Tikrit Journal for Agricultural Sciences
ISSN:1813-1646 (Print); 2664-0597 (Online)
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

TJAS
Tikrit Journal for Agricultural Sciences

DOI: https://doi.org/10.25130/tjas.25.2.13

Chitosan nanoparticles improve corn yield in differentiating maize hybrids under drought stress

Bekhal Mustafa Hama

Biotechnology & Crop Science Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaimani, KRG, Iraq

Corresponding author: E-mail: ***bekhal.mustafa@univsul.edu.iq***

KEY WORDS:
water stress; Chitosan NPs; field capacity; antioxidant; productivity.

Received: 19/11/2024
Revision: 09/02/2025
Proofreading: 26/03/2025
Accepted: 25/05/2025
Available online: 30/06/2025

ABSTRACT

Drought is considered an important constraint that hampers the growth, development, and productivity of *Zea mays* crops worldwide. The study is intended to determine changes in the morphological, physiological, antioxidant, and yield parameters of corn crops caused by drought, and assess the effects of chitosan NPs in reducing physiological and biochemical changes and overcoming drought-induced yield losses. The drought was maintained during the vegetative phase on two drought-contrasting maize hybrids, ZP6666 (tolerant) and Drami (sensitive), by limiting irrigation and maintaining 50% field capacity (moderate DS), and 25% field capacity (severe DS). Chitosan NPs were sprayed with 100, 200, and 300 mg L^{-1} corn leaves. Using 100 mg L^{-1} chitosan NPs significantly increased most characteristics, except RWC, which showed a non-significant response in drought-prone maize leaves. The recovery of drought was notable in both hybrids. Water stress as moderate and severe drought stress conditions reduced kernel yield/pot, while spraying chitosan nanoparticles on maize leaves increased yields by 42 and 10 percent for tolerant and 8.5 and 9 percent for sensitive hybrids at both stress conditions respectively. This study suggests that chitosan NPs with concentrations of 100 mg L^{-1} play a remarkable role in combating the negative effects of drought. These nanoparticles can improve the plant's osmotic state, activate ROS elimination enzymes to maintain membrane integrity and cell protection, and increase yields in drought conditions.

جسيمات النانو الجيتوسان تحسن إنتاجية هجن الذرة المتناقضة تحت ظروف الجفاف

بيخال مصطفى حمه
قسم بايوتكنلوجى وعلم المحاصيل – كلية علوم الهندسة الزراعية – جامعة السليمانيه

الخلاصه

يعتبر الجفاف عائقاً هاماً يعيق نمو وتطور وإنتاجية محصـــول الذرة في جميع أنحاء العالم. تهدف هذه الدراســـة إلى تحديد التغيرات في العوامل المورفولوجية والفسيولوجية ومضادات الأكسدة وإنتاجية محصول الذرة الناجمة عن الجفاف، وتقييم آثار جسيمات النانو الجيتوسان في الحد من التغيرات الفسيولوجية والكيميائية الحيوية والتغلب على خسائر الغلة الناجمة عن الجفاف. تم الحفاظ على الجفاف خلال المرحلة الخضرية على اثنين من هجن الذرة المتناقضة مع الجفاف ZP6666(متحمل)، ودرامي (حساس)، عن طريق الحد من الري والحفاظ على السعة الحقلية بنسبة 50٪ (DSمعتدلة)، والسعة الحقلية 25٪ DS)شديدة). تم رش الجيتوسـان NPs بأوراق الذرة 100 و 200 و 300 ملغم/لتر. أدى اسـتخدام 100 ملجم/لتر من الجيتوسـان NPs إلى زيادة كبيرة في معظم الخصـائص، باستثناءRWC ، الذي أظهر استجابة غير معنوية في أوراق الذرة المعرضـة للجفاف. كان انتعـاش الجفـاف ملحوظًـا في كلا الهجينين. أدى الإجهـاد المـائي بســـبـب الجفـاف المعتـدل والشـــديـد إلى انخفـاض إنتـاج الحبوب/اصـيص، في حين أدى رش جسـيمات النانو الجيتوسـان على أوراق الذرة إلى زيادة الغلة بنسـبة 42 و10 في المائة للهجن المتحملة و8.5 و9 في المائة للهجن الحســـاســـة في كلتا ظروف الإجهاد على التوالي. تشـــير هذه الدراســـة إلى أن الجيتوسـان NPs بتركيزات 100 ملغم/لتر تلعب دوراً ملحوظاً في مكافحة الآثار السلبية للجفاف. هذه الجسيمات النانوية قادرة على تحسين الحالة الاسموزية للنبات ، وتنشيط إنزيمات إزالة ROS للحفاظ على سلامة الغشاء وحماية الخلايا، وزيادة الغلة في ظروف الجفاف.

الكلمات المفتاحية: الإجهاد المائي، الجيتوسان NPs ، السعة الحقلية، مضادات الأكسدة، الانتاجيه.

INTRODUCTION

Maize (*Zea mays* L.) or corn, is one of the most important crops in the world to its variety, high adaptability, and excellent nutritional value, and is considered to be the third most essential cereal after wheat and paddy around the world, it accounts for 4.8% of the total acreage and is attributed to 3.5% of the world's crop value in agricultural production (Deryng *et al*., 2014). It is a high-yield crop with the highest rate of photosynthesis among all food crops, and as a C4 plant, it can accumulate dry matter faster than rice, wheat, or other grains (Afrad *et al*., 2019). Sustainability of crop output is attained mostly by overcoming diverse eco-stressors, including high CaCO3 and salinity or drought. Cultivation of disreputable soils restrains agricultural productivity due to their low fecundity, nutritional imbalance, high ECe, and unavailability of water and nutrients (Belal *et al*., 2019). Calcareous soils are predominant in dry climates; the common characteristics of calcareous soils (e.g., availability of water and nutrients) are harmfully affected by high pH value (7.5−8.5) and carbonate content (Belal *et al*., 2019). These unfavorable situations prohibit plant growth and production through overproducing ROS, adversely influencing physio-biochemical traits, osmoregulation, and antioxidant defense systems (Belal *et al*., 2019). Otherwise, saline soils are common, predominantly in dry districts. Salinity stress causes starvation, "physiological drought", and osmotic stress. It constrains plant growth and production via the influences of overproducing ROS on physio-biochemical indices and defense systems in plants (Belal *et al*., 2019). The reverse effects on plants are aggravated by the incorporation of saltiness and calcareousness (saline-calcareous soil), and the soil becomes unproductive. Therefore, it is necessary to use plants that are tolerant of such soils along with treating them foliarly with bio-stimulators to raise their tolerance further to stress environments.

As one of several harmful influences of climatic variation, long-term drought is a major challenge in the 21st century. In the coming years, more droughts and water shortages are expected to occur in many countries (Spinoni *et al*., 2021). Each year, drought causes major damage to agriculture, urban landscapes, and pasture sand forests annually. Drought stress (DS) inhibits plant growth and hurts plant physiology, morphology, and productivity (Bayat & Moghadam, 2019). Water deficit conditions affect plant physiological and biochemical processes and result in lower yields. (Daryanto *et al*., 2016) reported that crop yields declined by more than 60% due to a water shortage based on many soil and plant factors. Shortage of water reasons osmotic pressure, which can cause damage to tissue oxidation, membrane damage, proteolysis, and oftentimes high concentrations of reactive oxygen (ROS) (Popović *et al*., 2016). Water shortage stress has significantly reduced growth, turgor, water potential, osmotic pressure modification, photosynthesis, and stomatal conductivity (Sheshbahreh *et al*., 2019). Furthermore, stress on water shortages has reduced cell membrane stability, chlorophyll content, and nutrient absorption, as well as increased plant oxidative injury (Nadeem *et al*., 2019). Plants adapt various physiological and chemical mechanisms to alleviate the detrimental impacts of drought (Ali *et al*., 2021). For example, the accumulation of osmolytes like proline, carbohydrate, and amino acids mentions cell swelling and osmotic modification (Hassan *et al*., 2021). The reduction in stomata and the increase in activity of enzymatic antioxidants are other mechanisms for eradicating ROS and reducing DS in plants (Ali *et al*., 2021). In conditions of severe water stress, these mechanisms are inadequate, acquiring external applications of certain substances, comprising natural and organic composites, and increasing crop resistance (Hassan *et al*., 2018).

One of the pioneering approaches that have gained momentum is taking on the chitosan nanoparticles (CNPs) as a nutriment that also decreases crop water loss. Chitosan NPs are attained by deacetylation of Chitin, are a little poisonous, uncomplicated to gain, and a cheap composite largely used in agriculture and medicine (Morin-Crini *et al*., 2019). Numerous studies have displayed that CNPs-foliate has many advantages, comprising a reduction in leaf water loss (Attaran *et al*., 2022), improved plant growth and development underneath nonliving pressures (Coelho and Romano, 2022), and the capability to prevent adverse impacts on crops under critical environments. In addition, chitosan NPs have been shown to improve crop growth and productivity by ameliorating moisture and necessary nutrient absorption (Makhlouf *et al*., 2022). The plant that received chitosan nanoparticles had higher quantities of amino acids on the leaves, which meant that the absorption of nitrogen or mobilization was increased, or the utilization of this nutrient was more efficient, and this nutrient was advantageous by the exogenous response of chitosan nanoparticles (Li *et al*., 2017). However, it is not well understood how CNPs affect adverse conditions, although there is evidence that chitosan and its derivatives improve crop tolerance to drought stress by reducing the negative impact of water shortage on harvest indexes and yields (Makhlouf *et al*., 2022). The size of chitosan NPs is smaller than that of ordinary chitosan (less than 100 nm), has a high aspect proportion, and a larger surface area (Hassan *et al*., 2021). They improve plant metabolic activity and more efficiently transport active chemicals through the cell membrane (Bandara *et al*., 2020). CNPs have proven to be advantageous to plant quality and yield, but report restricted capacity to stimulate plant defense schemes under abiotic stress, such as water stress (Hassan *et al*., 2021). Therefore, we used biopolymer to test the assumption that exogenous application of chitosan nanoparticles would boost the main, antioxidant, and osmoregulation metabolisms of maize and consequently mitigate the harmful influence of drought stress on kernel yield.

MATERIALS AND METHODS

(1) Plant Materials:

In this study, two water shortage contradicting maize hybrids, ZP6666 (tolerant) and Drami (sensitive), used, based on the results of *in vitro* tests of 17 maize hybrids to water shortage by polyethylene glycol-MW 6000 (20 gm L^{-1}) (additional data are given in supplementary table 1), were gathered from the Agricultural Research Center, Ministry of Agriculture, Sulaimani, Kurdistan region, Iraq.

(2) Nanoparticles Characterization and Preparation of its Suspension:

Chitosan nanoparticles were commercially purchased from Iranian Nanomaterials Pioneers Company, NANOSANY (Mashhad, Iran). The characteristics of CNPs were 50 nm in size, with a molecular weight of 161g/mol and 99% purity. To prepare a suspension of CNPs, the nanoparticles dissolved in 1% acetic acid, then diluted with distilled water and heated for 2 hours at 90°C at 1200 rpm. The pH of the solution was adjusted with 1N sodium hydroxide (pH=6.5–7) (Li *et al*., 2008). Finally, different doses of the chitosan NPs (100, 200, and 300 mg L^{-1}) were prepared for the pot test.

(3) Experimental Design Components, Plant Treatment, Cultivation Conditions:

The experiment involved three groups. The plants in the first group were well-watered (with 100% field capacity), the plants in the second group stressed with 50% field capacity (moderate DS), and the third category included plants that stressed with 25% field capacity (severe DS). To conduct this study, two factors were used in a completely randomized design (CRD). The first constituent represented maize hybrids (one sensitive and one tolerant) and the second represented the treatment group, consisting of Chitosan nanoparticles prepared at four concentrations (0, 100, 200, and 300 mg L^{-1}). The experiment was conducted on April 13th, 2023 at the College of Agricultural Engineering Sciences—University of Sulaimani (latitude 35o 33" N, 45o 27" E, altitude 884.8 masl). Each treatment consists of four replications. Seeds of two hybrids planted in plastic trays in a plastic house. Then the seedlings transplanted on April 25th into the plastic pots, one plant per pot (diameter 30 cm, height 40 cm) filled with an equal amount of soil containing Silty Clay loam in texture, with an EC of 0.25 dSm^{-1}, a pH of 8.15, an organic carbon content of 1.55%, a total nitrogen content of 0.25%, Available Phosphate P of 8.836 $mgkg^{-1}$, a Soluble Potassium K^+ of 4.496 $mgkg^{-1}$, and CaCo3 of 26%. Soil preparation was proposed according to the needs of soil and crop, with amounts of 5.652 gpot-1 of each phosphorus as triple superphosphate and nitrogen as urea (46% N) fertilizer. Soil moisture content was measured according to the gravimetric method (Datta *et al*., 2009) and monitored daily until the end of the experiment using a soil moisture meter (SOIL MOISTURE MONTOR WITH TIME DISPLAY, MODEL NO: WH0291). The pots are irrigated with tap water (when the humidity drops below a certain level) to keep the moisture content at the desired level. After 45 days after seeding (45 DAS), the solutions were supplied via foliar application through a coastal sprayer, whose spray pressure was obtained using a CO2 cylinder and controlled by a low-pressure manometer at a flow rate of 102 L ha^{-1} and a pressure of 3 BAR two times with four-day intervals at three stages, knee stage (V5), tasseling stage (VT), and milk stage (R3) which considered critical period for abiotic stress. All measures were taken to prevent the contact of the sprayed CNPs solutions to neighboring plants. The experiment was carried out until harvest.

(4) Growth Parameters Evaluation:

At growth stage R3, three plants per treatment were used for measuring growth characteristics, such as plant height (cm) using a tape meter, root fresh weight (g), root dry weight (g), total plant fresh weight (g), total plant dry weight (g) and leaf area index (%) using the method described by (Sanderson, Daynard, & Tollenaar, 1981).

$$LA = Max\ Length \times Max\ Widith \times 0.75$$

$$LA/\ plant = LA \times Number\ of\ leaf$$

$$LAI = (LA/\ plant)\ /\ area\ of\ the\ pot \qquad (1)$$

(5) Physiological Characteristics Evaluation:

For each treatment at milk stage R3, physiological characteristics measured such as Total Chlorophyll Content (mgg^{-1}) using a SPAD-meter.

Relative Water Content (RWC) was determined according to (Reynolds, Pask, & Mullan, 2012) for each sample following the formula.

$$\text{RWC (\%)} = [(FW - DW)/TW - DW)] * 100 \qquad (2)$$

Where, FW: leaf fresh weight; DW: leaf dry weight; TW: leaf turgid weight.

Relative Electrical Conductivity or Electrolyte Leakage (EL) was calculated by the formula described by (Chang *et al*., 2016).

$$\text{EL (\%)} = (EC1/EC2) * 100 \qquad (3)$$

Where, EC1: initial electrical conductivity of the solution at 32 °C; EC2: final electrical conductivity of the solution at 100 °C

Membrane stability index (MSI) was calculated following the equation proposed by (Basu, Kumari, Kumar, Kumar, & Rajwanshi, 2021).

$$\text{MSI (\%)} = [1 - (EC1/EC2)] * 100 \qquad (4)$$

(6) Measurement of Biochemical Traits:

At milk stage R3, Soluble sugar content (SSC in μgg^{-1}), and Proline Content (PC in μgg^{-1}) in leaf samples was determined following the method defined by (Lateef, Mustafa, & Tahir, 2021).

The content of total phenolic compounds (TPC in μgg^{-1}) using the Folin–Ciocalteu method and Antioxidant capacity by DPPH (μg $Troloxg^{-1}$ FM) in each extract was measured according to (Lateef *et al*., 2021).

(7) Yield and its Component Characters Evaluation:

At the harvest time, yield and yield components were recorded such as No. of kernel per ear, kernel yield per Pot (g), Biological Yield per Pot (g), and Harvest Index (%). The harvest index (HI) was accounted for following (Ion *et al*., 2015):

$$\text{HI} = (Kernel\ yield/Biological\ yield) * 100 \qquad (5)$$

(8) Statistical Analysis:

For all analyzed parameters, the means and the standard error (SE) were calculated. For the statistical analysis of the results, the two-way variance analysis (ANOVA) and Duncan multi-range test with 0.05% of the XLSTAT 2019 version (Boston, USA) for assessing the data obtained in this study. Pearson correlation was carried out using XLSTAT version 2019 was used for performing principal component analysis (PCA) and agglomerative hierarchical clustering (AHC).

RESULTS AND DISCUSSION

Table 1 shows the variance analysis of the traits studied. All attributes investigated revealed significant differences in all stress treatments. When maize materials were subjected to stress treatment, significant reactions between maize hybrids for the tested parameters were observed, except for relative water content (RWC). Similar reactions have been reported from maize hybrids as a result of current treatment. As far as the interaction between hybrids and characteristics is concerned, most characteristics have shown significant changes, except for RWC, which showed a non-significant response.

Table (1) Variance analysis of all studied traits and it is interaction with two maize hybrids under the presence of drought stress, foliar application of chitosan nanoparticles (CNPs).

Traits	Hybrids		Treatments		Hybrids*Treatments	
	F	Pr > F	F	Pr > F	F	Pr > F
PH	2873.892	< 0.0001	12687.013	< 0.0001	532.253	< 0.0001
RFW	388394.898	< 0.0001	432096.638	< 0.0001	77133.877	< 0.0001
RDW	69262.837	< 0.0001	79139.862	< 0.0001	15626.276	< 0.0001
TPFW	1635500.340	< 0.0001	2700212.125	< 0.0001	272212.053	< 0.0001
TPDW	771997.800	< 0.0001	876329.453	< 0.0001	303619.471	< 0.0001
LAI	505.449	< 0.0001	301.384	< 0.0001	11.407	< 0.0001
RWC	0.222	0.641	1.948	0.082	1.317	0.266
EL	588.782	< 0.0001	1896.596	< 0.0001	181.516	< 0.0001
MSI	588.782	< 0.0001	1896.596	< 0.0001	181.516	< 0.0001
TCC	6199.204	< 0.0001	3523.638	< 0.0001	185.375	< 0.0001
SSC	3759.900	< 0.0001	828.506	< 0.0001	144.491	< 0.0001
PC	2745.033	< 0.0001	440.268	< 0.0001	150.829	< 0.0001
TPC	35.826	< 0.0001	244.395	< 0.0001	22.723	< 0.0001
DPPH	3853.477	< 0.0001	667.004	< 0.0001	140.298	< 0.0001
K/E	2656.457	< 0.0001	2233.743	< 0.0001	117.593	< 0.0001
KY/P	12595.657	< 0.0001	10778.736	< 0.0001	576.498	< 0.0001
BY/P	11626.833	< 0.0001	84522.533	< 0.0001	8604.368	< 0.0001
HI	3321.675	< 0.0001	3042.413	< 0.0001	451.007	< 0.0001

PH: Plant Height; RFW: Root Fresh Weight ;RDW: Root Dry Weight; TPFW: Total Plant Fresh Weight; TPDW: Total Plant Dry Weight; LAI: Leaf Area Index; RWC: Relative Water Content; EL: Electrolyte Leakage; MSI: Membrane Stability Index; TCC: Total Chlorophyll Content; SSC: Soluble Sugar Content; PC: proline content; TPC: total phenolic content; DPPH: Antioxidant assay (DPPH); K/E: Number of Kernel per Ear; KY/P: Kernel Yield per Pot; BY/P: Biological Yield per pot; HI: Harvest Index.

Moderate drought stress (50% FC) reduced plant height, root fresh weight, root dry weight, total plant fresh weight, total plant dry weight and leaf area index by 38.73%, 86.08%, 87.09%, 81.59%, 86.40% and 62.06% respectively, while severe drought stress (25% FC) decreased these growth parameters by 68.15%, 91.18%, 94%, 88.94%, 96.41% and 74.63% respectively compared to control condition. Application of chitosan NPs on foliage at a concentration of 100 mg L^{-1}

increased all growth characteristics in both stress conditions by 20.04%, 61.82%, 64.95%, 54.70%, 55.16% and 37.83% in MS and by 44.40%, 35.44%, 63.78%, 55.95%, 76.51% and 52.87% in SS for each PH, RFW, RDW, TPFW, TPDW, and LAI respectively (table 2). Maize hybrids reduce plant height under drought stress, while preserving plant height with the availability of chitosan NPs (MS+CNPs) and (SS+CNPs). Under current drought conditions (MS) and (SS), root dry matter accumulation reductions of more than 78 and 84 grams respectively were detected compared to control conditions, and the reduction in all available applications of CNPs was reduced. Similar reactions of total plant dry weight accumulation were observed, and reactions to CNPs application detected a higher accumulation of 11 and 23 grams in response to both stress conditions. The maximum value of the leaf area index was recorded at 0.485% under the control conditions, followed by MS+CNP100 mg L^{-1} (0.296%), MS+CNP100 mg L^{-1}, SS+CNP200 mg L^{-1} and MS+CNP200 mg L^{-1}, which recorded 0.261%, 0.256% and 0.255% respectively, while the minimum value of this property was recorded under current drought conditions (SS and MS) with 0.123% and 0.184% respectively.

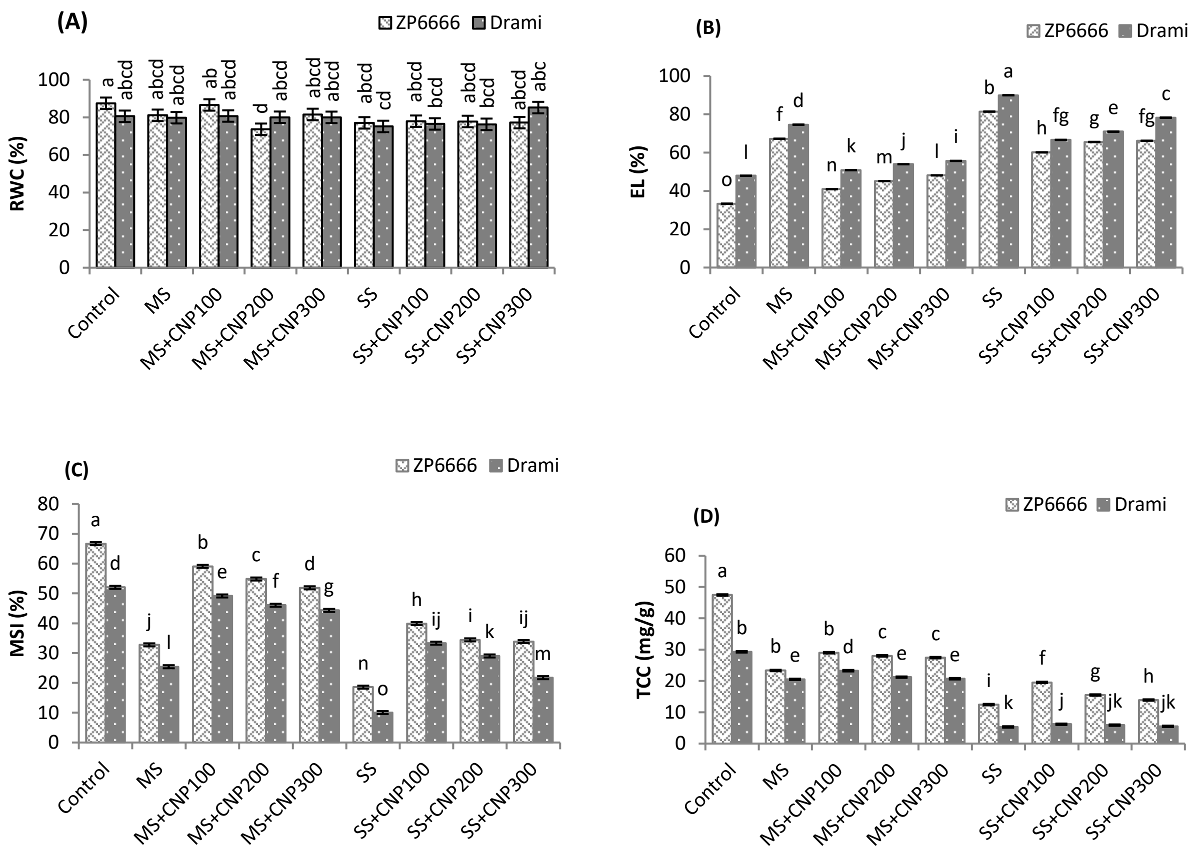
Table (2) Growth parameters during stress and chitosan nanoparticles (CNPs) application on two drought contrasting hybrids (ZP6666 and Drami) subjected to the various treatments.

Treatments	PH (cm)	RFW (g)	RDW (g)	TPFW (g)	TPDW (g)	LAI (%)
Control	138.000 a	232.882a	90.383 a	557.372a	197.969 a	0.485 a
MS	84.550 e	32.415g	11.665 f	102.587f	26.904 f	0.184 e
MS+CNP100	105.750 b	84.913b	33.283 b	226.475b	60.006 b	0.296 b
MS+CNP200	100.067 c	71.022c	21.597 c	174.477c	48.886 c	0.255 c
MS+CNP300	97.133 d	37.085d	17.457 d	134.457e	32.303 d	0.218 d
SS	43.950 i	20.533g	5.420 i	61.617h	7.092 i	0.123 f
SS+CNP100	79.050 f	31.807f	14.968 e	139.885d	30.196 e	0.261 c
SS+CNP200	69.200 g	20.512g	10.447 g	81.110g	19.302 g	0.256 c
SS+CNP300	52.633 h	9.453h	8.795 h	36.738i	15.456 h	0.195 e
Pr > F(F2)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes

PH: Plant Height; RFW: Root Fresh Weight, RDW: Root Dry Weight; TPFW: Total Plant Fresh Weight; TPDW: Total Plant Dry Weight; LAI: Leaf Area Index; Control: untreated plants with CNPs grown under normal condition ; MS: Moderate Stress (untreated plants with CNPs grown under stress condition 50% FC); MS+CNP100: Moderate Stress + CNPs100 mg L^{-1}; MS+CNP200: Moderate Stress + CNPs200 mg L^{-1}; MS+CNP300: Moderate Stress + CNPs 300 mg L^{-1}; SS: Sever Stress (untreated plants with CNPs grown under stress condition 25% FC); SS+CNP100: Sever Stress + CNPs100 mg L^{-1}; SS+CNP200: Sever Stress + CNPs200 mg L^{-1}; SS+CNP300: Sever Stress + CNPs300 mg L^{-1}. Different letters represent a significant difference between the mean values according to Duncan's Multiple-Range Test ($p \leq 0.0001$).

According to the results of the interaction between treatments and hybrids, there was not significant difference between the two hybrids in relative water content (fig. 1). Other important characteristics that have been widely used to study the performance of the studied materials and their response to any available stress conditions are electrolyte leakage and membrane stability. Under control condition, leakage rate were 33.3 and 47.9 percent for the maize hybrids ZP6666 (tolerant) and Drami (sensitive) respectively, while under both conditions of water shortage (MS and SS) these materials suffered a large leakage rate were 67.2, 81.4, 74.5 and 98.9 percent respectively. However, due to the availability of exogenous applications, the leaking of electrolytes by maize hybrids has reduced by nearly more than 20 percent compared to moderate and severe stress conditions alone. As indicated in figure 1, the maximum stability of the membrane was achieved under control condition at 66.6% and 52% for both hybrids, while poor performance of this trait was noted under stress conditions without exogenous treatments. However, exogenous CNPs application at both stress conditions improved stability, which around 20% of the reduction compared to untreated circumstances, was documented. The content of total chlorophyll was sharply reduced by both maize hybrids to reach value at MS to 23.33, 20.5 mgg^{-1} and at SS to 12.4, 5.3 mgg^{-1} respectively compared to the control condition, while exogenous application of CNPs eliminated this reduction.

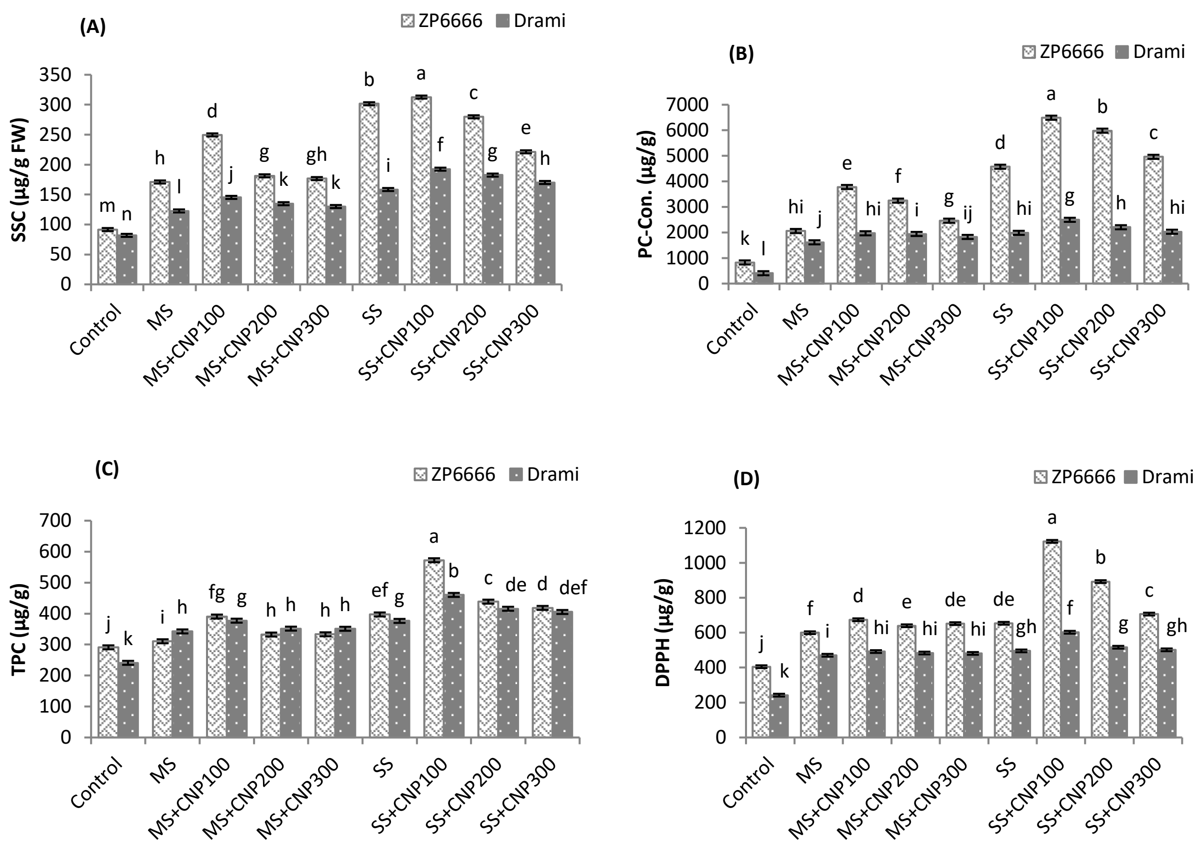
Figure 1. Physiological characteristics during stress and chitosan nanoparticles (CNPs) application on two drought contrasting hybrids (ZP6666 and Drami) subjected to the various treatments, RWC (Relative Water Content, A), EL (Electrolyte Leakage, B), MSI (Membrane Stability Index, C), and TCC (Total Chlorophyll Content, D).



ZP6666: Tolerant Hybrid; Drami: Sensitive Hybrid; Control: untreated plants with CNPs grown under normal condition ; MS: Moderate Stress (untreated plants with CNPs grown under stress condition 50% FC); MS+CNP100: Moderate Stress + CNPs100 mg L^{-1}; MS+CNP200: Moderate Stress + CNPs200 mg L^{-1}; MS+CNP300: Moderate Stress + CNPs 300 mg L^{-1}; SS: Sever Stress (untreated plants with CNPs grown under stress condition 25% FC); SS+CNP100: Sever Stress + CNPs100 mg L^{-1}; SS+CNP200: Sever Stress + CNPs200 mg L^{-1}; SS+CNP300: Sever Stress + CNPs300 mg L^{-1}. Different letters represent a significant difference between the mean values according to Duncan's Multiple-Range Test ($p \leq 0.0001$).

In this study, four basic biochemical traits were studied, including SSC (µg/g PW), PC (µg/g), TPC (µg/g), and DPPH (µg/g). Drought stress and exogenous applications of CNPs affected the activity of biochemical properties significantly ($p < 0.0001$) (fig. 2). CNPs at a concentration of 100 mg L^{-1} at severe drought stress was promoting accumulation of SSC, PC, TPC, and DPPH with values of 312.716, 6491.923, 572.247, and 1122.702 µg/g respectively in ZP6666 hybrid (tolerant). Regarding the hybrid Drami (sensitive), CNPs at 100 mg L^{-1} at severe stress also improved the accumulations in all studied traits: SSC (192.345 µg/g), PC (2495.769 µg/g), TPC (460.262 µg/g), and DPPH (602.432 µg/g) compared to rest treatments. The response of the two drought contrasting hybrids to stress conditions without external application of CNPs, similar accumulation pattern in all studied traits with respect to control conditions were observed, with values of SSC (158.395 µg/g), PC (1986.153 µg/g), TPC (376.367 µg/g), and DPPH (495.675 µg/g) (fig. 2).

Figure (2) Biochemical attributes during stress and chitosan nanoparticles (CNPs) application on two drought contrasting hybrids (ZP6666 and Drami) subjected to the various treatments, SSC (Soluble Sugar Content, A), PC (Proline Content, B), TPC (Total Phenolic Content, C), and DPPH (Antioxidant assay, D).



ZP6666: Tolerant Hybrid; Drami: Sensitive Hybrid; Control: untreated plants with CNPs grown under normal condition ; MS: Moderate Stress (untreated plants with CNPs grown under stress condition 50% FC); MS+CNP100: Moderate Stress + CNPs100 mg L^{-1}; MS+CNP200: Moderate Stress + CNPs200 mg L^{-1}; MS+CNP300: Moderate Stress + CNPs 300 mg L^{-1}; SS: Sever Stress (untreated plants with CNPs grown under stress condition 25% FC); SS+CNP100: Sever Stress + CNPs100 mg L^{-1}; SS+CNP200: Sever Stress + CNPs200 mg L^{-1}; SS+CNP300: Sever Stress + CNPs300 mg L^{-1}. Different letters represent a significant difference between the mean values according to Duncan's Multiple-Range Test ($p \leq 0.0001$).

Table 3 shows the yield characteristics of plants grown under water-stressed and fully irrigated conditions. Spray of CNPs at a concentration of 100 mg L^{-1}, under both water stress conditions (MS+CNP100 and SS+CNP100), successfully increased almost all studied yield parameters in comparison to the respective treatments of untreated plants with CNPs (MS and SS). This treatment effectively enhanced number of kernel per ear (575.000; 284.667) and (375.667; 195.333), kernel yield per pot (204.670 g; 100.070 g) and (132.933 g; 69.823 g), and biological yield (161.500 g; 60.553 g) and (185.620 g; 52.647 g), respectively for both tolerant hybrid (ZP6666) and sensitive hybrid (Drami) compared to corresponding stress conditions (MS and SS). The availability of stress conditions without application of CNPs has significantly reduced all the yield characteristics evaluated, as opposed to the conditions fully irrigated for both hybrids except harvest index (HI). Application of 300 mg L^{-1} CNPs at severe stress condition (SS+CNP300) did not increase kernel yields for sensitive hybrid (Drami). Therefore, it can be inferred that the sprinkle of 100 mg L^{-1} CNPs on the leaves not only mitigates the consequences of drought, but also increases the yield of maize under water stress.

Table (3) Production characteristics in contrasting drought tolerant maize hybrids (ZP6666 and Drami) grown under different treatments.

Treatment	Control	MS	MS+CNP 100	MS+CNP 200	MS+CNP 300	SS	SS+CNP100	SS+CNP 200	SS+CNP300
					ZP6666				
KE	622.667a	332.000g	575.000b	482.667c	404.333d	253.000j	284.667h	266.667i	256.000ij
KY (g)	221.667a	121.633g	204.670b	170.050c	143.073d	89.257k	100.070i	94.063j	90.737k
BY (g)	372.333a	139.527f	161.500d	139.607f	129.630g	55.487j	60.553i	50.343l	45.417n
HI (%)	59.533o	84.473l	126.730g	121.811h	110.372j	160.874d	165.273c	186.855b	199.796a
					Drami				
K/E	482.000c	343.333f	375.667e	372.667e	353.000f	178.667l	195.333k	182.667l	166.333m
KY (g)	171.640c	117.860h	132.933e	131.303e	124.690f	63.367m	69.823l	65.073m	59.240n
BY (g)	217.640b	144.973e	185.620c	115.473h	116.027h	51.363l	52.647k	48.217m	40.630o
HI (%)	78.864m	83.902l	71.617n	113.711i	107.470k	123.419h	132.638f	134.962f	145.812e
Pr > F(F1*F2)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

ZP6666: Tolerant Hybrid; Drami: Sensitive Hybrid; Control: untreated plants with CNPs grown under normal condition ; MS: Moderate Stress (untreated plants with CNPs grown under stress condition 50% FC); MS+CNP100: Moderate Stress + CNPs100 mg L^{-1}; MS+CNP200: Moderate Stress + CNPs200 mg L^{-1}; MS+CNP300: Moderate Stress + CNPs 300 mg L^{-1}; SS: Sever Stress (untreated plants with CNPs grown under stress condition 25% FC); SS+CNP100: Sever Stress + CNPs100 mg L^{-1}; SS+CNP200: Sever Stress + CNPs200 mg L^{-1}; SS+CNP300: Sever Stress + CNPs300 mg L^{-1}. Different letters represent a significant difference between the mean values according to Duncan's Multiple-Range Test ($p \leq 0.0001$).

Pearson's Correlation Coefficient (PCC) and Principal Component Analysis (PCA):

Pearson's correlation analysis of parameters such as morphological, physiological, biochemical properties, and yield characteristics was evaluated and presented in table 4. The results indicated significant direct correlations between the leaf area index and physiological characteristics like relative water content, membrane stability index, total chlorophyll content, and harvest parameters such as number of kernels per ear, kernel yield per pot, and biological yield per pot. In addition, the inverse relationship between the total soluble sugar and harvest parameters such as the number of kernels per ear, kernel yield per pot, and biological yield per pot have been observed in maize subjected to drought stress conditions.

Different treatment groups observed a clear separation and control of yield parameters subjected to drought stress. Principle component 1 dealt with a 94.16 % deviation, while principle component 2 described a 4.32 % deviation in the results (Fig. 3 A). The control (untreated plants with CNPs grown under normal conditions) and moderate stress with 100 (MS+CNP100) and 200 mg L^{-1} CNPs treatments (MS+CNP200) in clade 2 were far away from the moderate stress (MS) and moderate stress with 300 mg L^{-1} CNPs treatment (MS+CNP300) located in the clade 4. Interestingly, the treatments were located in different directions, indicating different performances in response to yield characteristics. In clade1, severe stress with 100 (SS+CNP100), 200 (SS+CNP200) and 300 mg L^{-1} CNPs treatments (SS+CNP300) only show a strong connection to harvest index (HI), while only severe stress treatment (SS) placed in clade 3 don't show any association with yield parameters. Dendograms cluster the different treatment groups into three main groups (Fig. 3 B). According to cluster analysis, the first group was implicated the control treatment, while moderate stress (MS), moderate stress spraying with 100 (MS+CNP100), 200 (MS+CNP200), and 300 mg L^{-1} (MS+CNP300) CNPs were grouped into the same cluster, and severe stress (SS), severe stress with spraying 100 (SS+CNP100), 200 (SS+CNP200), and 300 mg L^{-1} (SS+CNP300) CNPs were placed in another cluster. The analysis of both samples and groups shows that the concentration of CNPs at 100 mg L^{-1} helped recover maize plants from drought stress.

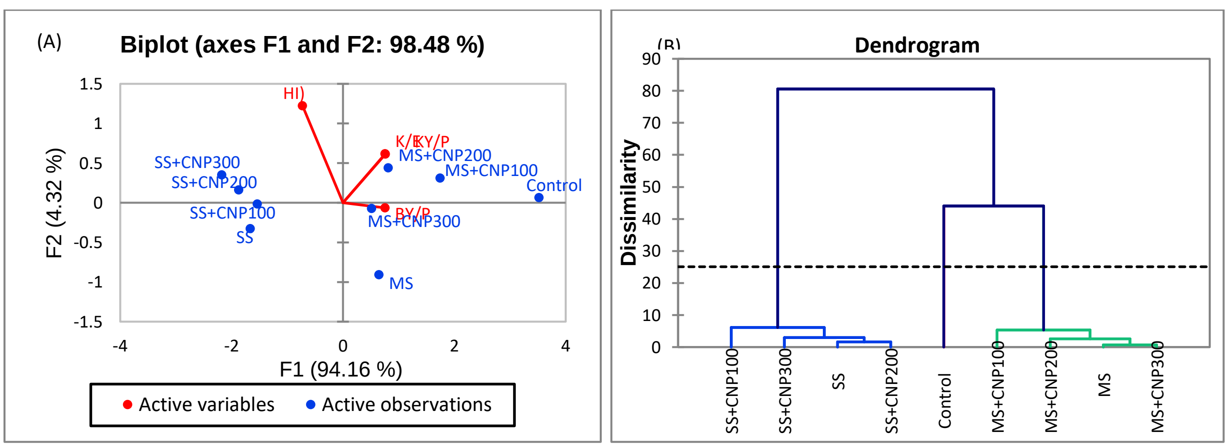


Figure (3) Biplot projection of Principal components 1 as well as 2 (A) and dendogram clustering of different yield parameters during moderate and severe drought stress and CNPs at different concentrations (B).

Table (4) Pearson's Correlation-coefficient among various growth, physiological, biochemical as well as yield traits during moderate and severe stress conditions. Blue color show positive correlation; red color shows inverse correlation

Variables	PH	RFW	RDW	TPFW	TPDW	LAI	RWC	EL	MSI	TCC	TSS	PC	TPC	DPPH	K/E	KY/P	BY/P	HI
PH	1																	
RFW	0.779	1																
RDW	0.758	0.994	1															
TPFW	0.822	0.980	0.974	1														
TPDW	0.727	0.968	0.963	0.967	1													
LAI	0.779	0.898	0.895	0.935	0.871	1												
RWC	0.454	0.558	0.595	0.528	0.525	0.379	1											
EL	-0.914	-0.724	-0.709	-0.755	-0.665	-0.748	-0.471	1										
MSI	0.914	0.724	0.709	0.755	0.665	0.748	0.471	-1.000	1									
TCC	0.879	0.843	0.824	0.863	0.811	0.818	0.546	-0.862	0.862	1								
TSS	-0.489	-0.374	-0.350	-0.396	-0.388	-0.138	-0.288	0.279	-0.279	-0.357	1							
PC	-0.431	-0.342	-0.320	-0.345	-0.336	-0.091	-0.337	0.176	-0.176	-0.260	0.938	1						
TPC	-0.575	-0.479	-0.440	-0.455	-0.419	-0.283	-0.314	0.355	-0.355	-0.547	0.774	0.771	1					
DPPH	-0.346	-0.311	-0.291	-0.293	-0.283	-0.046	-0.268	0.102	-0.102	-0.184	0.881	0.935	0.809	1				
K/E	0.879	0.826	0.804	0.820	0.735	0.776	0.541	-0.888	0.888	0.953	-0.339	-0.276	-0.564	-0.242	1			
KY/P	0.881	0.830	0.808	0.823	0.738	0.777	0.546	-0.887	0.887	0.952	-0.343	-0.281	-0.566	-0.247	1.000	1		
BY/P	0.867	0.898	0.881	0.920	0.894	0.793	0.602	-0.746	0.746	0.913	-0.597	-0.545	-0.660	-0.479	0.869	0.872	1	
HI	-0.669	-0.520	-0.490	-0.563	-0.528	-0.390	-0.426	0.431	-0.431	-0.591	0.798	0.839	0.715	0.694	-0.565	-0.567	-0.785	1

Soil water shortage (also known as drought stress) is one of the most common abiotic stresses affecting plant photosynthesis and agricultural production worldwide through various biochemical, physiological, and molecular processes (Anjum *et al*., 2016). Water stress has a negative influence on plant growth, BY, RWC, membrane integrity, and photosynthetic rate (Moolphuerk *et al*., 2022). Under this situation, various plant reactions occur, including biochemical and physiological changes (Toscano *et al*., 2016). Based on variance analysis of the traits studied, Scarcity of water decreases all growth, physiobiochemical and yield parameters of maize (Table 1) may be due to lack of nutrients from the soil and nutrient uptake by the roots, in part, because the drop in soil moisture results in a reduced rate of diffusion of nutrients from the soil matrix to the absorbing root surface (Waraich *et al*., 2011). Additionally, in this study based on soil analysis, maize crop were grown in defective soil and faced some stresses. Among them, osmotic stress, physiological drought, high pH (8.15), high $CaCO_3$ content (26 %), low EC (0.25 dSm^{-1}), nutrient deficiency, etc. These stressors definitely make the soil lower productive. Consequently, to get an appropriate and pleasing crop production from this tested defective soil, a tolerant crop such as maize hybrid (ZP6666) should be used and treated (leafy spraying) with a biopolymer, especially the tested CNPs (100 mg L^{-1}). This has been used successfully to overcome many stresses and have been documented as effective treatments for stressed plants (Alzahrani and Rady, 2019). Under both drought stress conditions (MS and SS), a significant ($p < 0.001$) decrease in physiological parameters and productivity (Fig. 1 and Tables 2 and 3) may be due to a lessening in soil moisture absorption and the ensuing reduction in cell division, extension, and plant growth (Yadav *et al*., 2021).

The oxidative damage caused by the water shortage disrupted chloroplast and chlorophyll synthesis. Water-deficit stress also destabilized membrane structures due to increased oxidative damage, as evidenced by reduced membrane stability index in water-deficit stress. Others report that oxidative damage caused by water deficit stress may be the main cause of reduced chlorine and MSI content (Ahmadi *et al*., 2010). The foliar application of chitosan alleviated oxidative damage, and the subsequent improvement of chlorophyll content, and MSI. (Dehghanipoodeh *et al*., 2018) also observed a substantial rise in chlorophyll and MSI from chitosan. In contrast to the MSI results, the EL of plants exposed to severe DS was 2.442 times greater than in plants with regimes irrigated regularly. The outcomes of this study show that plants treated with 100 mg L^{-1} CNPs had a 26.151% lesser EL than untreated plants underneath severe DS. These results are compatible with those described by other authors (Hafez *et al*., 2020; Liu *et al*., 2020). Hidangmayum *et al*., (2019) informed that the use of chitosan could be a better option to improve the growth of plants in a stressed environment, especially under non-biological pressure. Various strategies have been implemented to combat the harmful effects of climate change, from irrigation management to genetic engineering of crops through technological interventions (Liu *et al*., 2021). The recent advances in nanobiotechnology have a large potential to improve agricultural productivity by improving plant tolerance mechanisms (Liu *et al*., 2021). Low concentrations of nanomaterials (100 mg L^{-1}) have been shown to improve plant resistance to abiotic stresses, including activating plant cell signals because of great ROS manufacture and/or reactive nitrogen species (RNSs), motivating plant defense mechanisms that include enzymes and non- enzyme antioxidants (Khan *et al*., 2017). Some earlier studies have shown that low concentrations of CNPs (100 mg L^{-1}) stimulate plant growth and DS defense reactions (Bakhoum *et al*., 2022). In the test, three concentrations of CNPs (100, 200, and 300 mg L^{-1}) were studied in maize under water stress and in comparison with unstressed plants (CNP= 0 mg L^{-1}). When plants are prone to water shortage and other stressors, they stimulate the production of antioxidant enzymes, such as TPC, antioxidants, SGS, and PC, neutralize reactive oxygen species (ROSs), which otherwise damage plants (Yadav *et al*., 2021). In our study, both drought stress (MS and SS) increased SSC, PC, TPC, and DPPH compared to control treatment (Fig. 2). One of the physiological reactions of corn plants that tolerate water shortages is to activate enzyme antioxidant systems that absorb ROS (Noman *et al*., 2015). However, sensitive corn species avoided show this capability to scavenge ROS, not only by showing low photosynthesis rates when exhibited to prolonged stress (de Souza *et al*., 2014). However, studies have displayed that the use of chitosan increases carbon assimilation in plants, in addition to inducing these antioxidant defenses. Proline and sugar are important osmoregulators of corn, and even under water-deficiency conditions (Sun *et al*., 2016), water can remain within the foliar cells. In addition, under drought conditions, polysaccharides decompose, forming osmolytes such as soluble sugar, thereby enabling plants to maintain cell turgor (Nazarli *et al*., 2010), and is considered to be a mechanism for adapting to drought stress conditions. Chitosan and its derivatives led to a more aggregation of these composites, thereby improving the tolerance of sensitive hybrids (Drami) to water shortages. Under conditions of drought, the foliar application of chitosan in Trifolium Rema triggersa cascade of reactions that lead to more tolerance of water shortage. In this study, chitosan produced a larger gathering of sugars, proline and phenolic content. These compounds are related to the osmotic adjustment of plants and antioxidant protection under stress (Li *et al*., 2017).

CONCLUSION

This study revealed for the first time information about the mechanisms of *Zea mays* tolerance caused by CNPs in moderate and severe drought stress. CNPs (especially 100 mg L^{-1}) improve drought resistance of corn, which shows a significant increase in growth, physiological biochemical and yield responses under drought stress. CNPs can provide excellent alternatives to agrochemicals to mitigate water stress due to their ecological advantages (e.g. natural source, non-toxicity, safety, biodegradability, etc.).

ACKNOWLEDGMENTS

The Sulaimani University / College of Agricultural Engineering Sciences is acknowledged by the author for providing facilities for this study.

REFERENCES

Afrad, S. I., Wadud, F., and Babu, S. C. (2019). Reforms in agricultural extension service system in Bangladesh *Agricultural extension reforms in South Asia* (pp. 13-40): Elsevier.

Ahmadi, M., Malvar, R., and Campo, L. (2010). Biochemical changes in maize (Zea mays L.) seedlings exposed to drought stress at different nitrogen levels. *J. Crop Sci, 50*, 51-58.

Ali, E., El-Shehawi, A. M., Ibrahim, O., Abdul-Hafeez, E., Moussa, M., and Hassan, F. (2021). A vital role of chitosan nanoparticles in improvisation the drought stress tolerance in Catharanthus roseus (L.) through biochemical and gene expression modulation. *Plant Physiology and Biochemistry, 161*, 166-175.

Alzahrani, Y., and Rady, M.M. (2019). Compared to antioxidants and polyamines, the role of maize grain-derived organic biostimulants in improving cadmium tolerance in wheat plants, Ecotoxicol. *Environ. Saf*., 182, 109378.

Anjum, N. A., Sharma, P., Gill, S. S., Hasanuzzaman, M., Khan, E. A., Kachhap, K., . . . Vasudhevan, P. (2016). Catalase and ascorbate peroxidase—representative H 2 O 2-detoxifying heme enzymes in plants. *Environmental science and pollution research, 23*, 19002-19029.

Attaran Dowom, S., Karimian, Z., Mostafaei Dehnavi, M., and Samiei, L. (2022). Chitosan nanoparticles improve physiological and biochemical responses of Salvia abrotanoides (Kar.) under drought stress. *BMC Plant Biology, 22*(1), 364.

Bakhoum, G., Sadak, M., and Tawfic, M. (2022). Chitosan and chitosan nanoparticle effect on growth, productivity and some biochemical aspects of Lupinustermis L plant under drought conditions. *Egyptian Journal of Chemistry, 65*(5), 537-549.

Bandara, S., Du, H., Carson, L., Bradford, D., and Kommalapati, R. (2020). Agricultural and biomedical applications of chitosan-based nanomaterials. *Nanomaterials, 10*(10), 1903.

Basu, S., Kumari, S., Kumar, P., Kumar, G., and Rajwanshi, R. (2021). Redox imbalance impedes photosynthetic activity in rice by disrupting cellular membrane integrity and induces programmed cell death under submergence. *Physiologia Plantarum, 172*(3), 1764-1778.

Bayat, H., and Moghadam, A. N. (2019). Drought effects on growth, water status, proline content and antioxidant system in three Salvia nemorosa L. cultivars. *Acta Physiologiae Plantarum, 41*(9), 149.

Belal, E.E., El Sowfy, D.M., and Rady, M.M. (2019). Integrative soil application of humic acid and sulfur improves saline calcareous soil properties and barley plant performance, Commun. *Soil Sci. Plant Anal.*, 50, 1919–1930.

Chang, Z., Liu, Y., Dong, H., Teng, K., Han, L., and Zhang, X. (2016). Effects of cytokinin and nitrogen on drought tolerance of creeping bentgrass. *PloS one, 11*(4), e0154005.

Coelho, N., and Romano, A. (2022). Impact of chitosan on plant tissue culture: recent applications. *Plant Cell, Tissue and Organ Culture (PCTOC), 148*(1), 1-13.

Daryanto, S., Wang, L., and Jacinthe, P.-A. (2016). Global synthesis of drought effects on maize and wheat production. *PloS one, 11*(5), e0156362.

Datta, A., Sindel, B. M., Kristiansen, P., Jessop, R. S., and Felton, W. (2009). The effects of temperature and soil moisture on chickpea (Cicer arietinum L.) genotype sensitivity to isoxaflutole. *Journal of Agronomy and Crop Science, 195*(3), 178-185.

de Souza, T. C., Magalhães, P. C., de Castro, E. M., Carneiro, N. P., Padilha, F. A., and Júnior, C. C. G. (2014). ABA application to maize hybrids contrasting for drought tolerance: changes in water parameters and in antioxidant enzyme activity. *Plant Growth Regulation, 73*, 205-217.

Dehghanipoodeh, S., Ghobadi, C., Baninasab, B., Gheysari, M., and Shiranibidabadi, S. (2018). Effect of silicon on growth and development of strawberry under water deficit conditions. *Horticultural Plant Journal, 4*(6), 226-232.

Deryng, D., Conway, D., Ramankutty, N., Price, J., and Warren, R. (2014). Global crop yield response to extreme heat stress under multiple climate change futures. *Environmental Research Letters, 9*(3), 034011.

Hafez, Y., Attia, K., Alamery, S., Ghazy, A., Al-Doss, A., Ibrahim, E., . . . Abdelaal, K. (2020). Beneficial effects of biochar and chitosan on antioxidative capacity, osmolytes accumulation, and anatomical characters of water-stressed barley plants. *Agronomy, 10*(5), 630.

Hassan, F., Ali, E., and Alamer, K. (2018). Exogenous application of polyamines alleviates water stress-induced oxidative stress of Rosa damascena Miller var. trigintipetala Dieck. *South African Journal of Botany, 116*, 96-102.

Hassan, F., Ali, E., Gaber, A., Fetouh, M., and Mazrou, R. (2021). Chitosan nanoparticles effectively combat salinity stress by enhancing antioxidant activity and alkaloid biosynthesis in Catharanthus roseus (L.) G. Don. *Plant Physiology and Biochemistry, 162*, 291-300.

Hidangmayum, A., Dwivedi, P., Katiyar, D., and Hemantaranjan, A. (2019). Application of chitosan on plant responses with special reference to abiotic stress. *Physiology and molecular biology of plants, 25*, 313-326.

Ion, V., Dicu, G., Dumbravă, M., Temocico, G., Alecu, I. N., Băşa, A. G., and State, D. (2015). Harvest index at maize in different growing conditions. *Romanian Biotechnological Letters, 20*(6), 10951.

Khan, M. N., Mobin, M., Abbas, Z. K., AlMutairi, K. A., and Siddiqui, Z. H. (2017). Role of nanomaterials in plants under challenging environments. *Plant Physiology and Biochemistry, 110*, 194-209.

Lateef, D., Mustafa, K., and Tahir, N. (2021). Screening of Iraqi barley accessions under PEG-induced drought conditions. *All Life, 14*(1), 308-332.

Li, B., Wang, X., Chen, R., Huangfu, W., and Xie, G. (2008). Antibacterial activity of chitosan solution against Xanthomonas pathogenic bacteria isolated from Euphorbia pulcherrima. *Carbohydrate Polymers*, *72*(2), 287-292.

Li, Z., Zhang, Y., Zhang, X., Merewitz, E., Peng, Y., Ma, X., . . . Yan, Y. (2017). Metabolic pathways regulated by chitosan contributing to drought resistance in white clover. *Journal of proteome research, 16*(8), 3039-3052.

Liu, C., Zhou, H., and Zhou, J. (2021). The applications of nanotechnology in crop production. *Molecules, 26*(23), 7070.

Liu, Z., Liu, T., Liang, L., Li, Z., Hassan, M. J., Peng, Y., and Wang, D. (2020). Enhanced photosynthesis, carbohydrates, and energy metabolism associated with chitosan-induced drought tolerance in creeping bentgrass. *Crop Science, 60*(2), 1064-1076.

Makhlouf, B. S. I., Khalil, S. R. A. E., and Saudy, H. S. (2022). Efficacy of humic acids and chitosan for enhancing yield and sugar quality of sugar beet under moderate and severe drought. *Journal of Soil Science and Plant Nutrition*, *22*(2), 1676-1691.

Moolphuerk, N., Lawson, T., and Pattanagul, W. (2022). Chitosan mitigates the adverse effects and improves photosynthetic activity in rice (Oryza sativa L.) seedlings under drought condition. *Journal of Crop Improvement*, *36*(5), 638-655.

Morin-Crini, N., Lichtfouse, E., Torri, G., and Crini, G. (2019). Fundamentals and applications of chitosan. *Sustainable agriculture reviews 35: chitin and chitosan: history, fundamentals and innovations*, 49-123.

Nadeem, M., Li, J., Yahya, M., Sher, A., Ma, C., Wang, X., and Qiu, L. (2019). Research progress and perspective on drought stress in legumes: A review. *International journal of molecular sciences, 20*(10), 2541.

Nazarli, H., Faraji, F., and Zardashti, M. (2010). Effect of drought stress and polymer on osmotic adjustment and photosynthetic pigments of sunflower.

Noman, A., Ali, S., Naheed, F., Ali, Q., Farid, M., Rizwan, M., and Irshad, M. K. (2015). Foliar application of ascorbate enhances the physiological and biochemical attributes of maize (Zea mays L.) cultivars under drought stress. *Archives of Agronomy and Soil Science, 61*(12), 1659-1672.

Popović, B., Štajner, D., Ždero-Pavlović, R., Tumbas-Šaponjac, V., Čanadanović-Brunet, J., and Orlović, S. (2016). Water stress induces changes in polyphenol profile and antioxidant capacity in poplar plants (Populus spp.). *Plant Physiology and Biochemistry*, *105*, 242-250.

Reynolds, M., Pask, A., and Mullan, D. (2012). *Physiological breeding I: interdisciplinary approaches to improve crop adaptation*: CIMMYT.

Sanderson, J., Daynard, T., and Tollenaar, M. (1981). A mathematical model of the shape of corn leaves. *Canadian Journal of Plant Science, 61*(4), 1009-1011.

Sheshbahreh, M. J., Dehnavi, M. M., Salehi, A., and Bahreininejad, B. (2019). Effect of irrigation regimes and nitrogen sources on biomass production, water and nitrogen use efficiency and nutrients uptake in coneflower (Echinacea purpurea L.). *Agricultural Water Management, 213*, 358-367.

Spinoni, J., Barbosa, P., Cherlet, M., Forzieri, G., McCormick, N., Naumann, G., . . . Dosio, A. (2021). How will the progressive global increase of arid areas affect population and land-use in the 21st century? *Global and Planetary Change*, *205*, 103597.

Sun, C., Li, M., Gao, X., Liu, L., Wu, X., and Zhou, J. (2016). Metabolic response of maize plants to multi-factorial abiotic stresses. *Plant Biology*, *18*, 120-129.

Toscano, S., Farieri, E., Ferrante, A., and Romano, D. (2016). Physiological and biochemical responses in two ornamental shrubs to drought stress. *Frontiers in plant science, 7*, 186872.

Waraich, E.A., Ahmad, R.S., Ashraf, M.Y. (2011). Role of mineral nutrition in alleviation of drought stress in plants. *Aust. J. Crop Sci*, 5, 764–777.

Yadav, B., Jogawat, A., Rahman, M. S., and Narayan, O. P. (2021). Secondary metabolites in the drought stress tolerance of crop plants: A review. *Gene Reports*, *23*, 101040