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Chitosan application in the induction of water deficit tolerance in maize plants

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ABSTRACT. The present research seeks to elucidate the feasibility of chitosan (CHT) in the induction of water deficit tolerance in different maize hybrids, contrasting tolerance to water restriction, tolerance and sensitivity. The maize plants were subjected to water deficit and foliar application of different chitosan doses (60, 100, 140, and 180 mg L^{-1}) at the pre-flowering growth stage and evaluated during the stress period of fifteen days. To understand the induction behaviour of the tolerance to water restriction, biophysical parameters, such as water potential, relative water content and chlorophyll content, gas exchange, and biochemical assays, were quantified based on the activity of SOD, CAT, APX, and PAL antioxidant enzymes, lipid peroxidation activity and hydrogen peroxide content. Among the treatments, maize plants subjected to chitosan foliar application at a dose of 140 mg L^{-1} presented similar behavioural responses to plants under favourable irrigation conditions. Such positive responses are related to the high degree of activity of antioxidant enzymes, gas exchange and low levels of lipid peroxidation and hydrogen peroxide. The results support the potential use of CHT to increase tolerance to water stress.

Keywords: antiperspirant; antioxidant enzymes; water stress; gas exchange.

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Introduction

Maize (*Zea mays* L.) is a crop that grows in temperate to tropical zones during frost-free periods when average temperatures exceed 15°C. To obtain maximum production, maize requires 380 to 550 mm of water, depending on the weather. Off-season maize, which currently represents most of the Brazilian production, was named because of the less favourable cultivation conditions, especially regarding water availability and planting time after harvesting the summer crop. Under environmental conditions that cause water deficit, such as drought, the consequent impacts on productivity depend on the stress duration and intensity and progression rate, as well as genotype, developmental stage and interaction with other stresses (Chaves et al., 2016; Feng, Lindner, Robbins, & Dinneny, 2016). The pre-flowering period is considered the most sensitive and determinant stage of productive potential. Just two days of water stress, during flowering, are sufficient to decrease crop yield by 20%, whereas four to eight days result in a decrease of more than 50% (Magalhães & Durães, 2008).

In view of higher air temperature and lower precipitation, together with the increasingly limited availability of water resources in agricultural areas, it is necessary to adopt alternative strategies aimed at incorporating more knowledge of the biological and climatic factors related to water deficit tolerance. The use of antiperspirant chemicals, acting as a biostimulant, is a strategy that has been actively used since it enables maize plants to tolerate water deficit both in pre-flowering and post-flowering (Katiyar, Hemantaranjan, & Singh, 2015).

Chitosan (CHT), considered a biostimulant, can stimulate physiological responses to water deficit tolerance (Katiyar et al., 2015). The results of the last few decades have indicated that this biopolymer has the potential to be developed as a type of antiperspirant in situations of agricultural stress, inducing drought tolerance through increased defence against oxidative stress, without compromising the agronomic yield.

However, the potential of chitosan goes beyond that. It can reduce the environmental impacts of agricultural activity. Its non-toxic properties have made its use in agriculture due to its biocompatibility, biodegradability and bioactivity (Katiyar et al., 2015). Agriculture has a strong impact on the environment.

In this sense, the use of clean technologies, that can reduce the use of toxic chemicals, is necessary, especially in cash crops such as maize. This necessity makes chitosan a sustainable and clean alternative. Chitosan also induces mechanisms against various biotics stresses, such as fungi, bacteria and insects, and promotes the formation of protective barriers that increase plant productivity (Katiyar et al., 2015).

The present study had the objective of evaluating the performance of two hybrid maize species under drought conditions and subjected to different Chitosan doses to induce water deficit tolerance.

Material and methods

Plant material and growing conditions

Maize hybrids DKB 390 and BRS 1010 (tolerant and sensitive, respectively) to be tested for drought tolerance were grown under greenhouse conditions at Embrapa (Brazilian Agricultural Research Corporation), division of maize and sorghum in Sete Lagoas, Minas Gerais State, Brazil (19°28’ S, 44°15’08” W, 732 m latitude). The mean maximum and minimum temperatures recorded during the evaluation period were 29.2 and 22.5°C, respectively. Relative air humidity ranged from 52 to 78%. Both hybrids were grown in plastic pots containing 20 dm^3 of typical dystrophic Red-Yellow Latosol of fine to medium texture taken from the plateau area.

Soil moisture determination

The water content in the soil was monitored daily in the morning and afternoon (9:00 and 15:00), with the aid of a watermark humidity sensor (model tensiometer) model 200SS - 5 (Irrometer, California, USA) installed in the centre of the vessels of each repetition at a depth of 20 cm. These sensors detect the ground water voltage based on the electrical resistance and were coupled to watermark metres manufactured by the same company. Values range from 0 kPa (fully wet) to -200 kPa (fully dry). The water replacement was performed based on the readings obtained with the sensor and the water was returned to the field capacity (FC) during the period that preceded the application of the treatments. These calculations were performed on a spreadsheet, according to the water retention curve of the soil. In parallel, the crop and phytosanitary treatments needed were carried out according to Souza et al. (2014).

Treatment application

Upon reaching the pre-flowering, maize plants were subjected to the soil water deficit effects. This condition was imposed by the daily supply of 50% of the available water until the water potential in the soil reached approximately 138 kPa (Souza et al., 2014). The experimental design was a randomized complete block design with 14 treatments. The treatments were characterized by the different CHT doses at concentrations of 60, 100, 140, and 180 mg L^{-1} in acidified water, irrigated and water deficit conditions for both hybrids. CHT is characterized by having free aminic groups, solubility in acid solutions and insolubility in pH higher than 6.5 (Katiyar et al., 2015), thus justifying treatment with acidified water. The treatments were applied to both hybrids. For the delivery of CHT doses, a solution was prepared according to (Dzung, Khanh, & Dzung, 2011) by dissolving the CHT in 100 mL of 0.5% acetic acid for 12h. This solution was then diluted to the corresponding concentrations.

The solutions were supplied via foliar application through a costal sprayer, whose spray pressure was obtained using a CO_2 cylinder and controlled by a low pressure manometer at a flow rate of 102 L ha^{-1} and a pressure of 3 BAR. All measures to prevent the contact of the sprayed CHT solutions to neighbouring plants were taken. The treatment application was performed on the first day after exposure to water stress, which lasted 15 days. All evaluations were performed on the first day, the seventh day and at the end of 15 days (1, 7, and 15 DAA). The water supply was then restored and maintained at levels close to that of the field capacity. The experiment was carried out until harvest.

Parameters of water retention, photochemical efficiency and chlorophyll index

The leaf water potential (Ψw) was evaluated using a pressure chamber (model 1000, PMS Instrument Company, Albany OR, USA). The measurements were performed at 12 hours according to the methodology described by (Scholander, Hammel, Hemingsen, & Bradstreet, 1964). For the relative water content (RWC) determination, the methodology described by (Silveira, Araújo, Lima, & Viégas, 2009) was followed. The photosystem II (Fv/Fm) maximum quantum efficiency was determined using the fluorimeter (Plant

Efficiency Analyser, Hans a tech Instruments King's Lynn, UK). The chlorophyll index was evaluated using a SPAD - 502 chlorophyll meter (Minolta Corp, Tramsey, USA). All readings were performed in the morning on the first leaf below the flag leaf.

Gas exchange measurements

The gas exchange measurements were performed using a portable photosynthesis system (IRGA, Model LI-6400, Li-Color, Lincoln, Nebraska, USA) with an integrated fluorescence chamber (LI-6400-40 leaf chamber fluorometer, Li-Cor). All measurements were performed in the morning between 8 and 11 am on a fully expanded leaf (maize ear). The evaluated parameters were leaf photosynthetic rate (Pn), stomatal conductance (gs), respiration (E), CO_2, and intercellular concentration (Ci). The measurements were performed over a leaf area of 2 cm^2, with CO_2 flow controlled by cylinders (Liqueur) of 12 grams at a concentration of 380 μmol mol^{-1}. The photon flow density (PPFD) was 1,500 μmol m^{-2} s^{-1} and leaf temperature was controlled at 26°C.

Extraction and enzymatic activity

Enzyme extraction was performed by macerating 200 mg of leaf in liquid nitrogen and adding 1.5 mL of the extraction buffer consisting of 100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 2 mM DTT, 0.8 mM PMSF, 1% PVPP, and 1 mM ascorbic acid (ASC). The extract was centrifuged at 14,000 rpm for 30 minutes at 4°C, and the supernatant was collected and stored at -80°C in the analysis period. The collected supernatants were used in all enzymatic analyses (Biemelt, Keetman, & Albrecht, 1998).

The quantification of enzymatic activity followed established protocols. Superoxide dismutase (SOD) activity was evaluated by the ability to inhibit nitro blue tetrazolium (NBT) photoreduction (Giannopolitis & Ries, 1977). Catalase (CAT) was determined by H_2O_2 consumption at 240 nm for 3 minutes (Havir & Mchale, 1987). Ascorbate peroxidase (APX) was determined by monitoring the ascorbate oxidation at 290 nm for 3 minutes (Nakano & Asada, 1981). Phenylalanine ammonia lyase (PAL) was determined by the cinnamic acid formation at 290 nm (Zucker, 1965).

Extraction and quantification of hydrogen peroxide content (H_2O_2) and malondialdehyde (MDA)

Samples containing 200 mg of leaf tissue were macerated in liquid nitrogen, supplemented with 20% PVPP, homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged to 10,000 g for 10 minutes at 4°C. The supernatant was used to determine the hydrogen peroxide (H_2O_2) and MDA content. The H_2O_2 content was measured using a spectrophotometer according to (Velikova, Yordanov, & Edreva, 2000). Malondialdehyde content (MDA), which is a lipid peroxidation final product, was used to determine the membrane damage level. The method used was according to (Buege & Aust, 1978).

Agronomic parameters evaluation

At harvest, plant height, ear size and diameter were measured using a millimetre ruler and a slide caliper. We also evaluated ear number per row, grain number per row and final grain weight. Then, the plants were subjected to drying in forced convection oven at 70°C for 72h. The harvest index was estimated based on the total dry biomass ratio (grain dry mass/total plant dry mass)*100 (Magalhães & Durães, 2008).

Data analysis

For all analysed parameters, the means and the standard error (SE) were calculated. For the statistical analysis of the results, the analysis of variance (ANOVA) and the Skott-Knott averages comparison test were performed at a 0.05% significance level ($p \leqslant 0.05$) using Sisvar version 4.3 (Federal University of Lavras, Lavras, Minas Gerais State, Brazil).

Results and discussion

To understand the plant resilience and plasticity response to environmental pressures, it is necessary to understand the cellular and developmental mechanisms that determine the functional consequences on plant metabolism. The maize plants subjected to the CHT 140 mg L^{-1} dose and irrigated showed similar results, having the highest values for all treatments, in all analysed parameters and independent of the hybrid. The observed differences between hybrids are related to their genetic characteristics, which characterize them as tolerant or sensitive to the water limitation.

Irrigated treatments and the CHT application at 140 mg L^{-1} did not show differences over time between the two hybrids. However, for all other treatments there was an effective reduction of water potential (Figure 1). The DKB 390 (tolerant) hybrid presented, in general, a smaller water potential reduction than the BRS 1010 (sensitive) hybrid.

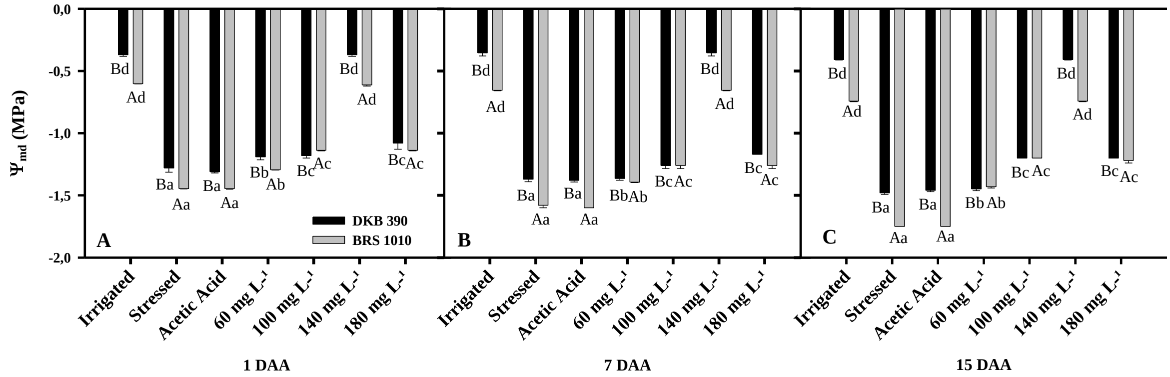

Figure 1. Leaf water potential at noon ψ_{md}, during the stress application of two drought contrasting hybrids (DKB 390 and BRS 1010) subjected to the different treatments. The averages followed by the same letter do not statistically differ from each other. Uppercase letters denote comparisons between genotypes, and lowercase letters denote comparisons between treatments within the same genotype by the Scott-Knott test at a 5% significance level ($p \leqslant 0.05$).

The relative water content (RWC) behaviour for irrigated treatments and CHT 140 mg L^{-1} in both DKB 390 and BRS 1010 did not present differences in the parameters measured during the stress period (Figure 2). However, for all other treatments, there was an effective reduction when compared to 15 days under water stress. On the first day of stress application, the greatest reduction of the relative water content was in the sensitive hybrid (BRS 1010). Throughout the stress days, the stressed BRS 1010 presented a significant reduction which was then followed by the DKB 390.

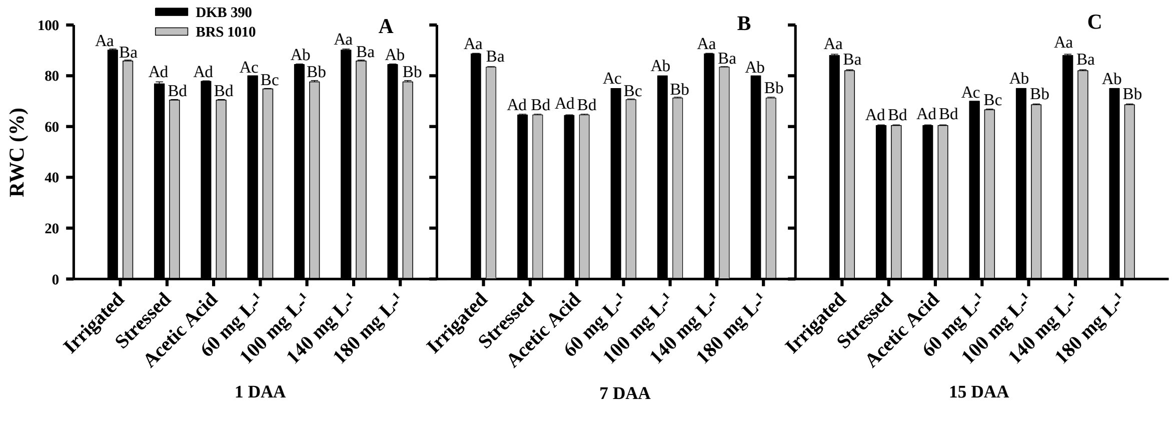

Figure 2. Relative water content (RWC %), during stress application on two drought contrasting hybrids (DKB 390 and BRS 1010) subjected to several CHT treatments. The averages followed by the same letter do not statistically differ from each other. Uppercase letters denote comparisons between genotypes, and lowercase letters denote comparisons between treatments within the same genotype according to the Scott-Knott test at a 5% significance level ($p \leqslant 0.05$).

Foliar application of CHT, especially at 140 mg L^{-1}, had a significant effect on the chlorophyll index in both hybrids compared to treatments under conditions of water deficit and acidified water spray (Figure 3). Over time, a decrease of this index was observed in the treatments, except in the irrigated plants with an application of CHT 140 mg L^{-1}. No differences were found between the hybrids.

The CHT chemical and physical structures are related to their strong bonding (β-1,4) and because it is a relatively water-insoluble polymer. Solubility can be achieved when a pH adjustment occurs by diluting acetic acid. These characteristics are critical in assessing the biological properties of this polymer, as the transfer of insoluble CHT would decrease cellular responses (Hadwiger, 2013). In the present study, it was observed that the CHT dilution in 0.5% acetic acid did not negatively affect the metabolic characteristics of maize plants, regardless of the hybrids analysed.

Studies that involve water stress monitor the soil moisture daily. In the present investigation, it was observed that during the 15 days of water restriction, soil moisture was equal to or less than 50% of the total available water capacity in the soil, resulting in water stress, which can be described as moderate in the early days and severe in the final days.

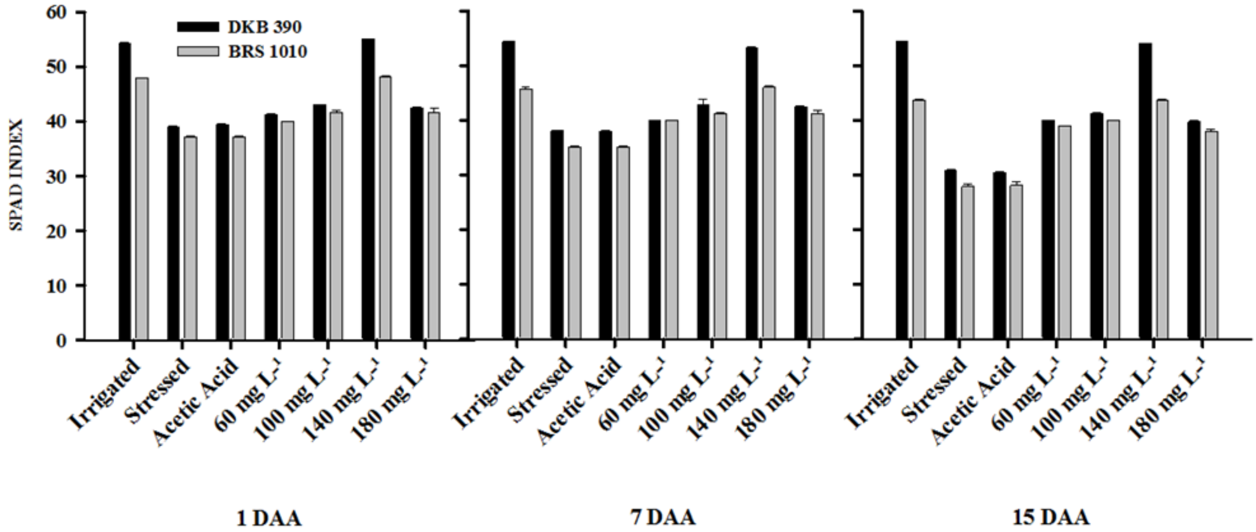

Figure 3. Chlorophyll index during the stress application on two drought contrasting hybrids (DKB 390 and BRS 1010) with and without different CHT application doses. The averages followed by the same letter do not statistically differ from each other. Uppercase letters denote comparisons between genotypes, and lowercase letters denote comparisons between treatments within the same genotype by the Scott-Knott test at a 5% significance level ($p \leq 0.05$).

The results obtained regarding chlorophyll content are related to the fact that under water restriction conditions, the decrease chlorophyll is commonly observed (Pirbalouti, Malekpoor, Salimi, & Golparvar, 2017). This behaviour can be attributed to a reduction in the synthesis of the major pigment-protein complexes, which protect the photosynthetic apparatus from oxidative damage to lipids and chloroplast proteins. The decrease in this pigment content is a negative consequence of water deficit; however, it is considered as an adaptive characteristic in plants growing under water restriction (Hadwiger, 2013). The CHT resembles cellulose, with the only difference being found in the amine group ($-NH_2$) at the C2 position instead of the hydroxyl group (-OH) pertaining to cellulose. However, unlike vegetable fibre, CHT has positive ionic charges, which gives it the ability to chemically bind with negatively charged macromolecules (Katiyar et al., 2015). This chemical characteristic suggests that an interaction between chlorophyll and CHT can occur through its protonated amino group, thus corroborating the maintenance of chlorophyll observed in the present investigation (Rizzi, Fini, Semeraro, & Cosna, 2016).

A significant decrease in the Fv/Fm ratio (maximum photochemical efficiency) was observed in both hybrids and notably in BRS 1010 in all treatments, except in plants under irrigated conditions and CHT dose of 140 mg L^{-1} (Table 1).

Table 1. Maximum photochemical efficiency of PSII (Fv/Fm) maize hybrids with contrasting drought tolerance characteristics for cultivating under different treatments.

				DKB 390*				
	Irrigated	Stressed	Acetic acid	60 mg L^{-1}	100 mg L^{-1}	140 mg L^{-1}	180 mg L^{-1}	CV%
1 DAA	0.80 a	0.69 b	0.69 d	0.69 c	0.73 b	0.79 a	0.70 b	15.1
7 DAA	0.81 a	0.63 c	0.65 c	0.69 b	0.72 b	0.79 a	0.68 b	16.2
15 DAA	0.80 a	0.58 d	0.59 d	0.60 c	0.70 b	0.75 a	0.66 b	14.3
				BRS 1010*				
1 DAA	0.79 a	0.68 b	0.69 b	0.70 b	0.72 b	0.78 a	0.69 b	14.0
7 DAA	0.78 a	0.62 c	0.64 c	0.71 b	0.3 b	0.77 b	0.69 b	14.5
15 DAA	0.74 a	0.55 c	0.59 c	0.67 b	0.68 b	0.72 a	0.68 b	14.1

*Tolerant hybrid DKB 390, sensitive hybrid BRS 1010.

The averages followed by the same letter do not statistically differ from each other. Uppercase letters denote comparisons between genotypes, and lowercase letters denote comparisons between treatments within the same genotype by the Scott-Knott test at a 5% significance level ($p \leqslant 0.05$).

A unique behaviour was observed in relation to the analysed gas exchange parameters, except for Ci (CO_2 intracellular concentration). On the first day, the water deficit significantly affected the stressed treatments, but there was a unique behaviour of the plants subjected to CHT 140 mg L^{-1}, presenting a similarity with the plants under irrigation conditions. Lower parameter values were more pronounced in the BRS 1010 hybrid (Figure 4). After 15 days of stress, both hybrids presented a reduction in the values of these parameters, however DKB 390 performed better than BRS 1010. By analysing the CO_2 intracellular concentration, it can be observed that the sensitive hybrid presented a superior behaviour to that of the tolerant hybrid; in addition, the lowest contents analysed occurred in maize plants subjected to treatments of CHT 140 mg L^{-1} and in favourable irrigation conditions.

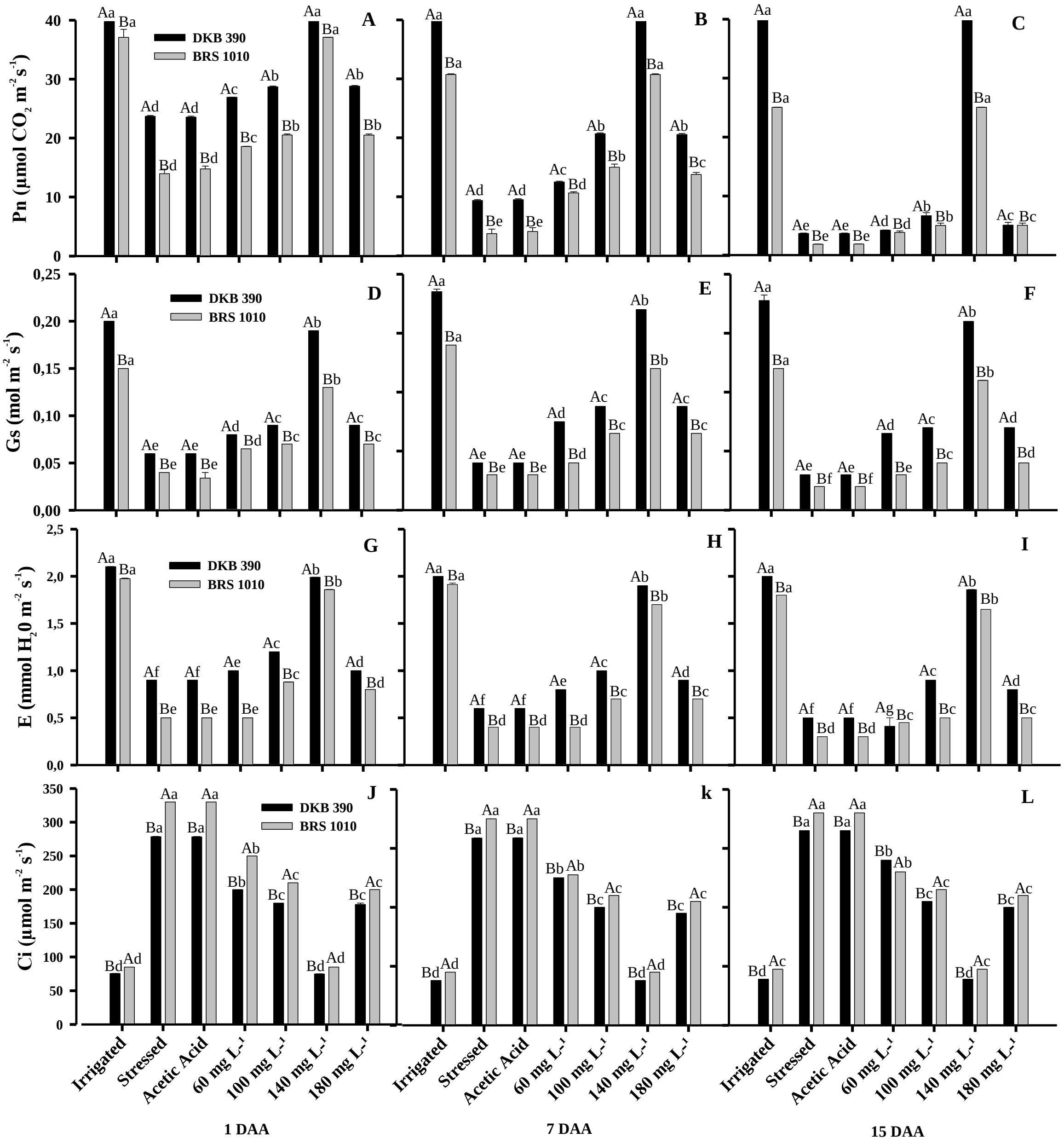

Figure 4. Gas exchange parameters during stress application on two drought contrasting hybrids (DKB 390 and BRS 1010) subjected to the various treatments, Pn (photosynthetic rate, A and B), gs (stomatal conductance, D, E, and F), E (perspiration rate, G, H, and I), and Ci (internal carbon, J, K, and L). The averages followed by the same letter do not statistically differ from each other. Uppercase letters denote comparisons between genotypes, and lowercase letters denote comparisons between treatments within the same genotype by the Scott-Knott test at a 5% significance level ($p \leqslant 0.05$).

The foliar application of CHT 140 mg L^{-1} and the irrigated treatments resulted in maintenance of the Fv/Fm ratio, thus indicating that photosynthetic electron transport was not affected, as probably no photoinhibition occurred in the photosystem II (PSII) complexes. CHT can stimulate the xanthophyll cycle, reflecting corresponding changes in the zeaxanthin/violaxanthin ratio (Iriti et al., 2009). CHT probably regulates 9 cis-epoxycarotenoid dioxygenase (NCED), a key enzyme of ABA biosynthesis through the carotenoid pathway. In fact, in the present investigation, it was observed that CHT 140 mg L^{-1} resulted in a decrease in stomatal conductance, thus acting as an antiperspirant, without negatively affecting the photosynthetic rate and the carbon intracellular concentration. This result suggests a decrease in the CO_2 assimilation and transport efficiency, but photosynthetic activity remains strong.

The longer duration of water stress resulted in lower enzyme activity in both hybrids (Figure 5). Irrigated treatments with CHT 140 mg L^{-1} showed similar behaviour regarding the activity of SOD, CAT, APX, and PAL enzymes, demonstrating a high activity than the other conditions. As other results showed, it was observed that the tolerant hybrid (DKB 390) presented a higher enzymatic activity than the sensitive hybrid (BRS 1010).

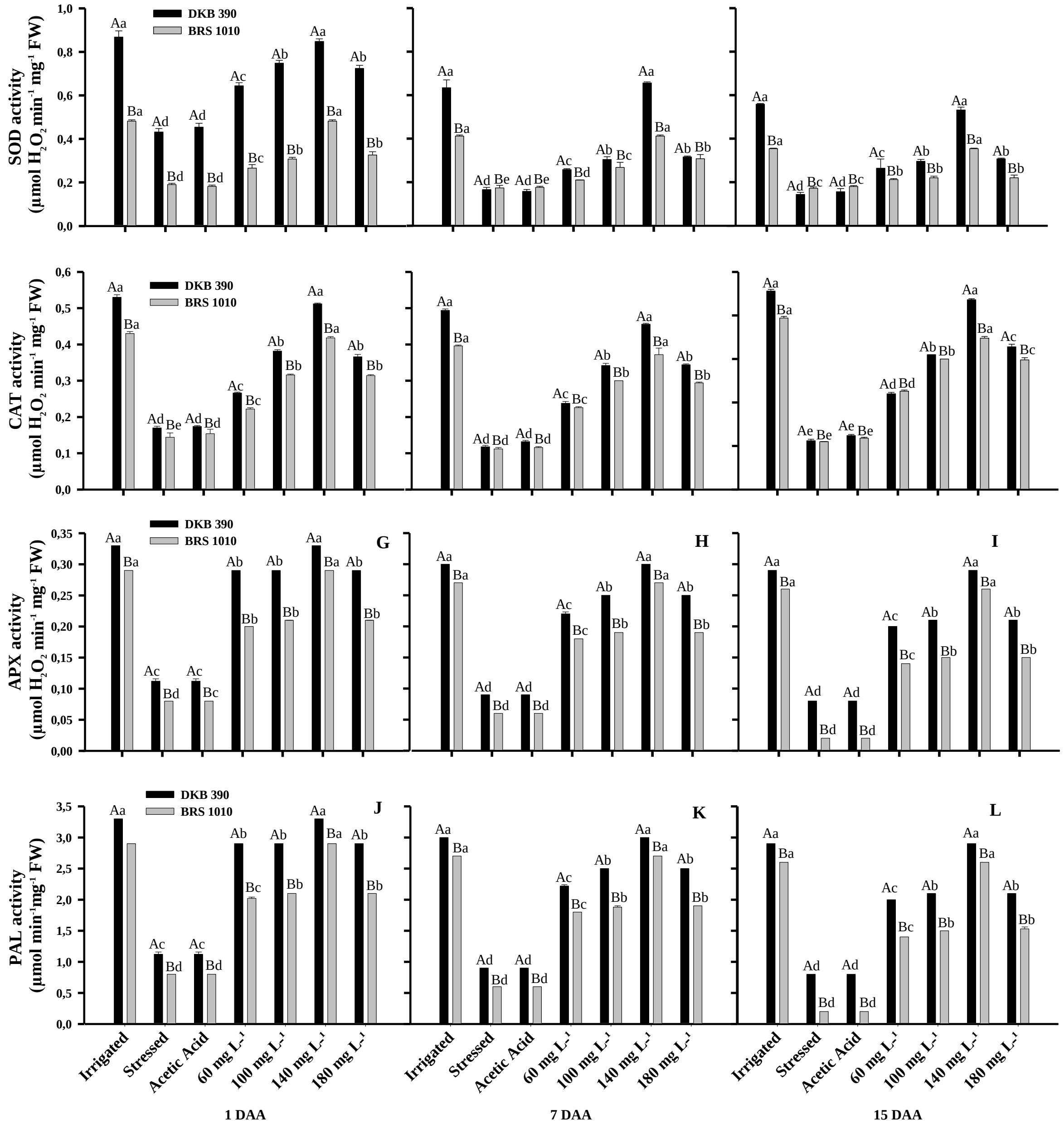

Figure 5. Antioxidant SOD enzymes activity (A and B), CAT (D, E, and F), APX (G, H, and I), and PAL (J, K and L) during the stress application on two contrasting hybrids (DKB 390 and BRS 1010) subjected to the different treatments. The averages followed by the same letter do not statistically differ from each other. Uppercase letters denote comparisons between genotypes, and lowercase letters denote comparisons between treatments within the same genotype by the Scott-Knott test at a 5% significance level ($p \leqslant 0.05$).

As expected according to the above results, under stress treatments and with the application of 0.5% acetic acid, in general and independent of the hybrids, there was an increase in the malondialdehyde (MDA) content and hydrogen peroxide, for which specific behaviour was observed throughout the water restriction days (Figure 6). It is observed that the irrigated treatments and CHT 140 mg L^{-1} resulted in the lower H_2O_2 and lipid peroxidation content. In relation to the hybrids, it was observed that the maize plants sensitive to the water deficit (BRS 1010) had higher concentrations of the analysed contents than the tolerant plants (DKB 390).

The H_2O_2 may play a role as a secondary messenger in response to abiotic stress, leading to a tolerance increase towards these unfavourable conditions, especially low water availability, by maintaining cellular homeostasis through the antioxidant enzymes SOD, CAT, and APX. The CHT defence response includes the improvement of secondary metabolism enzymatic activity, such as PAL. It is proposed that CHT receptors are present on the plasma membrane; however, through a signalling cascade, the chloroplast is the primary CHT action organelle (Hadwiger, 2013). Charge-charge interactions between positively charged CHT amine groups and negatively charged phospholipids promote a signal that will lead to the octadecanoid pathway activation; this metabolic pathway is directly related to the decreased H_2O_2 formation as well as the PAL enzyme activation (Pichyangkura & Chadchawan, 2015).

Most likely, the high content of MDA found in plants subjected to water stress, except for the treatment of 140 mg L^{-1}, resulted in damage to the phospholipids, resulting in a low interaction between plant cells and CHT, promoting a decrease in the antioxidant enzyme activity.

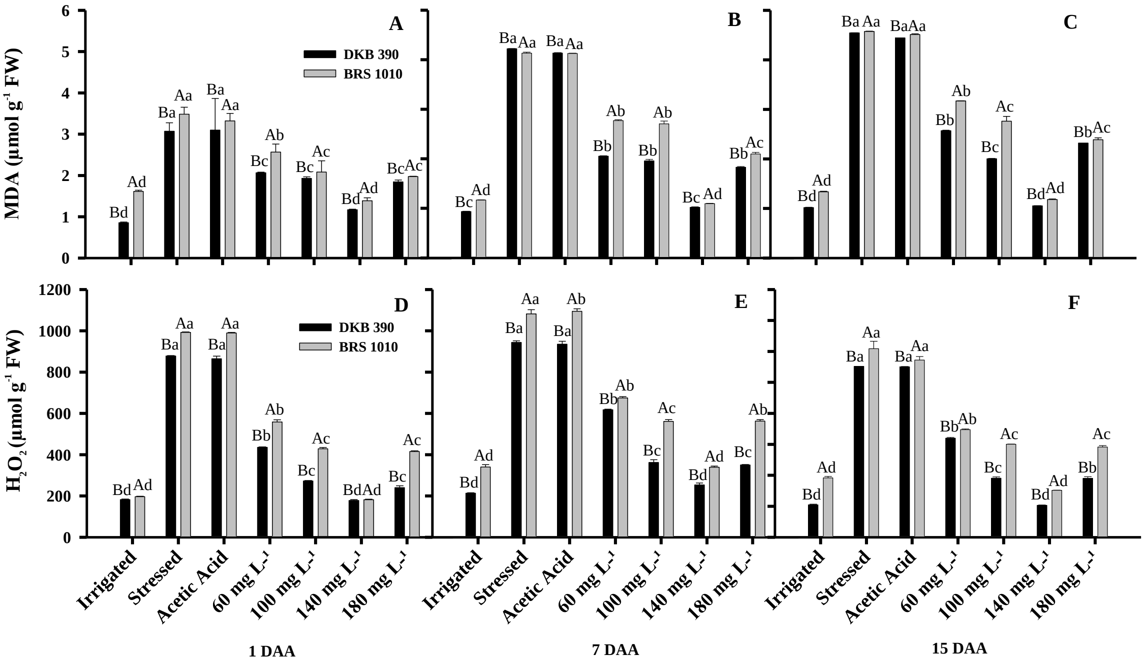

Figure 6. Malondialdehyde content (MDA) (A, B, and C) and H_2O_2 (D, E, and F) during the stress application on two drought contrasting hybrids (DKB 390 and BRS 1010) subjected to several treatments. The averages followed by the same letter do not statistically differ from each other. Uppercase letters denote comparisons between genotypes, and lowercase letters denote comparisons between treatments within the same genotype by the Scott-Knott test at a 5% significance level ($p \leq 0.05$).

The stress effect caused by lack of water was observed under stressed conditions different CHT doses and under irrigated production conditions. The results found in the DKB 390 hybrid confirmed a higher tolerance than in BRS 1010, where it was observed that the maize plants of the sensitive hybrid did not yield excessive grain. It was verified through the analysed parameters that maize plants grown in CHT 140 mg L^{-1} and those under irrigated conditions showed similar responses (Table 2).

The averages followed by the same letter do not statistically differ from each other. Uppercase letters denote comparisons between genotypes, and lowercase letters denote comparisons between treatments within the same genotype by the Scott-Knott test with a 5% significance level ($p \leq 0.05$).

The CHT stimulating effect on plant growth can be attributed to an increase in the availability, water absorption and essential nutrients by adjusting the cellular osmotic pressure and reducing the free radical accumulation by increasing the antioxidant enzymes activity (Pirbalouti et al., 2017). The adverse consequences reflected by the agronomic parameters in plants under water restriction become an adaptive resource for the plants survival under stress. One of the reasons that may have led to a better yield in the agronomic parameters analysed, especially for the application of CHT 140 mg L^{-1} and irrigated plants of the genotype DKB 390, is the harvest index (HI) increase, that is, a larger differential of photoassimilates allocated to the ear during its life cycle.

Table 2. Production characteristics in contrasting drought tolerant maize hybrids grown under different treatments.

	Irrigated	Stressed	Acetic acid	60 mg L^{-1}	100 mg L^{-1}	140 mg L^{-1}	180 mg L^{-1}	CV%
DKB 390*								
PD	2.47Aa	2.02 Ad	2.03 Ad	2.31 Ab	2.31 Ab	2.46 Aa	2.26 Ac	13.8
ED	49.10 Aa	35.00 Ad	35.00 Ad	46 Ab	46 Ab	49 Aa	46.1 Ab	14.7
NGR	26 Aa	10.0 Ac	10.8 Ac	24.2 Ab	24.8 Ab	26.8 Aa	24.8 Ab	14.6
NRE	15.8 Aa	8.4 Ad	8.4 Ad	13.4 Ac	13.2 Ac	15.8 Aa	12.4 Ac	15.2
TDB	174.84 Aa	91.00 Ad	91.04 Ad	144.90 Ab	145.10 Ab	173.90 Aa	139.10 Ac	12.3
DGB	61.89 Aa	28.2 Ac	28.4 Ac	51.30 Ab	50.9 Ab	61.12 Aa	51.2 Ab	14.6
HI	0.35% Aa	0.30% Ac	0.30% Ac	0.32% Ab	0.31% Ab	0.35% Aa	0.33% Ab	14.1
BRS 1010								
PD	2.18 Ba	1.88 Bd	1.89 Bd	2.02 Bc	2.14 Bb	2.19 Ba	2.15 Bb	13.9
ED	43.31 Ba					43.1 Ba		12.4
NGR	17.20 Ba					16.9 Ba		15.9
NRE	13.20 Ba					13.1 Ba		13.6
TDB	129 Ba	37.5 Bd	37.3 Bd	58.4 Bc	61.1 Bb	128.1 Ba	60.9 Bb	15.8
DGB	25.3 Ba					25 Ba		13.5
HI	0.31% Ba					0.31% Ba		15.5

Tolerant hybrid DKB 390, sensitive hybrid BRS 1010. PH: plant height (m); ED: spike diameter (mm); NGR: number of grains per row; NRE: number of ears per row; TDB: total dry biomass (g); DGB: total grain biomass (g); HI: harvest index (g.g-1). * There was no production.

The CHT mode of action is not well elucidated yet. The diversity of potential action pathways of this biopolymer can be unlimited due to the uniformity of positive charges along its length, thus allowing a vast interaction with the other molecules in the cell matrix. The role of CHT as an antiperspirant in agriculture may be related to the fact that when deposited in the cell wall, the CHT promotes a decrease in stomatal conductance, increasing the leave's resistance to water vapor loss, thus improving the water use in plants to assimilate carbon, and in turn, the biomass production. Another approach to reduce H_2O through perspiration is the leaves solar reflection increase through the reflective type of antiperspirant action, thus limiting the loss of water vapor, providing evaporative cooling to the leaves (Emam, Khattab, Helal, & Deraz, 2014). With this, the transpiration efficiency would be increased without negatively affecting the photosynthesis and the harvest agronomic yield, thereby translating into a tolerance mechanism under conditions of water deficit.

Conclusion

Chitosan leaf application at a dose of 140 mg L^{-1} resulted in similar responses in maize plants grown under favourable irrigation conditions. The difference obtained in relation to the hybrids under study is also related to the genetic differences that differentiate them regarding sensitivity and tolerance.

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