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Do chitosan and its derivatives have the same protective effect on drought-contrasting maize genotypes? An analysis of physiological and production processes

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Abstract

Water stress is among the most severe abiotic stress factors for maize production. The application of chitosan causes various responses in plants, as a function of its structure and concentration. Therefore, chemical modifications were proposed in this study to enhance the biological effects on plants. Hybrid maize plants with drought-contrasting characteristics, were subjected to water deficit and spraying with chitosan (CHI) and semi-synthesized chitosan derivatives, *N*-Succinyl (SUC) and *N,O*-Dicarboxymethyl (MCA). The obtained data show that the application of CHI and its derivatives (0.5 mg.plant^{-1}) led to an increase in production for the two evaluated hybrids in comparison with the control under stress. Regarding leaf gas exchange, over the stress period, it was observed that the application of the MCA derivative yielded greater P_n than the other treatments in plants subjected to drought, in both hybrids. In the evaluation of chlorophyll content, there was an increase in this content through the application of CHI and its derivatives for both maize hybrids under study. With water recovery in plants, the water potential (Ψ_{md}) of those treated with chitosan derivatives was greater than that of the irrigated control plants. In the quantification of proline concentration, higher values were observed in plants treated with MCA derivatives for the drought-sensitive hybrid. Chitosan derivatives, SUC and MCA, were responsible for higher starch concentrations in both maize hybrids. Evaluating the morphological characteristics of roots, the drought-tolerant hybrid showed higher means for all parameters evaluated when subjected to drought, and MCA was responsible for longer root length and greater mean root diameter. The results support the potential use of chitosan and its derivatives to increase tolerance to water deficit in maize.

Keywords: drought stress; *N*-succinyl; *N,O*-dicarboxymethyl; physiological characteristics; *Zea mays*.

Introduction

Chitin is considered the second polysaccharide in greater abundance in nature, comprising the cell walls of fungi, insect exoskeletons and crustacean shells. Chitosan is the deacetylated form of chitin, produced by the exposure of crustacean exoskeletons to high temperatures and alkaline conditions, which deacetylate the polymer and help remove proteins and calcium minerals (Roosen et al., 2016). Chitosan has a nucleophilic behavior, making it suitable for chemical and structural modifications such as acetylation, alkylation, carboxylation, sulfonation and amidation. The reactivity of primary amine groups and/or hydroxyl groups of the glucosamine units brings great versatility to it, allowing the alteration of physicochemical and biological aspects. With the insertion of functional groups in the polymeric chain of chitosan, it is possible to produce derivatives with different properties, thus allowing its use in medical, biotechnological and agricultural areas (Saharan, 2016).

The application of chitosan affects various plant responses, as a function of its structure and concentration (Kananont et al., 2010; Almeida et al., 2020), the species treated (Ohta et al., 2004) and the development stage of these plants (Pornpienpakdee et al., 2010). Chitosan serves several purposes in agriculture, and its benefits can be broadly divided into four main areas: 1. Direct antibiosis against crop pests and pathogens; 2. Conditioning of beneficial microorganisms, both in plant defense and growth; 3. Stimulation of plant defense responses against biotic stress; 4. Regulation of plant growth, development, nutrition and tolerance to abiotic stress (Russell, 2013).

Despite the various studies on the use of chitosan in agriculture, there are few that explore its application and responses related to individual characteristics of different plant genotypes, such as maize genotypes with drought-contrasting characteristics, as already observed for several other substances (Souza et al., 2013), besides few studies based on the development of chitosan derivatives, which is an important strategy that should be considered when using this biopolymer in agriculture, since most commercial chitosans are soluble only at acidic pH values, causing adverse reactions with the treated surface and hindering its applications.

Water deficit is the main environmental factor that limits crop growth and yield worldwide. In order to increase agricultural yield in areas with water deficiency, it is extremely important to induce drought tolerance and several agronomic and physiological practices have been used in order to meet this purpose (Anjum et al., 2011). Strategies such as the study and production of drought-tolerant genotypes (genetic improvement) and the development of products known as biostimulants which, applied to plants, induce water deficit tolerance (Souza et al., 2013; Patrick, 2015; Martins et al., 2018), have been adopted.

Water stress is among the most severe abiotic stress factors for maize production, as a function of its intensity, phenological stage and genetic material. Maize plants subjected to water stress during pre-flowering reduce grain yield by up to 60% (Magalhães et al., 2008). The primary plant response to the lack of water is stomatal closure, in order to prevent excessive water loss through leaf transpiration. However, this response also decreases CO_2 fixation for photosynthesis, causing a breakdown in the entire photosynthetic apparatus, as in chlorophylls and accessory pigments that protect photosystems from oxidative damage. Consequently, there is a reduction in plant development, biomass accumulation and production (Dos Reis et al., 2019).

In search of sustainable agricultural techniques, with lower impact on the environment and that present effective solutions to climate changes and the limitations of water use in agriculture, the objective of this study was to evaluate the effects of the addition of *N*-succinyl (SUC) and *N,O*-dicarboxymethyl (MCA) groups to the structure of chitosan, and to understand their actions and effects on gas exchange, accumulation of osmoregulators, morphological characteristics of roots and production of different maize genotypes, with drought-contrasting characteristics, subjected to water deficit.

Results

Production parameters

In the analysis of production parameters (EL, ED, EW), it was possible to observe that the application of chitosan (CHI) and the MCA derivative in drought-sensitive hybrid maize plants (BRS 1030), led to higher means in all parameters under study, being statistically superior or equal, even those found in irrigated plants (IC) ($P \leq 0.05$). In relation to the hybrid with drought tolerance characteristics (DKB 390), CHI led to an increase in the characteristics studied when compared to the control under stress (SC) (Table 1). In the evaluation of harvest index (IDC), CHI was responsible for providing a higher mean for the drought-sensitive genotype. On the other hand, for plants with tolerance to water deficit, the control under stress (SC) was statistically superior to the other treatments in the evaluation of this attribute (Table 1).

Gas exchange measurements

The application of chitosan and its derivatives led to significant effects on leaf gas exchange in the maize hybrids (BRS 1030 and DKB 390) under study. Regarding the photosynthetic rate (P_n), at the beginning of the water deficit period (1DAA), the application of CHI and its derivatives caused a decrease in the means of treatments compared to the control plants (IC and SC), in both hybrids. Over the stress period (15DAA), it was observed that the application of MCA yielded greater P_n than the other treatments in plants submitted to drought, as well as in both hybrids. For the tolerant genotype (DKB 390), it was possible to observe that plants of this hybrid maintained higher photosynthetic rates, when compared to BRS 1030, even under exposure to the 15-day water deficit period, and the application of MCA improved these results even more, reaching an average statistically equal to the irrigated control (IC) (Fig. 1A, B and C).

In terms of stomatal conductance (g_s), the derivatives SUC and MCA enabled high g_s values (0.46 and 0.41 respectively) in plants of the BRS 1030 hybrid, even after they passed a period of 15 days of water restriction (15DAA). For the drought-tolerant hybrid, DKB 390, there were no major highlights between treatments in relation to the increase in stomatal conductance, when compared to the irrigated control and that under stress (Fig.1D, E and F).

During water deficit (1DAA and 15DAA), CHI and its derivatives reduced the transpiration rate (E) in plants of the DKB 390 hybrid compared to the irrigated control (IC) and that under stress (SC) (Fig.1 G, H). Regarding BRS1030, in the same period, there were, in general, no statistical differences between treatments. With plant rehydration, there was a great reduction in E for the treatments using CHI and its derivatives for the two hybrids (Fig.1 G, H and I).

With the beginning of the water deficit period (1DAA), the application of CHI and its derivatives resulted in a lower water use efficiency (WUE) in the plants of the two hybrids under study (Fig.1 J). However, the results show that, after the maximum stress period (15DAA), as well as in rehydration, CHI and its derivatives generally led to a higher WUE than that found in controls (IC and SC) (Fig. 1 K and L).

Leaf water potential and chlorophyll contents

In the evaluation of chlorophyll content (Chl A + Chl B), an increase was observed with the application of chitosan and its derivatives for both maize hybrids under study (Fig. 2 A). For the drought-sensitive genotype (BRS1030), the use of CHI led to higher means in plants under stress, even when compared to plants irrigated during the whole experimental period (IC). Similar results were found for the analysis of the content of xanthophylls and carotenes (XANT + CARO), where the application of CHI and its derivatives increased these contents, with CHI once again responsible for the highest means, mainly for hybrid BRS1030 (Fig. 2 B).

Regarding leaf water potential (Ψ_{md}), the application of CHI and its derivatives did not lead, in general, to a higher water status compared to plants that underwent stress without spraying (SC), during the dry period (1DAA and 15DAA), and this is probably due to the accumulation of proline and others, besides osmotically active solutes (Fig 3.A and B). With water recovery in the plants, the water potential of those treated with CHI derivatives was greater than that measured in plants that did not undergo stress (IC) (Fig. 3 C).

Proline and starch concentration

In the quantification of proline (Table 2), the maize hybrid DKB 390 yielded higher concentrations in all treatments when compared to BRS 1030. The treatments showed different results in the two genotypes studied, and MCA was statistically equal to IC, and superior to the other treatments for the drought-sensitive hybrid (BRS 1030). With respect to the tolerant hybrid (DKB 390), the treatments that resulted in the highest concentration of proline were SC, CHI and SUC.

Chitosan derivatives SUC and MCA were responsible for higher concentrations of starch in the leaves of both maize hybrids, which were higher than those observed in the irrigated control (IC). The drought-sensitive hybrid (BRS 1030) showed means statistically higher than those of the hybrid characterized as tolerant (DKB 390), reaching a maximum concentration of 12.45 µmol g^{-1} against the maximum of 8.99 µmol g^{-1} for DKB 390 (Table 2).

Root morphology

In the analysis of the morphological characteristics of roots (Fig. 4 A, B, C and D), the drought-tolerant hybrid (DKB 390) presented higher means for all parameters evaluated when subjected to drought, and MCA was responsible for a greater root length (Fig. 4 A) and mean root diameter (Fig. 4 C). The hybrid BRS 1030 behaves differently from DKB 390 and, in general, there was a decrease in the means of the parameters analyzed, in plants exposed to drought, when compared to the irrigated control (IC). MCA stood out among the other treatments in the evaluation of root length (Fig. 4 A) and volume (Fig. 4 D).

Discussion

Drought is considered a “multidimensional stress”, as it affects a series of cellular processes in cultivated plants, resulting in considerable yield losses. In this study, there was a correlated behavior between the two hybrids evaluated when treated with chitosan (CHI), with an increase in production parameters compared to the control under stress (SC). However, an interesting result was observed for hybrid BRS 1030, where CHI and MCA led to better results than those observed in irrigated plants (IC). Nonetheless, it is observed that MCA does not show the same increments in the drought-tolerant material (DKB 390). Previous studies have shown that chitosan improves yield and crop quality in several cases (Cabrera et al., 2013). Mondal et al. (2013) obtained an increase in most grain yield components by spraying chitosan at a concentration of 100 ppm in maize.

Harvest index (IDC) is a parameter widely used to assess partitioning efficiency, that is: the plant capacity to allocate carbon to organs of commercial interest which, in the case of maize, is the grain (Durães et al., 2002). The improvement in partitioning efficiency is directly related to the drain ability to channel a greater sucrose flow for its growth. The drought-sensitive hybrid (BRS 1030) showed a higher IDC when treated with CHI; for the tolerant genotype, it was observed that the use of CHI and its derivatives did not add up to SC. According to Avila et al. (2016), the higher harvest index observed in tolerant genotypes may be related to a greater cell wall invertase activity. Sucrose can be hydrolyzed to glucose and fructose in the apoplast by invertase, a sucrose cleavage enzyme; thus, they could enter the drain cells.

After exposure to water deficit conditions, plants may undergo processes that cause a reduction in their photosynthetic rate due to stomatal and non-stomatal limitations. Apparently, in this case, there was a stomatal limitation for both hybrids under study, at the beginning of water deficit (1DAA) and plant behavior in the first evaluation showed a lower stomatal conductance (g_s), as well as a lower photosynthetic rate (Pn) in maize plants treated with chitosan and its derivatives. However, over drought and plant rehydration, photosynthetic rate and conductance increased, mainly in plants treated with MCA. Almeida et al. (2020) observed that spraying 140 mg L^{-1} of chitosan on maize resulted in a decrease in stomatal conductance, thus acting as an antitranspirant, without negatively affecting the photosynthetic rate and intracellular carbon concentration.

CHI and its derivatives proved to be antitranspirant molecules, generally reducing transpiration (E) in maize plants submitted to drought, mainly for hybrid DKB 390. The role of chitosan as an antitranspirant in agriculture may be related to the fact that, when deposited on the cell wall, chitosan causes a decrease in stomatal conductance, increasing leaf resistance to the loss of water vapor, thus improving water use in plants for carbon assimilation and, in turn, biomass production. Another approach that can lead to a reduction in water loss through transpiration is to increase solar reflection of the leaves, thus limiting the loss of water vapor, providing evaporative cooling to the leaves (Emam et al., 2014).

Chlorophylls and carotenoids play an important role in plant photosynthesis, such as the production of sugars and organic molecules by fixing CO_2 and water. In this study, the application of CHI and its derivatives in leaves of both maize hybrids increased the content of photosynthetic pigments (Chlorophyll a + b), as well as that of accessory pigments (Xanthophylls + Carotenes). However, it is worth mentioning that, in the drought-sensitive hybrid (BRS 1030), CHI increased the levels of pigments in relation to those found in plants grown under optimal irrigation conditions (IC). Chlorophyll is one of the main chloroplast components for photosynthesis, and the relative content of chlorophyll has a positive relationship with the photosynthetic rate. Chitosan increases the stability of chlorophyll and can stimulate the expression of genes that are involved in the chlorophyll biosynthetic pathway (Naderi et al., 2015). Carotenoids are critical components of photosynthesis and antioxidant systems in plant cells (Zhou et al., 2015). During photosynthesis, carotenoids collect light energy and protect photosynthetic organelles from excess light energy through the xanthophyll cycle (Nisar et al., 2016).

The accumulation of proline is frequently known as the primary response of many plants to drought stress (Hong et al., 2000). Higher concentrations of proline are known to reduce the water potential of plant tissues, especially in leaves, allowing them to avoid water loss and/or continue to acquire water from the soil under drought conditions. Spraying chitosan derivatives resulted in a greater proline accumulation in the drought-sensitive hybrid (BRS 1030), which may be related to the lower water potential observed in plants under stress treated with chitosan and its derivatives. In a study developed in 2017, Bistgani et al. found a synergy between water stress and chitosan application in relation to proline levels, so that the highest

Table 1. Ear length (EL), ear diameter (ED), ear weight (EW), grain weight (GW) and harvest index (IDC) of two drought-contrasting maize hybrids (BRS1030 and DKB390), submitted to water stress and treated with chitosan and its derivatives.

Trat	EL (cm)		ED (mm)		EW (g)		IDC	
	BRS1030	DKB390	BRS1030	DKB390	BRS1030	DKB390	BRS1030	DKB390
IC	10.50Bb	12.80Aa	55.86Bb	92.02Aa	55.86Bb	92.02Aa	0.71Ba	0.70Ba
SC	11.46Ba	12.50Aa	53.48Bb	65.78Ca	53.48Bb	65.78Ca	0.68Bb	0.82Aa
CHI	12.76Aa	13.20Aa	76.99Aa	75.34Ba	76.99Aa	75.34Ba	0.80Aa	0.71Ba
SUC	10.74Ba	11.76Ba	45.09Ba	42.58Da	45.09Ba	42.68Da	0.44Ca	0.41Ca
MCA	11.26Ba	11.40Ba	82.28Aa	63.24Cb	82.28Aa	63.24Cb	0.70Ba	0.68Ba

*Means followed by the same uppercase letter in the column do not differ for treatments. Means followed by the same lowercase letter in the row do not differ in the comparison between hybrids by the Skott-Knott test at 5% probability (P≤0.05). Irrigated control (IC), control under stress (SC), Chitosan (CHI), Hemi-succinyl (SUC), *N*-carboxymethyl (MCA).

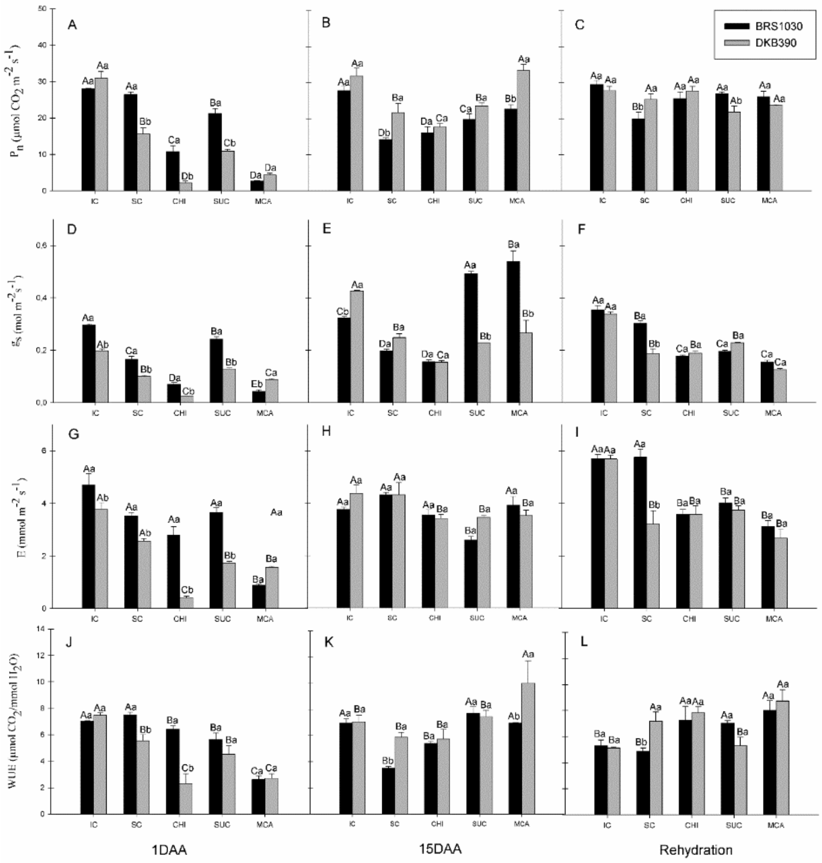

Fig 1. Gas exchange parameters during the imposition of stress and water recovery in two drought-contrasting hybrids (BRS 1030 and DKB 390) with the application of chitosan and its derivatives: (**A**, **B** and **C**), photosynthetic rate (P_n); (**D**, **E** and **F**), stomatal conductance (g_s); (**G**, **H** and **I**), transpiration (**E**); and (**J**, **K** and **L**), water use efficiency (WUE). Means followed by the same uppercase letter do not differ for treatments with chitosan and its derivatives. Means followed by the same lowercase letter do not differ in the comparison between hybrids by the Skott-Knott test at 5% probability (P ≤ 0.05). Irrigated control (IC), control under stress (SC), Chitosan (CHI), Hemi-succinyl (SUC), *N*-carboxymethyl (MCA). One day after application (1DAA), 15 days after application (15DAA), water replacement (Rehydration). Bars correspond to ± standard error (SE).

Table 2. Concentration of proline and starch in two maize hybrids (BRS 1030 and DKB 390) submitted to water deficit and treated with chitosan and its derivatives.

Treatment	Proline (µmol g^{-1})		Starch (µmol g^{-1})	
	BRS1030	DKB390	BRS1030	DKB390
IC	4.12Ab	5.87Ba	9.40Ba	6.75Bb
SC	2.53Bb	8.74Aa	3.93Da	4.49Ca
CHI	3.84Ab	7.70Aa	6.64Ca	4.71Cb
SUC	2.57Bb	7.92Aa	12.45Aa	6.80Bb
MCA	4.58Aa	4.92Ba	10.01Ba	8.99Ab

*Means followed by the same uppercase letter in the column do not differ for treatments. Means followed by the same lowercase letter in the row do not differ in the comparison between hybrids by the Skott-Knott test at 5% probability (P ≤ 0.05). Irrigated control (IC), control under stress (SC), Chitosan (CHI), Hemi-succinyl (SUC), N-carboxymethyl (MCA).

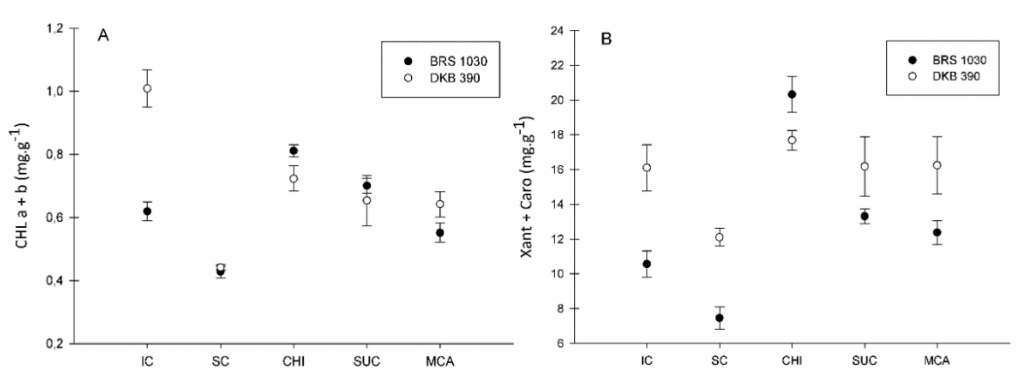

Fig 2. (**A**) Contents of chlorophyll (CHL A+CHL B) and (**B**) xanthophylls + carotenes (XANT + CARO), at 15 days of water stress in maize hybrids BRS 1030 and DKB 390, sprayed or not with chitosan (CHI) and its derivatives (SUC and MCA). Irrigated control (IC), control under stress (SC), Chitosan (CHI), Hemi-succinyl (SUC), *N*-carboxymethyl (MCA).

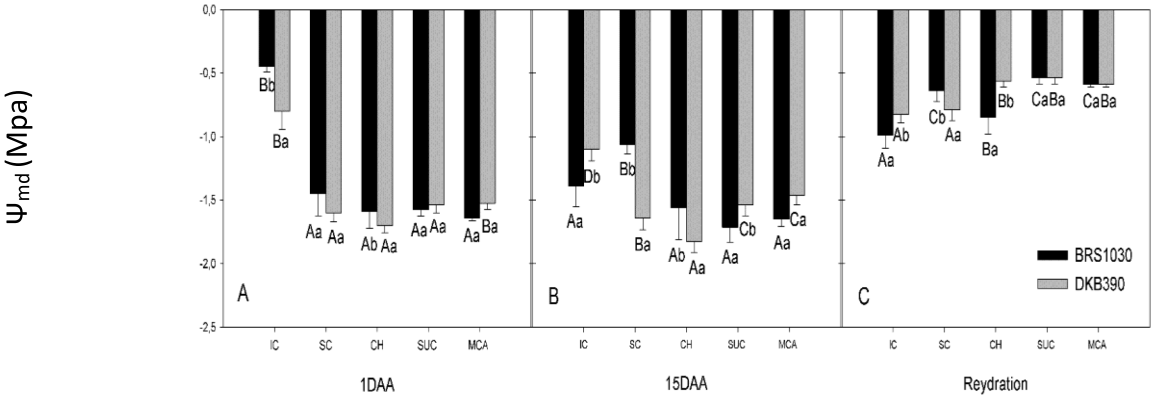

Fig 3. Leaf water potential at noon, of two maize hybrids (BRS 1030 and DKB 390), during the water stress period, submitted to different treatments. Means followed by the same letter do not differ by the Skott-Knott test at 5% probability ($p \leq 0.05$). Uppercase letters show a comparison between treatments with chitosan and its derivatives and lowercase letters show a comparison between genotypes. Bars correspond to ± standard error (SE). Irrigated control (IC), control under stress (SC), Chitosan (CHI), Hemi-succinyl (SUC), *N*-carboxymethyl (MCA). One day after application (1DAA), 15 days after application (15DAA), water replacement (Rehydration).

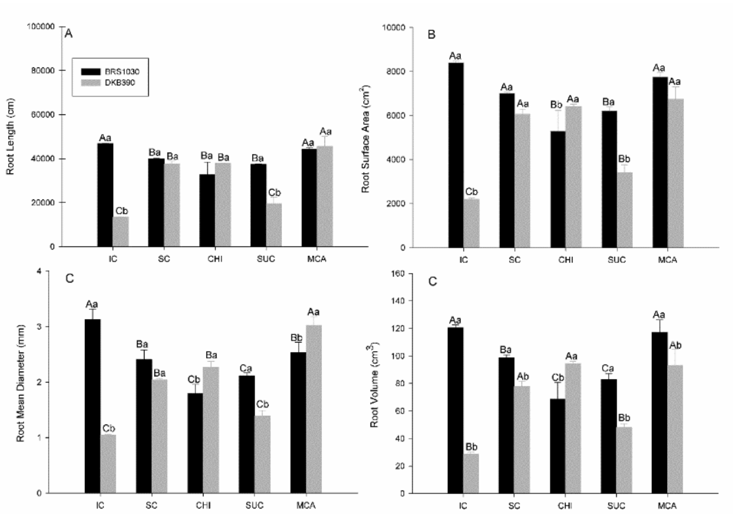

Fig 4. Root morphological characteristics of two maize hybrids (BRS 1030 and DKB 390) submitted to drought and treated with chitosan and its derivatives. (**A**) Root length, (**B**) root surface area, (**C**) mean root diameter, (**D**) root volume. Means followed by the same uppercase letter do not differ for treatments with chitosan and its derivatives. Means followed by the same lowercase letter do not differ for the hybrids by the Skott-Knott test at 5% probability ($p \leq 0.05$). Each value indicates the treatment mean ± SE. Irrigated control (IC), control under stress (SC), Chitosan (CHI), Hemi-succinyl (SUC), *N*-carboxymethyl (MCA). One day after application (1DAA), 15 days after application (15DAA), water replacement (Rehydration).

level of proline was accumulated in plants grown under severe stress and sprayed with 400 µL L^{-1} chitosan. Thus, the effect of chitosan in reducing water stress was due at least in part due to its stimulating influence on proline accumulation.

The light absorbed by photosynthesis is converted into chemical energy, part of which accumulates in the form of starch within the chloroplasts. Maize and oat plants, however, reserve part of the excess carbohydrates inside the vacuoles in the form of sucrose, accumulating little starch (Sparla, 2015). Starch has the function of supporting plant metabolism. In this context, starch is an efficient form of carbohydrate storage by plants, which can be used in situations where there is no light or even under stress conditions (Zanella et al., 2016). Chitosan derivatives SUC and MCA were responsible for leading to higher concentrations of starch in the leaves of both maize hybrids.

A wide variety of factors can influence different processes related to plant growth and development. They can be natural and endogenous, such as plant hormones, or synthetic and exogenous, such as elicitors and plant growth regulators (Taiz et al., 2014). Among the elicitors, polysaccharides such as chitosan and its derivatives effectively lead to growth, increasing fresh and dry weights and plant root length (Ali et al., 2014; Dar et al., 2015). Assessments of the root system and its efficiency in absorption say a lot about yield gains in dryland cultivation. The efficiency of the root system to absorb water and nutrients depends on its depth, volume, density, root hair profusion, longevity and other attributes. As in this study, Santo Pereira et al. (2017) observed an increase in the development of the root and leaf area of French beans, when treated with chitosan nanoparticles.

Materials and Methods

Synthesis of derivatives (SUC and MCA)

Chitosan derivatives were synthesized as described by Reis et al. (2019). The chitosan used for the obtention of derivatives has a deacetylation percentage (DDA%) of 63.5% (Martins et al., 2018). Both synthesized derivatives have chains with carboxylic acid groups, which were inserted into the starting chitosan via its amine (SUC) or amine+alcohol (MCA) groups.

Environmental conditions, plant material and experimental design

The experiment was carried out in a greenhouse located at Embrapa Milho e Sorgo, in the city of Sete Lagoas - MG (19°455' 241''S latitude, 44°17'31''W longitude and 732 m altitude); 20 L pots were used, filled with Oxisol. The plant materials used were the hybrids: BRS 1030, from the Embrapa Breeding Program, characterized as drought-sensitive and DKB 390, characterized as drought-tolerant (Souza et al., 2013; Souza et al., 2014). Three maize seeds were planted in each pot and, after germination, thinning was carried out, thus leaving two plants per pot. Fertilization was performed according to the soil chemical analysis recommendation applying, at planting, 10g of 08-28-16 to every 20 Kg of soil. Coverage was performed by applying 6 g of ammonium sulfate per pot 30 and 60 days after planting. The plants were regularly irrigated, maintaining optimum soil moisture until stress was imposed. All phytosanitary treatments necessary for the crop were carried out during the experiment. The average temperatures recorded during the period were a maximum of 20.3 °C and a minimum of 18.4 °C, and relative humidity was between 71% and 64%.

The experimental design was completely randomized (CRD), comprising 5 conditions (Irrigated Control, Control Under Stress, Chitosan, SUC and MCA), 2 maize hybrids (BRS 1030 and DKB 390) and 6 replications, totaling 60 pots.

Stress imposition and application of chitosan and derivatives

The water content in the soil was monitored daily in the morning and in the afternoon (9 a.m. and 3 p.m.), with the aid of a watermark humidity sensor (tensiometer) (200SS – 5'', IRROMETER, California – USA), installed in the center of the pots of each replication, at a depth of 20 cm. These sensors detect the water tension in the soil based on the electrical resistance and were coupled to digital meters (Watermark meter) from the same company. Values ranged from 0 kPa (fully wet) to -200 kPa (fully dry). Water was replaced, based on the readings obtained with the sensor and replenished until field capacity (FC) during the period that preceded the treatments. These calculations were performed with the aid of an electronic spreadsheet, based on the soil water retention curve.

When the plants reached the pre-flowering stage, treatments were imposed. The irrigated treatment consisted of daily irrigation until the soil reached moisture contents close to field capacity (water tension in the soil of approximately -18 kPa) whereas, for the non-irrigated treatments, irrigation was performed by applying 50% of the total water available, that is, until the water tension in the soil reached -138 kPa, whose value corresponds to the specified soil.

When the plants reached water stress (water tension in the soil -138 kPa), spraying was performed with chitosan and its derivatives (SUC and MCA) at a concentration of 0.5 $mg.plant^{-1}$ and flow rate of 120 $L.ha^{-1}$, using a 20 L^{-1} manual knapsack sprayer.

Water stress was maintained for 15 days. Ecophysiological analyses were carried out 24 hours after spraying, at the end of the water stress period (15 days of stress), and 24 hours after water recovery, in which the soil received irrigation again until reaching potential values close to field capacity. During this period, the following variables were analyzed: leaf gas exchange, total chlorophyll content, xanthophylls and carotenoids, leaf water potential, leaf area, proline and starch content, root morphological characteristics and, at the end of the crop cycle, production parameters.

Gas exchange measurements

Gas exchange was measured using a portable photosynthesis system (IRGA, LI-6400 XT, Li-Cor, Lincoln, Nebraska, USA). All measurements were carried out in the morning, between 8 and 12 noon, on a fully expanded leaf (ear leaf). The variables evaluated were photosynthetic rate (P_n), stomatal conductance (g_s), transpiration (E), intercellular CO_2 concentration (C_i) and water use efficiency (WUE). The measurements were taken in a leaf area of 6 cm^2, with controlled CO_2 flow using 12 g cylinders (Licor) at a concentration of 380 $µmol.mol^{-1}$. Photon flow density (PPFD) was 1500 µmol $m^{-2}.s^{-1}$ and leaf temperature was controlled (30 °C).

Total chlorophyll, xanthophylls and carotenes

Chlorophylls and carotenoids were extracted from 100 mg of leaf tissue macerated in grail and pistil in the presence of 3.0

mL of 80% acetone according to Arnon (1949).

Leaf water potential measurements
Leaf water potential was determined using a Scholander pressure chamber (1000, PMS Instrument Company, Albany SE, USA) on two fully expanded leaves by replication/treatment. Mean water potential (*midday*, Ψ_{md}) was measured at 12 noon. The first analysis of water potential was carried out on the seventh day after application (7DAA); a second analysis was performed 15 days after application (15DAA) and the last, after plant rehydration (Rehydration).

Proline and starch content
Proline content was quantified spectrophotometrically according to Bates et al. (1973). Starch was quantified according to the methodology described by Fernie et al. (2001).

Root morphology
For the evaluation of root morphology, the WinRhizo Pro 2007 image analysis system (Regent Instruments, Sainte-Foy, QC, Canada), coupled to a professional scanner (Epson, Expression 10000 XL, Epson America, Inc., USA) was used, equipped with an additional light unit (TPU). The procedures for obtaining the images were performed according to Souza et al. (2012) after 15 days of plant stress. The following characteristics were determined: mean root length (MRL), mean root surface area (MRSA), mean root diameter (MRD), root volume (RV).

Production parameters
Among the yield indicators, ear length (EL) and diameter (ED) were evaluated, besides ear weight (EW) and Harvest Index (IDC).

Data analysis
For statistical analysis, the means were compared between the treatments applied and between maize hybrids, on each day after application and after water recovery (1DAA = one day after application, 15DAA = 15 days after application and rehydration). Analysis of variance (ANOVA) and the Scott Knott test were used, at 0.05% significance ($P \leq 0.05$), besides the software Sisvar, version 4.3 (Universidade Federal de Lavras, Lavras, Brazil).

Conclusion

This study illustrated the protective effect of chitosan and its derivative produced from the addition of the *N,O-dicarboxymethyl* (MCA) group to the structure of chitosan, which resulted in a significant increase in growth, physiological parameters and yield of the maize crop, even when subjected to drought conditions. There are more marked effects with the use of these biomolecules in the treatment of hybrid maize sensitive to water deficit (BRS 1030) compared to the genotype that already has a certain tolerance to this environmental stress (DKB 390). Chitosan and its derivatives overcame the stress caused by drought by increasing water availability and absorption, leading to the development of the root system and the adjustment of cellular osmotic pressure. The experimental results also showed a significant increase in chlorophyll concentration, indicating that chitosan can improve photosynthetic performance and, therefore, yield.

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