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Research Article

Influence of distinctive Osmoprotectnats foliar spray in alleviating the harmful effects of water stress at sensitive growth stages of Maize (*Zea mays* L.)

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Abstract
Water availability is becoming a significant concern for crop production worldwide. In light of this, a study was conducted in maize crop to explore the effectiveness of various osmoprotectants including sodium nitroprusside nanoparticles (SNP NP) at a concentration of 90 ppm, melatonin (MEL), at 25 ppm and salicylic acid (SA) at 100 ppm in mitigating the adverse effects of drought, by evaluating their impact on morpho-physiological, biochemical and yield attributes of maize (*Zea mays* L.). Drought stress was induced by withholding irrigation during both the vegetative and reproductive stages of maize and then drought-stressed plants were foliar sprayed with different osmoprotectants. Results revealed that among the osmoprotectants tested, foliar application of salicylic acid at 100 ppm exhibited the most substantial improvement in morpho-physiological parameters (plant height, stem diameter, leaf number, root length, leaf area index, relative water content, leaf chlorophyll and carotenoid content) as well as biochemical parameteters like proline and soluble protein content increased, and enhanced membrane stability under drought conditions. The use of SA proved outstanding as it led to a remarkable 75% higher biological yield than plants subjected to drought stress. On the other hand, the SA foliar spray was successful, resulting in a 78.8% in grain yield. However, the extent of improvement varied depending on the growth stage at which the osmoprotectants were applied. While the foliar application of osmoprotectants showed promising results during the vegetative phase than the reproductive phase of maize. Nonetheless, the osmoprotectants' foliar spray exhibited a yield advantage by preserving photosynthetic pigments and the maize plants' ability to produce seeds under drought stress.

Keywords: Drought, Maize, Melatonin, Reproductive stages, Salicylic acid, Sodium nitroprusside nanoparticles, Vegetative stages.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most significant cereal crops worldwide. Due to maize importance as a primary source of food, animal feed, and biofuel, its consumption and demand are rising on a global scale (Cassman *et al.,* 2003). After rice and wheat, maize is India's third-most significant food crop. According to

Indiastat (2022), 9.9 million hectares of maize is cultivated in India, with an annual yield of 31.51 million tones. However, maize is highly vulnerable to drought stress, particularly during crucial growth stages compared to other cereals like wheat (Daryanto *et al.,* 2016). The average yearly yield loss in maize owing to drought is about 15% of its potential output (Ziyomo and Bernardo, 2013). Drought, climate change and increasing global population worsen the situation and serious risks to agricultural production and global food security.

Drought stress causes a variety of physiological changes in the plant cell system it results more production of Reactive Oxygen Species (ROS) in cells (Abid *et al.,* 2018; Qaseem *et al.,* 2019). It will causes lipid peroxidation, DNA fragmentation, damage to the cell membrane, photosynthetic apparatus, reduces cell growth, cell elongation, photosynthetic rate and stomatal conductance, as well as degradation of biomolecules like pigments and protein will also occur which ultimately result in cell death (Abid *et al.,* 2018;). To counterattack ROS, plants increased the activity of antioxidant enzymes such Ascorbate Peroxidase (APX), Glutathione Reductase (GR), Peroxidase (POD), Superoxide Dismutase (SOD), and Catalase (CAT) for quenching of ROS produced during drought stress (Hussain *et al.,* 2020; Sohag *et al.,* 2020). Along with the activation of defense system, variations in gene expression and the synthesis of certain proteins such as chaperones, produce a response to drought stress (Hasan *et al.,* 2018).

Several techniques are being employed to protect the crop against moisture stress, such as selection, breeding, genetic engineering, and the exogenous application of various chemicals through various mechanisms. Compared to others exogenous treatments of osmoprotectants can significantly improve drought tolerance (Ahammed *et al.,* 2020). In this context we want to examine the effect of osmoprotectants (sodium nitroprusside, melatonin and salicylic acid) foliar sprays in alleviating the harmful effects of water stress at sensitive growth stages of maize.

A little, common molecule called Nitric Oxide (NO) serves as a stress signaling molecule in plants under various stress conditions. Several enzymes, including nitrate reductase, nitrate synthase, and xanthine oxidase, are responsible for producing it in plants (Desikan *et al.,* 2002; Wendehenne *et al.,* 2003). NO improves membrane stability, water relations, photosynthetic capacity (Farooq *et al.,* 2009), decreases lipid peroxidation (Laspina *et al.,* 2005), decreases water loss in leaves (Hao *et al.,* 2008), and reduces ion leakage (Garcia- Mata & Lamattina, 2001), raises the ferritin accumulation (Li *et al.,* 2008), boosts the superoxide dismutase, peroxidase, and ascorbate peroxidase activities, and accelerates ion absorption/transport (Hao *et al.,* 2008; Song *et al.,* 2006) under drought stress.

The most popular NO donor for plants is sodium nitroprusside (SNP), which has a low molecular weight and cost, and is simple to handle. But SNP application was limited due to its short lifespan and photosensitivity and their actions are impacted by temperature, pH, and light (Silveira *et al.,* 2019). In light of this, in the present study, SNP was successfully transformed in to nanoformulations by ionic gelation method for slower release of nitric oxide and to improves SNP's efficiency.

Salicylic acid (SA), a phytohormone, is a potential substance that can control the antioxidant defense system, transpiration rates, stomatal movement, and photosynthetic rate in plants to decrease their sensitivity to drought stress (Nazar *et al.,* 2015). SA is a stress-signaling molecule that increases the production of biosynthetic enzymes and proteins in plants under environmental stress (Wang *et al.,* 2019). By enhancing seedling development, leaf gas-exchange features, and the activity of APX, CAT, and SOD enzymes, while lowering the MDA and H_2O_2 contents, the SA has been found to help reduce the detrimental impacts of drought stress (Maruri *et al.,* 2019).

The potential involvement of the ubiquitous biostimulating chemical melatonin (N-acetyl-5-methoxytryptamine) in plant growth, development, and stress responses has been largely explored in recent studies (Chen *et al.,* 2020). Melatonin plays a key role in developing physiological functions in plants, including photosynthesis, senescence, and reproduction (Sharma *et al.,* 2020). Melatonin's primary role during stressful situations is to enhance plant tolerance by lowering oxidative damage, sting organelle antioxidant defense capacity (Bhat *et al*., 2022).

Nevertheless, no study has been conducted to appraise the relative effect of these compounds (SNP nano formulation, salicylic acid and melatonin) in improving maize tolerance against drought stress. The present study aimed to i) examine the response of morpho-physiological, biochemical and yield components of maize to the foliar application of sodium nitroprusside nano formulations, salicylic acid, and melatonin under different soil water conditions during critical growth stages, ii) Compare the relative efficacy of different spraying applications for ameliorating the harmful effects of drought stress.

MATERIALS AND METHODS

Study area

The field experiment was conducted under the Rain house shelter, Department of Soil and Water Conservation, Agricultural Engineering College, Tamil Nadu Agricultural University, Coimbatore during the Rabi season in the year 2022 with the objectives of examining the response of morpho-physiological, biochemical and yield components of maize to the foliar application of

sodium nitroprusside nano formulations, salicylic acid, and melatonin under different soil water conditions during critical growth stages and compare the relative efficacy of different spraying applications for ameliorating the harmful effects of drought stress. The soil of the experimental site was red sandy loam texture with available soil moisture at field capacity and the permanent wilting point was 23.1 per cent and 9.6 per cent respectively and normal in pH (7.6), non-saline in EC (0.34 dS/m), low in available N (215.0 kg ha^{-1}), medium in available P (14.0 kg ha^{-1}), high in available K (452.0 kg ha^{-1}). The organic carbon status in the experimental soil was 0.51%. The total precipitation during the growing period was 334.3 mm. The minimum and maximum mean monthly temperatures were 29.4^{0}C and 20.8^{0}C, respectively. The maximum (85.2%) and minimum (56.4%) relative humidity was recorded during cropping. weather data was collected manually from the Agro Meteorological observatory at Tamil Nadu Agricultural University in Coimbatore.

Plant culture

Field experiment was laid out in a split plot design with three replication. The main plot consisted of three levels of irrigation regimes *viz*., Irrigation as per recommendation (I_1), Withholding irrigation at the vegetative stage (I_2), Withholding irrigation at the reproductive stage (I_3) and in sub plot consisting foliar spray of osmoprotectants *viz*., sodium nitroprusside nanoformulation (SNP NP) @ 90 ppm (F_1), melatonin @ 25 ppm (F_2), salicylic acid (SA) @ 100 ppm (F_3), and control (water spray) (F_4). Maize hybrid Co (H) 8 was used for the field experiment and dibbled on ridges with 60 x 20 cm spacing. To avoid seepage between main plots 1.5 m spacing followed. The recommended dose of NPK (200:125:125 kg ha^{-1}) was applied. All P_2O_5 and K_2O were applied at sowing time and nitrogen was applied in three equal splits i.e., sowing, knee height and tasselling stage using the source of urea, single superphosphate and murate of potash. All other agronomic practices were kept uniform throughout the conduct of the experiment. In the experiment, Plots designated as recommended irrigation (control) were maintained at 100 % field capacity. Drought treatment plots were maintained by keeping soil moisture at 50% field capacity by regular monitoring with theta probe moisture meter (Delta T. Sensor type). Drought stress (DS) treatment was imposed by skipping irrigation at different growth stages *viz.,* at the vegetative (30 - 45 DAS) and the reproductive (50 - 65 DAS) stages. The sodium nitroprusside nanoformulation (SNP NP), melatonin (ML), salicylic acid (SA), and control (Water spray) were applied three days after drought imposition, times, i.e., at 33 DAS to vegetative stage and 53 DAS to reproductive stage. Drought-stressed plants were given optimum moisture from 46 & 66 DAS for the vegetative and reproductive stages respectively. The morphological and physiological parameters were recorded 12 days after treatment *viz.,* 45 and 65 DAS, by randomly selecting three representative samples. Yield parameters were recorded at harvest, including, test weight, cob length, cob weight, cob girth, grain yield and harvest index.

Synthesis of sodium nitroprusside nanoformulation

SNP loaded chitosan nanoparticles (SNP CSNPs) were produced by using the ionotropic gelation method (Silveira *et al.,* 2019). Ionotropic gelation occurs due to positively charged chitosan amino groups and negatively charged sodium tripolyphosphate (TPP). To get chitosan to a final concentration of 1 mg/ mL^{-1}, it was first dissolved in acetic acid at a 1% concentration, then 50 mmol L^{-1} of SNP were added to the solution. The mixtures were held in magnetic stirring for 90 minutes at 25°C with a rotating speed of 400 rpm. Sodium hydroxide aqueous solution 1 mol/L was used to adjust the emulsion pH to 5.3. After adjusting pH then TPP (0.6 mg/mL^{-1}) was added to solution in drops, and magnetic stirring was performed about 30 min with 400 rpm, then Tween 80, a surfactant, was added to solutions at a rate of 0.5% (v/v) to stop particle aggregation. The resultant SNP-loaded chitosan nanoparticle suspension underwent a 15-minute centrifugation at 10,000 rpm. Before being used or subjected to analysis, the suspension was freeze-dried.

Characterization of sodium nitroprusside nanoparticles

SNP loaded chitosan nanoparticles were successfully created by using ionic gelation method. Utilizing a variety of material characterization techniques *viz.,* Dynamic Light Scattering (DLS), Fourier-Transform Infrared Spectroscopy (FT-IR) (Fig. 2b) and release kinetics (Fig. 2b). After characterization, it was observed that the SNP-loaded chitosan nanoparticles were stable and spherical in shape. SNP-loaded Chitosan Nanoparticles (SNP CSNPs) were 240 nm in size (Fig. 1a) as measured using the DLS method which had a zeta potential of 41.5 mV (Fig. 1b).

Plant growth attributes

From each replication, plant samples were taken. The plant parts, including the roots, shoots, and leaves, were separated, and their fresh biomasses were calculated using a precise electronic balance. A digital vernier calliper was used to measure stem diameter, and a measuring scale was used to measure plant height, root length, and shoot length. A sensitive electronic scale was used to determine the dry weight after the samples of roots, stems, and leaves were oven dried for 48 hours at a temperature of 70^{0}C. Using a ruler the

length and broadest breadth of the leaf were measured and multiplied with a correction factor of 0.70 to estimate the Leaf area (LA) and expressed in cm^2. For calculating the leaf area index (LAI) the leaf area was divided by the ground area.

Relative Water Content (RWC)

A fresh leaf was immediately weighed after being cut into 1.5 X 1.5 cm^2 sizes to get fresh weight (FW). The piece of the leaf that had been soaked in deionized water for 4 hours was weighed as turgid weight (TW). After 48 hours of drying at 70°C, the leaf fragment was weighed as dry weight (DW) (Turner, 1981). RWC was expressed as a percentage.

RWC = [(FW-DW) / (TW-DW)] ×100 ……Eq. 1

Chlorophyll and carotenoids determination

Wellburn's (1994) method was used to estimate the amounts of chlorophyll and carotenoids. Leaf tissues were soaked in dimethyl sulfoxide (DMSO) to extract chlorophyll. DMSO makes the plasmalemma permeable, which allows the pigments to leak out. The amount of chlorophyll in plants was calculated based on how efficiently chlorophyll extracts absorbed light in UV spectrophotometer. The absorbance of a known volume of fluid containing a known amount of leaf tissue was measured at three distinct wavelengths, 663 nm, 645 nm, and 480 nm, respectively, to determine the level of chlorophyll 'a', chlorophyll 'b', and total carotenoid. Arnon (1949) method was used to calculate the amounts of chlorophyll 'a', 'b' and total chlorophyll, while Lichtenthaler and Welburn (1983), formula was used to calculate the amount of carotenoids. Fresh leaf samples weighing 30 mg were put to test tubes containing 4ml DMSO. Tubes were kept at 65^0C in the dark for 4 hours. The samples were then removed, allowed to cool to ambient temperature, and the absorbance at 663, 645, and 480 nm was measured using DMSO as a blank. This value was reported as mg^{-1} FW.

Chlorophyll "a" = (12.7x A663 – 2.69xA645) x V/1000 x W …….Eq. 2

Chlorophyll "b" = (22.9 x A645 – 4.68 x A663) x V/1000 x W ……. Eq. 3

Total chlorophyll = (20.2 x A645 + 8.02 x A663) x V/1000 x W …….Eq. 4

Total carotenoids = (A480 + (0.114 x A663) – (0.638-A645)) x V/1000 x W …….Eq. 5

Where, A663 = Absorbance values at 663nm; A645 = Absorbance values at 645nm;

A480 = Absorbance values at 480nm, W = Weight of the sample in g and V = Volume of the solvent used (ml).

Membrane Stability Index (MSI)

Premchandra *et al.* (1990) approach was used to measure the Membrane Stability Index (MSI). 100 mg of leaf material, divided into two sets, was placed in test tubes with 100 ml of double-distilled water for the estimation of the membrane stability index. In a metabolic water bath, one set was heated to 40°C for 30 minutes, and the electrical conductivity of the solution was noted on a conductivity bridge (C1). The conductivity of the second batch was determined on a conductivity bridge (C2) after it had been heated at 100°C on a boiling water bath for 10 minutes. Membrane stability index (MSI) was calculated as:

MSI= [1-C1/C2] X 100 …….. Eq. 6

Proline estimation

Bates *et al.* (1973) approach was used to estimate the proline content. Leaf samples (0.5g) were homogenised in 10 ml of 3% sulphosalicylic acid and the extract was centrifuged at 5000 x g for 10 min. A test tube containing 2 ml of the supernatant was then mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid. The mixture was heated up on a water bath, for one hour at 100^0C. The tubes were taken out of the hot water bath and placed in the cold bath to cease the reaction. The mixture was mixed with 4 ml of toluene and vortexed for 15-20 second. The chromophore was aspirated from the aqueous phase. Then the absorbance of toluene phage was measure at 520 nm. A blank was run and its absorbance was subtracted from the sample absorbance. The proline content was calculated from the standard curve using L-Proline as standard. Proline concentration expressed in (micro gram $^{-1}$ F.W).

Total soluble proteins

The Bradford (1976) method was used to calculate the protein content, and bovine serum albumin was used as the standard. The Bradford's dye solution was added to 0.1 ml of the sample and gently stirred. A spectrophotometer was used to measure the absorbance at 595 nm after 5 minutes. Soluble protein expressed in (mg/g F.W).

Yield components and yield

Yield components, including cob length, cob girth, cob weight, number of rows per cob, number of grains per row, total number of seeds per cob and thousand-grain weight, were recorded as per the standard procedure. Three plants were randomly chosen and the average was worked out. For the thousand-grain weight, 100 seeds were randomly picked from grains of each treatment and multiplied by 10. Biomass and grain yield were recorded by harvesting six plants from three replicates of each treatment, and then they were converted to grams per plant. The harvest index was expressed as a percentage ratio of grain yield and biological yield.

Statistical analysis

Three replicates were used in the split plot design of the experiment. After making notes and entering data into

Excel, the MSTAT-C statistical tool was used to conduct a data normal test and convert numeric and percentage data. Using SAS (version 9.1), variance analysis and means comparison were carried out.

RESULTS AND DISCUSSION

Growth parameters

Water regimes and osmoprotectants foliar spray had a significant effect on plant height. Among water regimes under normal irrigation conditions maximum plant height (227 cm) was recorded which was 16.71% higher than achieved under drought conditions. Furthermore, drought imposed during the vegetative stage had a maximum impact on plant height and reduced up to 11% than the reproductive stage (Table 1). Among foliar applications, salicylic acid @ 100 ppm significantly improved plant height compared to water spray. Under normal irrigation settings, enhancement of plant height by foliar applications was non significant. These results agree with finding reported by Baroowa and Gogoi (2016) water stress during vegetative stage was most detrimental in terms of height compared to the reproductive stage in greengram and blackgram. According to Qasim *et al.* (2019). SA foliar spray in maize improved plant height by increasing cell division and cell elongation by raising endogenous gibberellic acid concentration under drought stress. According to Anosheh *et al*. (2012) foliar application of SA significantly increased plant height in wheat under drought stress.

Root length and stem girth of maize was significantly affected by water regimes and foliar application of osmoprotectants under drought stress ($p < 0.05$). Results indicated that among water regimes drought imposed at the vegetative stage resulted in more impact on root length and stem girth, the reduction up to 20% and 13% respectively compared with when drought was imposed at the reproductive stage to 14% and 9% respectively (Table 1). Among foliar applications SA @ 100 ppm significantly improved root length and stem girth under drought stress at water sensitive stages. The adaptation mechanisms of plants to drought stress are closely related to exceptional modifications in root morphology particularly root length and root surface area. These results are confirmatory with previous findings of Lathif *et al*. (2015) in that the foliar spray of SA significantly protected root length and root area of maize plants from adverse effects of drought stress. According to Hasan *et al*. (2022), foliar application of SA significantly improved the root length of wheat under drought stress. Jadhav and Bhamburedekar (2011), found significant increases in root growth when groundnut plants were applied with exogenous salicylic acid. Shemi *et al*. (2021) also found that SA was more effective in increasing maize stem diameter among osmoprotectants because SA increased stem girth by encouraging cell division.

Water regimes and foliar application of osmoprotectants significantly affected root and shoot fresh and dry weights. Results showed that maximum root and shoot fresh and dry weights were recorded under normal irrigation conditions than the drought at critical stages. Moreover, drought imposed at vegetative stage decreased maximum root and shoot fresh and dry weights of 20%, 15%, 27% and 17% respectively compared to when drought was imposed at the reproductive stage (Table 1). Among foliar applications, root and shoot fresh and dry weights improved significantly due to foliar application of salicylic acid @ 100 ppm at both vegetative 11%, 6%, 24% and 12% respectively and reproductive stages 7%, 4%, 22% and 10% respectively compared to water spay under drought stress. Salicylic acid foliar spray non-significant under no stress conditions. These results agree with the finding reported by Lathif *et al*. (2016) foliar application of salicylic acid in maize significantly improved root and shoot fresh and dry weights under drought stress. Zamaninejad *et al.* (2013) found that drought stress adversely affects the meristematic activity, cell elongation and premature abscission of maize leaves and roots, and reduces the photosynthetic activity and accumulation of dry matter.

The number of leaves per plant (LP) was considerably impacted by drought stress (Table 1). Compared to plants exposed to drought at critical growth stages, normal irrigation conditions were recorded the highest number of leaves (14.1) (Table 1). Both vegetative and reproductive growth stages were similarly responsive to water regimes. Among foliar sprays at both the vegetative and reproductive stages, a foliar application of SA @ 100 ppm produced the highest LP (13.77 and 13.9) compared with water spray (10.1) under drought stress. These findings concur with those of Baroowa and Gogoi (2016) who reported significant reduction in the leaf number of black gram and green gram plants were observed when subjected to stress for 15 days during the vegetative stage than stress at the reproductive stage.

Water regimes and osmoprotectants in foliar sprays significantly affected the leaf area index. Under normal irrigation conditions, the leaf area index (LAI) was recorded at its maximum value compared to drought stress at critical growth stages. Among water regimes, LAI was drastically decreased by (23%) when drought was imposed at the vegetative stage compared to the reproductive stage. Across various osmoprotectants, salicylic acid @ 100 ppm foliar treatment significantly improved LAI at both the vegetative (18%) and reproductive stages (14%) compared to water spray (Table 1) under drought stress. Effect of salicylic acid foliar spray on LAI was non-significant under normal irrigated conditions. These findings concur with those of Maswada *et al*. (2017) reported that SA foliar application im-

proved the leaf area of maize under drought conditions. According to Baroowa and Gogoi (2016), irrespective of genotypes, plants stressed during the vegetative stage (T2) showed the highest reduction of leaf area than the reproductive stage in greengram and blackgram.

Chlorophyll and carotenoids contents

Water regimes and osmoprotectants foliar sprays significantly affected pigments concentration ($p<0.05$). Results revealed that compared to irrigated settings, drought stress at critical growth stages significantly decreased the total chlorophyll and carotenoids content of maize leaves. Compared with drought induced at the reproductive stage, drought imposed at the vegetative stage had a greater influence on total chlorophyll (Chlorophyll 'a' and 'b') and carotenoid content up to (18% and 50%, respectively) (Table 2). Among of all osmoprotectants, foliar spry of salysilic acid (SA) @ 100 ppm significantly improved the Chl. 'a', Chl. 'b', total Chlorophyll, and carotenoid contents in both vegetative by 20%, 50%, 37%, and 14% respectively and reproductive stages by 15%, 33%, 35%, and 12% respectively compared with control (water spray) under drought stress. These findings concur with Arif *et al*. (2020), who reported SA foliar spray enhances various physiological processes like photosynthesis, chlorophyll and carotenoids pigment in maize. According to Maswada *et al*. (2017), drought lowers the amount of chlorophyll in maize leaves, and the production of chlorophyllase enzyme may be the reason for chlorophyll degradation but application SA application improved the pigment concentrations under drought stress. Bijianzadeh *et al*. (2019) reported that foliar application of SA significantly improved the chlorophyll 'a' and chlorophyll 'b' and carotenoid content in maize under drought stress.

Relative water content (RWC)

Water regimes and osmoprotectants in foliar sprays significantly affected relative water content ($p<0.05$). In comparison to no stress, relative water content (RWC) statistically decreased both the vegetative stage (23%) and stress at the reproductive stage (21%) of maize leaves and found to be similarly responsive to drought stress. The results showed that salicylic acid (SA) @ 100 ppm foliar spray improved the RWC by 10% at reproductive stage and 11% at vegetative stage compared to the control. These findings concur with those of Sedaghat *et al*. (2017) reported that the relative water content (RWC) of wheat cultivars exposed to drought conditions improved when these plants were subjected to exogenous SA application. According to Bijianzadeh *et al*. (2019), foliar application of salicylic acid significantly improved the leaf relative water content of maize.

Proline concentration

In comparison to no stress, drought stress at critical growth stages statistically ($p<0.05$) increased the proline content in maize leaves. Among water regimes, proline content increased both vegetative (73%) and reproductive (51%) stages under drought stress and

Table 1. Effect of different Osmoprotectants foliar spray on Growth parameters of maize exposed to normal irrigation and drought condition at vegetative and reproductive crop growth stages

	Growth parameters								
Treatments	**Plant height (cm)**	**Root length (cm)**	**Root fresh weight (g)**	**Root dry weight (g)**	**Shoot fresh weight (g)**	**Shoot dry weight (g)**	**Leaf number**	**Leaf area index**	**Stem girth (cm)**
Vegetative stage									
No stress	227.0	26.3	25.5	6.8	177.0	44.1	13.6	3.4	14.6
SNP NP	208.5	17.8	20.0	4.8	156.7	36.5	12.2	2.2	12.9
MEL	208.9	18.9	21.6	5.0	159.0	37.8	12.6	2.4	13.0
SA	212.3	21.0	22.9	6.2	165.7	39.5	13.0	2.8	13.5
Water spray	203.7	17.4	18.2	3.8	147.2	33.3	10.1	1.7	11.9
CD <(0.05)	3.1	1.03	1.1	0.9	4.2	1.5	0.8	0.4	0.7
Reproductive stage									
No stress	227.0	36.3	67.4	17.2	320.8	79.4	16	5.1	26.4
SNP NP	215.2	30.0	56.2	12.7	284.0	70.0	14.2	4.5	24.0
MEL	216.7	31.1	58.4	14.2	289.4	71.5	14.5	4.6	24.2
SA	219.0	33.0	59.2	15.8	301.6	75.1	15.3	4.9	24.9
Water spray	211.7	27.9	53.5	10.8	276.8	62.8	13.8	3.4	23.2
CD < (0.05)	3.1	1.1	1.5	0.9	5.1	2.9	0.8	0.6	1.4

NO stress= Irrigation to crop as per recommended, SNP NP = Sodium nitroprusside nano formulation @90ppm, MEL= Melatonin @25ppm, SA=Salicylic acid @ 100ppm, Water spray. Vegetative stage = Drought induced at vegetative stage, Reproductive stage = Drought induced at reproductive stage.

Table 2. Effect of different Osmoprotectants foliar spray on Chlorophyll and Relative water content of maize exposed to normal irrigation and drought condition at vegetative and reproductive crop growth stages

Chlorophyll and Relative water content								
Treatments	**Chloro-phyll 'a' (mg g^{-1} F.W)**	**Chloro-phyll 'b' (mg g^{-1} F.W)**	**Total Chloro-phyll (mg g^{-1} F.W)**	**Carote-noids (mg g^{-1} F.W)**	**Relative water content (%)**	**Soluble Protein (mg/g F.W)**	**Proline (micro $gram^{-1}$ F.W)**	**MSI (%)**
Vegetative stage								
No stress	3.0	0.8	3.9	0.60	92.6	23.7	92.2	85.4
SNP NP	2.5	0.6	3.1	0.30	70.6	30.3	159.8	70.5
MEL	2.7	0.6	3.3	0.37	73.1	31.8	166.0	72.6
SA	2.8	0.7	3.5	0.45	82.5	35.8	180.1	78.1
Water spray	1.9	0.3	2.6	0.25	63.0	24.2	134.3	62.4
CD < (0.05)	0.8	0.1	0.4	0.09	1.7	2.0	4.8	1.8
Reproductive stage								
No stress	5.1	1.0	6.1	1.00	91.8	33.3	103.3	81.2
SNP NP	4.4	0.6	5.0	0.67	71.0	40.6	160.9	69.9
MEL	4.5	0.6	5.1	0.70	74.6	42.3	162.7	69.8
SA	4.7	0.8	5.5	0.73	80.5	45.4	168.0	75.2
Water spray	4.0	0.4	4.4	0.60	63.4	37.1	133.1	57.3
CD < (0.05)	0.6	0.10	0.5	0.07	2.2	1.6	3.4	1.6

No stress= Irrigation to crop as per recommended, SNP NP = Sodium nitroprusside nano formulation @90ppm, MEL= Melatonin @ 25ppm, SA=Salicylic acid @ 100ppm, Water spray. Vegetative stage = Drought induced at vegetative stage, Reproductive stage = Drought induced at reproductive stage.

Table 3. Effect of different Osmoprotectants foliar spray on Yield attributes and Grain yield of maize exposed to normal irrigation and drought condition at vegetative and reproductive crop growth stages.

Yield attributes and Grain yield										
Treatments	**Cob length (cm)**	**Cob diame-ter (cm)**	**Cob weight (g)**	**No. of rows/ cob**	**No. of ker-nels / row**	**Total no. f kernels**	**100-seed weight (g)**	**kernel yield (t ha^{-1})**	**Stover yield (t ha^{-1})**	**Har-vest index (%)**
Vegetative stage										
No stress	19.0	14.2	219.5	14.7	33.7	460.0	36.8	7.2	10.5	0.409
SNP NP	16.6	13.0	212.1	13.4	29.3	385.2	34.4	6.4	9.7	0.400
MEL	17.6	13.6	215.3	13.4	31.0	412.4	34.8	6.6	9.9	0.402
SA	18.0	14.1	216.0	13.9	32.0	414.8	36.6	6.8	10.1	0.405
Water spray	16.0	12.6	210.2	12.4	29.0	365.0	33.6	6.4	9.5	0.395
Reproductive stage										
No stress	19.0	14.2	219.5	14.7	33.7	460.0	36.8	7.2	10.5	0.409
SNP NP	15.0	12.4	200.7	11.6	24.2	296.6	33.8	5.5	8.5	0.394
MEL	16.2	13.3	207.5	12.5	26.0	320.0	34.1	6.1	9.0	0.397
SA	17.0	13.6	210.1	12.8	28.0	338.0	35.0	6.3	9.2	0.400
Water spray	13.0	11.8	190.3	11.1	22.0	255.0	32.7	4.5	7.5	0.385
CD< (0.05)	0.8	1.0	3.5	0.8	2.4	11.3	NS	439.3	368	NS

No stress= Irrigation to crop as per recommended, SNP NP = Sodium nitroprusside nano formulation @90ppm, MEL= Melatonin @25ppm, SA=Salicylic acid @ 100ppm, Water spray. Vegetative stage = Drought induced at vegetative stage, Reproductive stage = Drought induced at reproductive stage.

was shown to be similarly sensitive. Exogenous application of various osmoprotectants considerably raised the leaf proline content further. As compared with control (water spray), salicylic acid (SA) @ 100 ppm foliar spray significantly increased the proline content in both vegetative (14%) and reproductive (13%) stages under stress (Table 2). Similarly, Shemi *et al*. (2021) reported that maize plants exposed to drought had much higher prolin, which increased further after SA application due to enzyme-controlled mechanism converting the amino acid glutamate into proline. Tayyab *et al*. (2020) reported that exogenous application of SA increased the proline content of maize under the drought stress conditions.

Soluble protein

Soluble protein had significant interaction with water regimes and osmoprotectants foliar sprays (Table 2). Results showed that, soluble protein greatly improved among water regimes and was equally responsive when stress imposed both at reproductive and vegetative stages. Among foliar sprays salicylic acid (SA) @

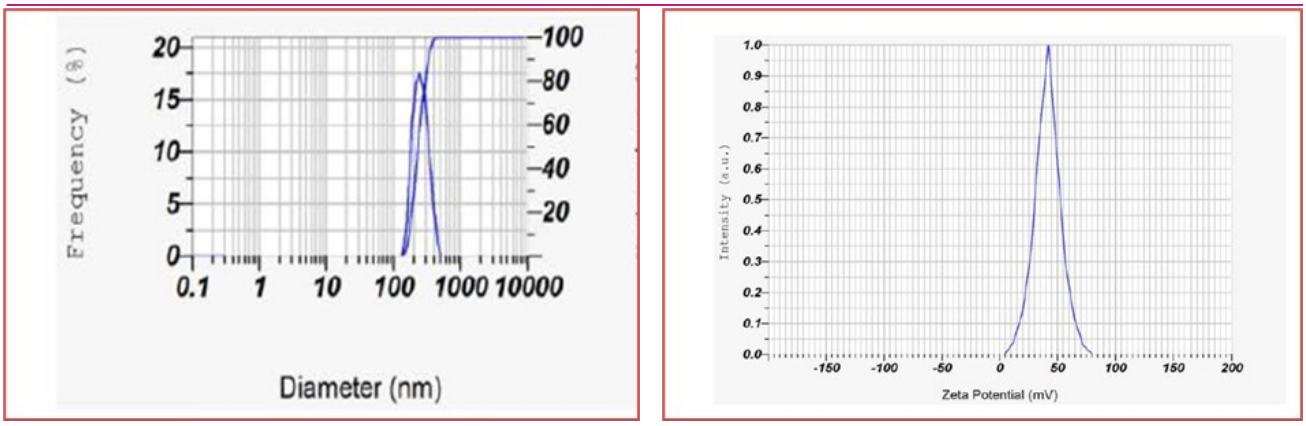

Fig. 1. *a) SNP Nano formulation particle size b) Zeta potential was analyzed by DLS method*

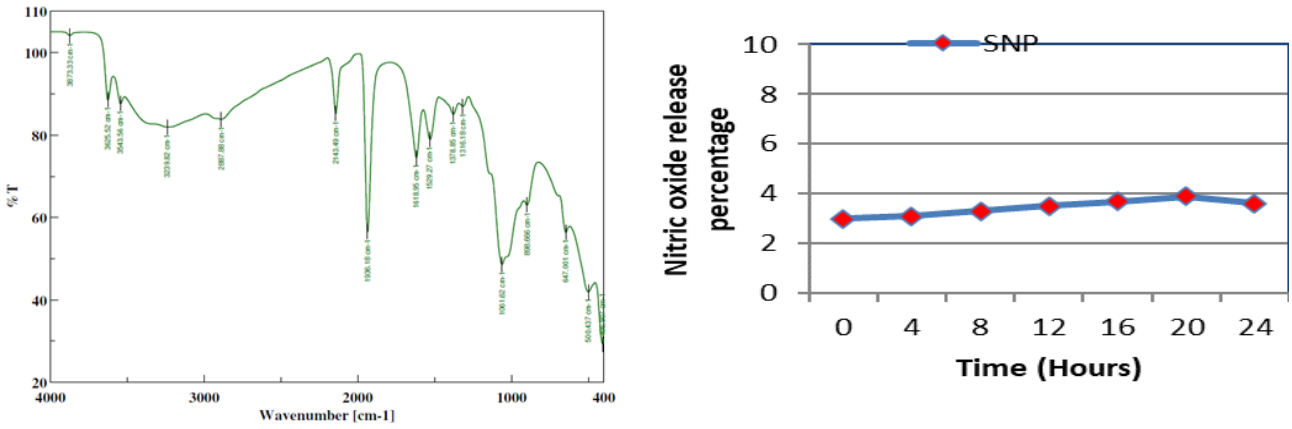

Fig. 2. *a) FTIR analysis of SNP loaded CSNP b) Kinetics of nitric oxide release from SNP Loaded CSNP*

100 ppm significantly increased the quantity of soluble protein in both the vegetative (21%) and reproductive stages (18%) as compared to the control (water spray) under drought stress (Table 2). Similar reports observed by Shemi *et al.* (2021) showed increases in soluble protein levels of maize during drought and further increased after SA application, indicating that further increased level of soluble protein in plants under drought stress may play a protective effect. According to Lathif *et al*. (2016), foliar application of salicylic acid significantly improved soluble protein content further in maize crops under drought stress.

Membrane stability index

Membrane stability index (MSI) which is an indicator of level of damage to cell membrane. The results revealed that among water regimes regular watering settings had the highest membrane stability index (MSI). Furthermore, the reduction of MSI was significantly more when drought was administered at the vegetative stage (27%) compared to the reproductive stage (17%). Among various osmoprotectants MSI significantly improved when plants treated with salicylic acid @ 100 ppm at both vegetative (11%) and reproductive stages (6%) compared to water spray under stress (Table 2). The present findings are consistent with those of Tayyab *et al*. (2020) who reported that exogenous application of SA increased the membrane stability index under drought stress in maize. According to Rao *et al.* (2012), SA positively impacted metabolic activity and grain production of maize under moisture stress due to enhanced membrane integrity.

Yield attributes and yield

Under various water regimes and osmoprotectants foliar sprays, statistically different Cob Diameter (CD) and Cob Length (CL) and Cob Weight (CW) were observed (Table 3). The results revealed that maximum CD and CL 14.2 cm, 19 cm and 219.5 g respectively were observed under a normal irrigation regime. Drought imposed at reproductive stage was more water sensitive in reduction of cob diameter, length and weight than the vegetative stage. Among foliar sprays, with comparison to water spray 11.8 cm, 13.0 cm and 190.3 g respectively, salicylic acid @ 100 ppm foliar application improved CD, CL, and CW readings at vegetative stage 14.1 cm 18.0 cm and 210.2 g respectively under drought stress. These findings are consistent with those made by Ghazi (2017), SA foliar application improved cob length cob diameter, cob weight number of grains/cob and 100-grains weight over the control under drought stress. Khodarahmpour and Hamidi (2012)

reported that water deficit stress strongly impacted CD and CL of maize crop. Additionally, they claimed that compared to the vegetative stage, the silking stage was more water sensitive in terms of CD and CL reduction.

The impact of water regimes and foliar application of osmoprotectants on number of kernel rows/cob, the number of kernels/row, and the total kernels/cob was significant ($p<0.05$), for the results maximum values of kernel rows/cob, kernels/row, and total kernels/cob were recorded as 14.7, 33.7, and 460.0 respectively in the irrigated treatment. The lowest number was found in drought stress at the reproductive stage as 11.1, 22.0, and 255.0 respectively (Table 3). Among foliar sprays, when compared to water spray, salicylic acid @ 100 ppm significantly increased the number of grain rows/cob, the number of grains/row, and the total number of grains/cob at the vegetative 13.9, 32.0, and 414.8 respectively and reproductive 12.8, 28, and 338.0 respectively stages under drought stress. These findings are consistent with those made by Ghazi (2017), in that the SA foliar application improved the no. of grains per cob in maize under drought stress conditions.

The results indicate that, water regimes at critical growth stages had a significant ($p<0.05$) impact on 100-seed weight but foliar spraying of osmoprotectants had non-significant effect on 100-seed weight. The recommended irrigation regime (control) resulted highest 100-seed weight (36.8 g) compared to stress at moisture sensitive stages. Among the two water sensitive growth stages, drought stress at vegetative stage affected minimum and recorded (33.6 g) of 100-seed weight than the drought stress at reproductive stage (32.7 g) (Table 3). According to Noreen *et al*. (2017), foliar spraying of SA at a rate of 200 mg L^{-1} allowed the wheat crop to withstand the challenges of water stress and resulted in increased 100-grain weight, grain yield. The application of SA and ABA increased in the number of flowers, pods per plant and 100-seed weight in faba bean plants (Abdelaal *et al*., 2015).

The results of the analysis of variance (ANOVA) revealed that both water regimes and osmoprotectants foliar sprays had a significant impact on kernel yield ($p<0.05$). The recommended irrigation regime (control) resulted in the highest kernel production (7.2 t ha^{-1}). Among the two sensitive growth stages, drought stress at the vegetative stage affected very minimum and produced the highest kernel yield (6.4 t ha^{-1}), than the drought stress at reproductive stage (4.5 t ha^{-1}) (Table 3). Among foliar sprays salicylic acid foliar spray at a concentration of 100 ppm improved the kernel yield at both the vegetative (6.8 t ha^{-1}) and reproductive growth stages (6.3 t ha^{-1}), compared to water spray. Similar to the present findings, Shemi *et al*. (2021) reported that salicylic acid significantly improved growth and kernel yield under both stress and non-stress environments. According to Noreen *et al*. (2017), foliar spraying of SA at a rate of 200 mg L^{-1} allowed the wheat crop to withstand the challenges of water stress and resulted in increased grain yield. This was achieved by improved physiological and biochemical processes that resulted in increased vegetative development and the translocation of active assimilation from source to sink.

Water regimes and osmoprotectants foliar sprays had a significant effect on Stover yield (SY) ($p<0.05$). SY was at its maximum (10.5 t ha^{-1}) when regular irrigation was employed, and at its minimum (7.5 t ha^{-1}) when a drought was imposed during its reproductive stage on maize in comparison to the vegetative stage. The reproduction stage was found to be more water sensitive. Among foliar treatments Salicylic acid @ 100 ppm showed a highest SY (9.7 t ha^{-1}) than water spray (Table 3). These findings concur with those made known by Azimi *et al*. (2013), who noted a decrease in biological yield in wheat during drought conditions but foliar application of SA significantly increased biological yield under moisture stress.

Water regimes and osmoprotecants foliar did not significantly influence harvest index (Table 3). Unlike the present results, Hasan *et al*. (2022) reported foliar application of higher concentrations (150 ppm) of salicylic acid significantly increased in value of harvest index in greengram under drought stress condition.

Conclusion

The study concluded that the morpho-physiological parameters of the vegetative stage in maize were more sensitive to water reduction than reproductive stage. Additionally, the reproductive stage of the maize CO (H) 8 variety exhibited higher sensitivity to water scarcity, particularly in terms of yield attributes and overall yield reduction. Applying SA at a concentration of 100 ppm during both the vegetative and reproductive stages resulted in improved drought tolerance in maize. This was achieved by the accumulation of higher levels of osmolytes, enhanced RWC and increased photosynthetic pigments in drought-stressed plants. It was observed that the vegetative stage of drought-stressed plants responded more favorably to osmoprotectants than the reproductive stage. Thus, the exogenous application of salicylic acid can be considered a key strategy to enhance plant growth, physiological and biochemical parameters, yield, and its components under drought-stressed conditions, followed by melatonin and sodium nitroprusside nanoformulation applications.

Conflict of interest

The authors declare that they have no conflict of interest.

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