when plants were submitted to water deficit (Table 1). In water deficit conditions there is an increase in the electrolyte leakage in plant cells (PIMENTEL et al., 2002). The soybean cultivars showed FC similar under normal irrigation management (Table 1). However, in water deficit the cultivars differed significantly, being that the cultivar CD 215 showed the highest free conductivity. This difference can be explained trough of analysis of Figure 2, where the cultivar CD 215 showed the lowest peroxidase enzyme activity, which is responsible for eliminating hydrogen peroxide from imbalance in redox reactions of cell promoted by the induction of water deficit. This probably caused more damage to the membrane, what justifies the lower percentage absolute integrity (PAI) evidenced in this cultivar (Table 1).

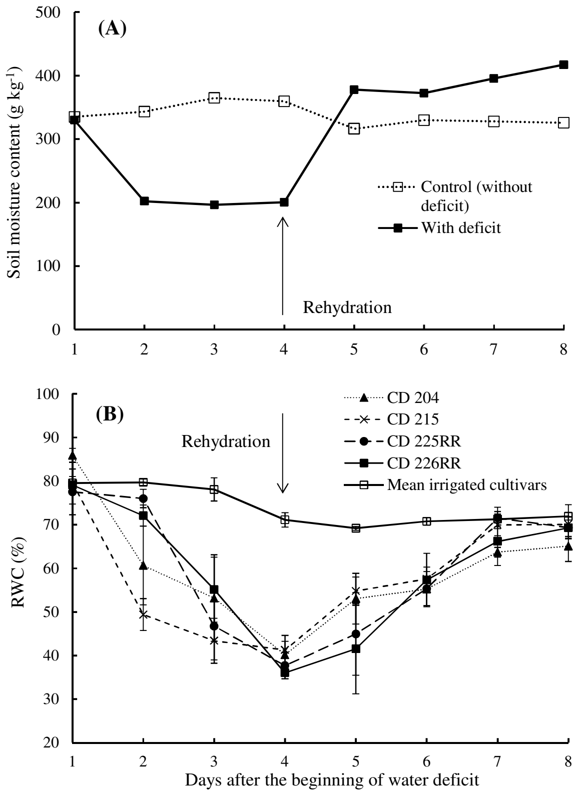

Figure 1. Soil moisture content (SMC, in A) and leaf relative water content (RWC, in B) in pre-down during the 4 days of exposure to water deficit and 4-d recovery of the soybean plants under control conditions. Vertical bars represent standard deviation of the mean (n = 4).

Table 1. Free conductivity (FC), total conductivity (TC), percentage absolute integrity (PAI), percentage relative integrity (PRI), and membrane damage (MD) in different soybean cultivars after 4 days of exposure to water deficit and after 4-d of recovery under control conditions.

Cultivar	FC		TC		PAI		PRI	PD
	Water deficit							
	Control	Deficit	Control	Deficit	Control	Deficit		
CD 204	3.9 Ba	13.4 Aab	51.0 Aa	30.3 Ba	0.93 Aa	0.56 Bab	1.65 a	98.2 a
CD 215	4.2 Ba	16.4 Aa	50.7 Aa	36.2 Ba	0.91 Aa	0.53 Bb	1.81 a	98.3 a
CD 225RR	3.0 Ba	12.3 Aab	36.6 Ab	27.6 Ba	0.92 Aa	0.55 Bab	1.69 a	98.3 a
CD 226RR	4.6 Aa	8.4 Ab	43.5 Aab	26.8 Ba	0.89 Aa	0.69 Ba	1.34 a	98.7 a
CV (%)	34.4		15.4		10.0		16.0	0.3
	Recovery 4 days							
	Control	Deficit	Control	Deficit	Control	Deficit		
CD 204	5.8 Aa	12.0 Aab	42.4 Ab	34.1 Aa	0.86 Aa	0.68 Aab	1.35 a	98.7 a
CD 215	5.2 Ba	18.4 Aa	57.4 Aa	48.4 Aa	0.91 Aa	0.59 Bb	1.70 a	98.3 a
CD 225RR	5.2 Aa	6.7 Ab	38.0 Ab	45.8 Aa	0.87 Aa	0.86 Aa	1.01 a	99.0 a
CD 226RR	5.1 Aa	7.3 Ab	47.5 Aab	40.8 Aa	0.89 Aa	0.82 Aab	1.13 a	98.9 a
CV (%)	68.1		16.6		15.4		31.3	0.4

Values represented by the same lower case letters, between the soybean cultivars and same upper case letters, into of each water regimes are not different according to the Tukey test and F test, both at the 0.05 level of confidence. C.V.: coefficient of variation.

Cultivar CD 226RR that showed lower conductivity presented higher peroxidase activity (Figure 2), indicating that this cultivar catalyzed the transfer of electrons to H_2O_2, forming $2H_2O$, maintaining so the integrity of the membrane, reflecting in higher value of PAI (Table 1). However on the fourth day of recovery, there wasn't significant difference for FC between the water regimes, except for the cultivar CD 215 (Table 1) which showed higher FC when submitted to water deficit. The bigger FC of cultivar CD 215 in conditions of water deficit can be attributed to the lower value of PAI (Table 1). These results evidence that there wasn't recovery of this cultivar, showing smaller tolerance to water deficit. The other cultivars presented similar values of FC between the water regimes, demonstrating the capacity of recovery of the plasma membrane of these soybean cultivars after passing for a period of water deficit.

The total conductivity (TC) differed among water regimes for all cultivars (Table 1). The plants conducted under normal irrigation management presented TC values greater than those plants subjected to water deficit, probably due to the higher production of photoassimilates by the plants well watered. The differences between the photosynthetic rates of stressed plants, and not stressed, can be attributed directly to damage of water deficit on photosynthesis (KRON et al., 2008; CHAVES et al., 2008). The TC of cultivars CD 204 and CD 215 was higher than other cultivars in the normal irrigation management. This occurred due to the difference between the photosynthetic rates, being this particularity of each cultivar. In the fourth day of plant recovery the TC was similar between the water regimes (Table 1). Showing the recovery of plants to water deficit occurred and the resumption of the photosynthesis process and, consequently, the accumulation of photoassimilates in the cells.

The peroxidase activity after 4 days of exposure to water deficit did not vary among cultivars subjected to water deficit (Figure 2A). But, the cultivars maintained in the normal irrigation management showed variation being that the cultivar CD 204 showed the highest peroxidase enzyme activity. Xiong et al. (2002) reported that in plants under water deficit, there is an increase in peroxidase activity that correlated with the increased to water deficit tolerance. In this study, this increase in peroxidase activity under water deficit was only evident for the cultivars CD 225RR and CD226RR (Figure 2A), indicating greater tolerance of these cultivars to water deficit.

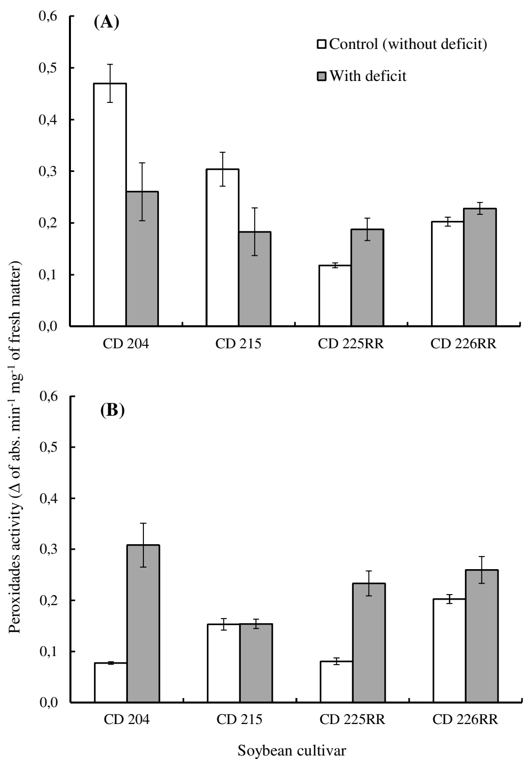

Figure 2. Peroxidase activity in different soybean cultivars after 4 days of exposure to water deficit (A) and after 4-d of recovery under control conditions (B). Vertical bars represents mean values (n = 4) ± standard error.

After 4 days of plant recovery under control conditions, there was significant difference of peroxidase activity between cultivars in both water regimes (Figure 2B). Cultivar CD 204 in water deficit showed higher peroxidase activity; however, this cultivar in normal irrigation conditions showed lower activity than the other cultivars. The cultivar CD 215 was the only that did not show difference among water regimes. The other cultivars under water deficit presented higher peroxidase activity than to normal irrigation management, which can be a defense mechanism against free radical formation resulting from water deficit.

The leaf area of plants under water deficit reduced only in the cultivar CD 215 (Table 2). This reduction is a defense mechanism of the plant, reducing water loss through evapotranspiration. The shoot dry matter production decreased with water deficit in all cultivars (Table 2). The root dry matter was not affected by water regimes.

There was interaction between cultivars and water regimes for the components of soybean production (Table 2). Except for the cultivar CD 215, other cultivars presented reduction in the number of pods per plant when subjected to water deficit. This reduction can be attributed to the abortion of flowers when the stress was imposed in the beginning of soybean flowering (R1-R2). The cultivar CD 226RR showed the higher number of pods per plant compared to other cultivars in the normal irrigation management; however, when it imposed water deficit, the cultivar CD 215 showed a higher number of pods per plant. All cultivars showed increments in thousand grain weight when it was imposed the water deficit (Table 2) probably due to reduction that occurred in the number of physiological drains (pods) of plant when it suffered water deficit, making that way happens the translocation of higher photoassimilate amounts to each drain. In water deficit conditions, the cultivar CD 225RR obtained the highest thousand grain weight, possibly because it was the cultivar that showed the highest decrease in the number of pods when under water deficit. These differences were not observed in treatment with normal irrigation.

Table 2. Leaf area (LA), shoot dry matter (SDM) and root dry matter (RDM), number of pods per plant (NPP), grains per pod (GP), thousand grain weight (TGW) and grain yield (GY) of four soybean cultivars under different water regimes

Cultivar	LA (cm^2 plant^{-1})		SDM (g plant^{-1})		RDM (g plant^{-1})	
	Control	Deficit	Control	Deficit	Control	Deficit
CD 204	439 Aab	311 Ba	32.5 Aa	20.5 Ba	18.2 Aa	14.3 Aa
CD 215	345 Ab	250 Ba	29.8 Aa	24.7 Ba	19.4 Aa	14.1 Aa
CD 225RR	442 Aa	279 Ba	32.8 Aa	22.0 Ba	11.8 Aa	7.5 Aa
CD 226RR	363 Aab	231 Ba	28.5 Aa	18.7 Ba	16.5 Aa	11.0 Aa
C.V. (%)	14.3		14.2		38.4	

Cultivar	NPP (unit)		GP (unit)		TGW (g)		GY (g plant^{-1})	
	Control	Deficit	Control	Deficit	Control	Deficit	Control	Deficit
CD 204	106 Ab	58 Bb	1.81 Ab	1.78 Aab	98 Ba	49,6 Aa	49.6 Aa	38.8 Bab
CD 215	113 Aab	102 Aa	2.09 Aab	2.21 Aa	115 Ba	39,2 Aa	39.2 Aa	32.8 Ab
CD 225RR	111 Aab	49 Bb	2.29 Aa	1.82 Bab	103 Ba	53,7 Aa	53.7 Aa	41.9 Ba
CD 226RR	133 Aa	70 Bb	2.03 Aab	1.44 Bb	101 Ba	51,3 Aa	51.3 Aa	40.6 Bab
C.V. (%)	12.9		12.3		15.2		12.6	

Values represented by the same lower case letters, between the soybean cultivars and same upper case letters, into of each water regimes are not different according to the Tukey test and F test, both at the 0.05 level of confidence. C.V.: coefficient of variation.

The grain yield, except for cultivar CD 215 was less when subjected to water deficit (Table 2). There was a reduction in grain yield in the order of: 21.6, 22.0 and 28.9% for the cultivars CD 204, CD and CD 225RR 226RR, respectively. Cultivars did not differ when conducted under normal irrigation management , however when subjected to water deficit the cultivar CD 225RR was significantly higher than other cultivars, and the cultivar CD 215 was the cultivar that showed the lowest grain yield per plant.

CONCLUSIONS

The soybean cultivars used in this study have different responses under water deficit conditions.

The CD 215 cultivar was the most sensitive to water deficit in the beginning of flowering stage.

Peroxidase enzymes activities can be a parameter used in the selection of soybean genotypes with greater tolerance to drought.

RESUMO: Este estudo teve como objetivo testar a hipótese de que a atividade das enzimas peroxidase poderia ser utilizada como um indicativo de tolerância à deficiência hídrica em cultivares de soja. O experimento foi realizado em vasos contendo 20L de um Latossolo vermelho argiloso em condições de casa-de-vegetação. Os tratamentos foram dispostos no delineamento de blocos casualizados em esquema fatorial 2 × 4, constituído de dois regimes hídricos (sem e com déficit hídrico) e quatro cultivares de soja (CD 204, CD 215, CD 225RR and CD 226RR), com quatro repetições. A soja foi cultivada até o início do florescimento com teor de água no solo próximo a capacidade de campo, e então, iniciou-se a diferenciação dos tratamentos com déficit hídrico, mediante a suspensão do fornecimento de água. Alteração no conteúdo relativo de águas nas folhas, tolerância protoplasmática, atividade das enzimas peroxidade e crescimento das plantas foram avaliados após quatro dias de exposição ao déficit hídrico e após quatro dias de recuperação das plantas em condições controle. As cultivares de soja utilizadas neste estudo apresentam respostas distintas em condições de déficit hídrico. A cultivar CD 215 foi mais sensível ao déficit hídrico imposto no inicio do florescimento. As cultivares de soja apresentaram respostas variáveis à atividade das enzimas peroxidase, e esta variável pode ser utilizada na seleção de genótipos de soja com maior tolerância à seca.

PALAVRAS-CHAVE: *Glycine max*. Radicais livres. Membrana plasmática. Antioxidantes.

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