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Synthesis and Standardization of Sodium Nitroprusside Nanoformulation on Germination and Early Seedling Growth of Maize under PEG- Induced Moisture Stress

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ABSTRACT

Background: Sodium nitroprusside (SNP) is a common substance used to decrease the harmful effects of abiotic stress and to improve the germination and early seedling growth of crops. However SNP use is limited by its short lifespan and photosensitivity. With this context, current study taken up to that synthesize of SNP loaded chitosan nanoparticles and to standardize for the seed treatment.

Methods: SNP loaded chitosan nanoparticles were successfully synthesized by using ionic gelation method. A variety of characterization techniques utilized *viz.,* dynamic light scattering (DLS), Fourier-transform Infrared spectroscopy (FT-IR) and release kinetics. A laboratory experiment was conducted to standardize the ideal SNP nanoformulation dosage for seed treatment in order to minimize the negative impacts of drought stress in maize. The treatments involved soaking seeds in solutions containing various concentrations of SNP nanoformulation, including 20, 40, 60, 80 and 100 µM including control.

Result: The experimental results indicated that, among SNP nanoformulation concentrations, seeds treated with @ 100 µM concentration showed the highest germination percentage (85%), promptness index (82.3%), vigor index (1624%), shoot and root length (8.4 cm and 11 cm), fresh and dry weight of shoot (123.7 and 61.6 mg/20 seedlings) and root (52.6 and 25.0 mg/20 seedlings) respectively, under PEG-induced drought stress conditions compared with control.

Key words: Chitosan, DLS, FTIR, Maize, PEG and sodium nitroprusside nanoparticles.

INDRODUCTION

Maize (*Zea mays L.*) is one of the most important grains crop in the world and also one of the most vulnerable crop to drought stress. Lack of water during the germination stage inhibits seedling emergence and establishment partially or entirely (Kaya *et al.,* 2006). It is emphasising the necessity of conducting scientific studies targeted at enhancing drought resistance.

Drought stress is a hazard to global crop output and a rapid climate change scenario has made this problem worse which could jeopardize food security (Shahbaz *et al.,* 2009). By 2025, it is predicted that the sparse precipitation and uneven distribution of rainfall, especially in dry and semiarid regions, will result in a 30% drop in worldwide crop production (Neufeldt *et al.,* 2013).

Plants under drought stress frequently display stomatal closure, which reduces CO_2 influx while preventing water loss. Consequently, this causes limited production of NADP and less carbon fixation during photosynthesis. Reactive oxygen species (ROS) are created as a result of electrons being transferred to oxygen, a different electron acceptor (Gill and Tuteja, 2010). Proteins, amino acids and nucleic acids are examples of crucial plant components that ROS, such as the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-), can directly damage (Mittler *et al.,* 2004).

The plant's ability to scavenge these elevated ROS levels is crucial to its ability to survive under stress. Stress-induced ROS accumulation is counteracted by enzymatic/ non-enzymatic antioxidant systems that include a variety of scavengers, such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POX), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), ascorbate and glutathione contents (Shi *et al.,* 2007). Additionally, osmotic regulators like proline (Pro) and ABA (absicic acid) can protect cells from the effects of oxidative damage. Several reports are employing a variety of tactics to battle the drought, including irrigation water utilization, agronomic management, cultural practices, breeding techniques and biotechnology instruments. Among

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variety of tactics using plant growth regulators (PGRs), a well known and affordable technique to counteract drought stress. PGRs are natural substances that are naturally present in plants and when applied exogenously, they have an impact on physiological process and foster growth and development.

Nitric oxide (NO) is a tiny, pervasive signal particle with hydrophobic characteristics that functions as a novel class of regulatory molecules in plants (Bhuyan *et al.,* 2020). Additionally, it functions as an intracellular signaling molecule and endogenous intermediary in plant systems. When crops are under stressful conditions, it plays a critical role in a variety of biochemical, physiological and developmental processes, such as maintaining water status, lowering ion leakage, germination, root growth, stomatal movement, recovering cell membrane and boosting photosynthetic capacity (Bhuyan *et al.,* 2020).

Sodium nitroprusside [$Na_2[Fe(CN)_5NO]$], is the most used NO donor for plants due to its low molecular weight, low price and ease of handling. As well as SNP application having fewer limitations as it is photosensitive, short half life (1-5 s), react quickly with oxygen and cellular components and releases cyanide result of its degradation, (Wang *et al.,* 2022). Despite this, NO donors are often unstable and high temperatures and light exposure speed up their decomposition rates. This causes a rapid release of NO, which might potentially have harmful effects and diminish the effectiveness of the signaling molecule (Sebra *et al.,* 2010). In this situation, the trapping of NO donors in nanomaterials has emerged as a tactic that could safeguard these molecules from degradation and allow a controlled release of NO, so extending its duration of activity (Mujtaba *et al.,* 2021). Despite their successful use for biomedical applications, potential applications of nanocarriers for NO delivery in plants have not yet been investigated. In this context, SNP is successfully encapsulated by using chitosan polymer.

In this context the goal of the current study was synthesis of sodium nitroprusside nanoformulation and to determine the effectiveness and mechanism of action of sodium nitroprusside nanoformulation on the germination and seedling development of maize under PEG-induced drought stress.

MATERIALS AND METHODS

Chemicals required for synthesis of SNP loaded chitosan nanoparticles

In the year 2022, the synthesis of sodium nitroprusside (SNP) nanoformulation was carried out at the laboratory of the Centre for Agricultural Nano Technology (TNAU) in Coimbatore. The materials required for the synthesis, including Chitosan (75% deacetylation, medium molecular weight), sodium nitroprusside (SNP), Tween-80 and sodium tripolyphosphate (TPP), were purchased from Sigma-Aldrich India. Additionally, the laboratory provided essential equipment and chemicals such as a magnetic stirrer, sonicator, centrifuge, pH meter, lyophilizer, glacial acetic acid, sodium hydroxide, distilled water and petri dishes for the research work.

Methods for incorporation of sodium nitroprusside in to chitosan nano particles

SNP-loaded chitosan nanoparticles (CSNPs) were prepared using the ionotropic gelation method (Silveira *et al*., 2019). Chitosan was dissolved in 1% acetic acid to achieve a final concentration of 1 mg/mL, followed by the addition of 50 mmol L^{-1} of SNP. The mixture was stirred magnetically for 90 minutes at 25°C and 400 rpm. The emulsion pH was adjusted to 5.3 using a 1 mol/L sodium hydroxide solution. Then, TPP (0.6 mg/mL^{-1}) was added dropwise to the solution and magnetic stirring was continued for 30 minutes at 400 rpm. To prevent particle aggregation, Tween 80 (0.5% v/v) was added. The resulting SNP-loaded chitosan nanoparticle suspension was then centrifuged at 10,000 rpm for 15 minutes and finally freeze-dried before further use or analysis.

Characterization of SNP nanoformulation

Dynamic light scattering (DLS)

The nanoparticle size, polydispersity index (PDI) and zeta potential of the synthesized SNP nanoformulation were measured using the Nanopartica SZ-100 instrument from Horiba Scientific. For the analysis, SNP nanoparticles were dispersed in deionized distilled water (5 mg of sample dissolved in 10 ml of deionized water) and then sonication was performed using a sonics vibra cell sonicator. The measurements were conducted at a scattering angle of 90°C and a temperature of 25°C.

FTIR analysis

The infrared (IR) spectra of the SNP nanoformulation were analyzed using FT-IR 6800 type spectroscopy with the assistance of portable attenuated total reflectance (ATR) Fourier transform infrared spectroscopy (ATRS-FTIR). The measurements were carried out at room temperature in the absorbance mode, with a resolution of 4 cm and 10 scans were performed. For the analysis, 1 mg of the sample was placed on the sensor of the instrument and the FTIR spectra of the SNP-loaded chitosan nanoparticles were recorded.

NO release profile from the SNP CSNPs

The kinetics of NO release from encapsulated SNP (sodium nitroprusside) was studied in two different conditions. In the dark, the NO release was observed over a 24-hour period at 30°C using UV-vis spectroscopy, with a final NO donor concentration of 50 mM. Researchers monitored the spectrum changes at 397 nm to assess the kinetics curve (Jassim *et al*., 2011; Oliveira *et al*., 2016). In the light, the kinetics of NO release from encapsulated SNP were observed over a 9-hour period at 30°C using UV-vis spectroscopy, under light circumstances with a photosynthetic photon flux density (PPFD) of 300 mol m^{-2} s^{-1}.

Plant growth conditions and treatments for standardization test

In the year 2022, a laboratory experiment was conducted at the Department of Agronomy, Tamil Nadu Agricultural University (TNAU), Coimbatore, to optimize the ideal concentration of SNP nanoformulation for mitigating the negative effects of drought stress on maize germination and early seedling growth. The maize hybrid CO (H) 8 was used for the experiment and obtained from the Department of Millets at TNAU. The seeds were first selected for uniformity and health, then surface-sterilized with 5% sodium hypochlorite for three minutes and rinsed with deionized water three times. Subsequently, the seeds were soaked in different concentrations of SNP nanoformulation (20, 40, 60, 80 and 100 µM) for 12 hours and placed separately in petriplates with 20 seeds per petriplate. Both absolute control (without PEG 6000) and control (with PEG 6000) treatments were included and utilized untreated SNP nanoformulation. To impose drought stress, polyethylene glycol (PEG 6000) was used at a concentration of -0.8 MPa. Each treatment had three replications. The petriplates were kept at room temperature and in dark conditions to promote germination. After 7 days, the percentage of germination was calculated following the International Seed Testing Association (ISTA) standard method (Jincy *et al*., 2021). Germination criterion was determined based on the when seed radicle length reaching at least 2 mm of emergence.

Monitoring seedling characteristics

For each treatment, several seedling growth characteristics were measured. The characteristics noted for evaluating the maize seedlings tolerance to drought stress are described below:

Percentage of germination

From the fourth to the seventh day, the proportion of seeds that germinated was measured each day, are seedlings with a plumule and radical length of 2 mm counted as germinated seeds which was then expressed as a percentage. The formula was used to compute the germination percentage, is

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds subjected to germination}} \times 100 \quad \text{Eq...(1)}$$

Root and shoot length

Shoot and root length of randomly chosen seedlings were measured from each replication. The length of the shoot was calculated from the collar region to the tip of the longest leaf and given in centimeters. The root length also expressed in cm and measured from the collar region to the longest root.

Fresh and dry weight seedlings

The fresh and dry weight of the seedlings was recorded from total seedlings of every replication. The fresh weight was recorded and then samples were kept in a hot air oven at 65°C for 48 h, the dry weight was taken and expressed as mg seedling^{-1}.

Vigor index

The seedlings vigor index was calculated in accordance with Abdul-Baki, (1973) description. The formula was used to calculate the vigor index, which was expressed as a percentage.

$$\text{Vigor index} = (\text{Shoot length} + \text{Root length}) \times \text{Germination percentage} \quad \text{Eq...(2)}$$

Germination stress tolerance index and Promptness index

The emerging seeds promptness index (PI) and germination stress tolerance index (GSTI) were calculated using the formulas provided by Bouslama and Schapaug, (1984) and Sapra (1991), respectively.

$$PI = nd2\ (1.0) + nd4\ (0.75) + nd6\ (0.50) + nd8\ (0.25) \quad \text{Eq...(3)}$$

Where:

nd2, nd4, nd6 and nd8= Percentage of seeds which germinate after 2, 4, 6 and 8 days after sowing, respectively.

$$\text{Germination stress tolerance index (GSTI)} = \frac{PIS}{PINS} \times 100 \quad \text{Eq...(4)}$$

Where:

PIS= PI under drought stress.

PINS= PI under normal condition.

Plant height stress index and root length stress index

The plant height stress index (PHSI) and root length stress index (RLSI) was estimated on the tenth day, calculated using the Ellis and Roberts (1981) formula and represented as a percentage.

$$PHSI = \frac{\text{Plant height stressed plants}}{\text{Plant height control plants}} \times 100 \quad \text{Eq...(5)}$$

$$RLSI = \frac{\text{Root length stressed plants}}{\text{Root length control plants}} \times 100 \quad \text{Eq...(6)}$$

Statistical analysis

The experiment design used was a completely randomized design (CRD) with three replications. And the data collected from various traits were statistically analyzed by using R software (version 4.1.2) with the analysis of variance (ANOVA). The critical difference (CD) was determined at 5% probability ($p<0.05$) and the least significant difference (LSD) test was performed to examine the differences in group averages. Microsoft office excel was used to create the figures.

RESULTS AND DISCUSSION

Dynamic light scattering (DLS)

DLS was used to measure hydrodynamic diameter, PDI and zeta potential of sample. The mean particle size of SNP

loaded CSNPs at selected concentration was 244 nm (Fig 1a) and zeta potential was 41.5 mV (Fig 1b). Similar results reported by Sebra *et al.* (2010).

Fourier transform infrared (FTIR) spectra

To validate the presence of functional groups and chemical bonds in a sample, infrared spectral analyses had been conducted. In Fig 2, the spectrum of SNP-loaded CSNPs was displayed. Peaks in the FTIR spectrum for chitosan seen at around 3239 cm^{-1} (corresponding to -OH and $-NH_2$ stretching vibrations), 2887.8 cm^{-1} (corresponding to CH_2 asymmetric stretching), 1618 cm^{-1} (corresponding to $-NH_2$ bending vibrations) and 1316 cm^{-1} (corresponding to C-O stretching vibrations). The peak of $PO4^{-2}$ group of TPP was visible in 1061 cm^{-1}. The SNP exhibited two absorption bands at 2143 cm^{-1} and 1936 cm^{-1} (Fig 2) which confirmed the formation of sodium nitroprusside nanoparticles. The absorption bands at 2143 and 1936 cm^{-1}, attributed to stretching of (Ca=N) and (NO), respectively. Similar results reported by Sebra *et al.* (2015).

NO release profile from CSNPs

The highest stability of SNP-CSNPs under the experimental conditions was demonstrated by the fact that they emitted the least amount of NO, with a peak of 3.3 mmol L^{-1} after 24 hours (Fig 3). In comparison to dark settings, light causes an increase in NO release. For SNP-CSNPs, light dramatically reduced the strength of the SNP absorption band at 397 nm (Fig 3). Similar results reported by Silvaria *et al.* (2019) SNP light reduced the effectiveness of nitric oxide.

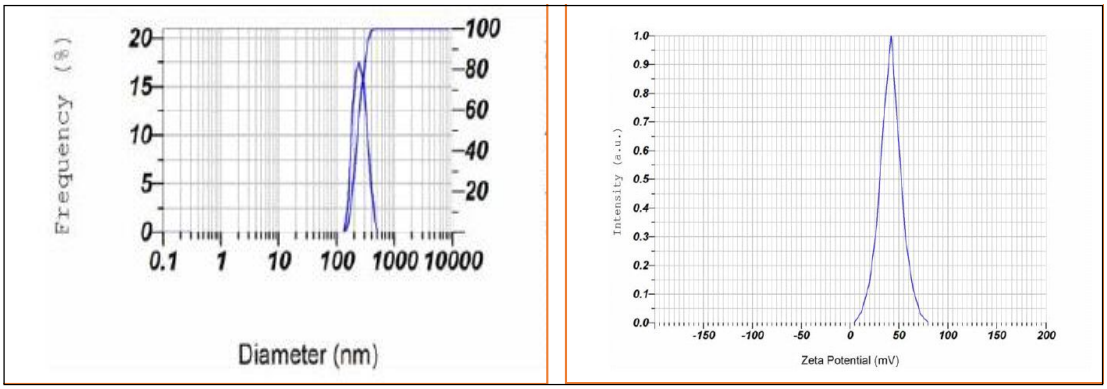


Fig 1: a) SNP Nano formulation particle size b) Zeta potential was analyzed by DLS method.

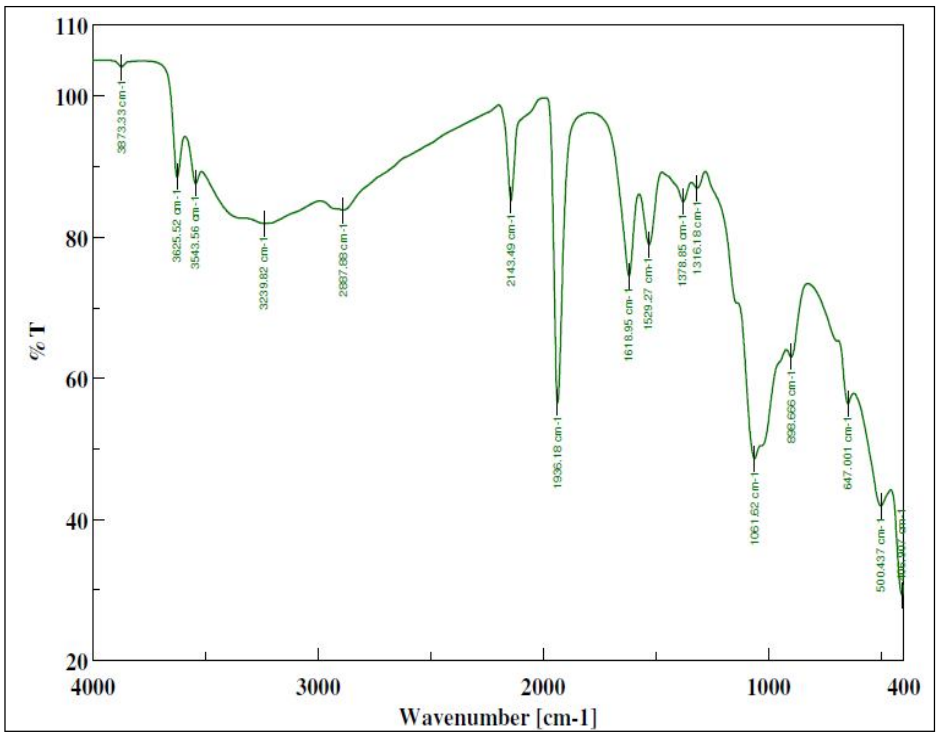


Fig 2: FTIR analysis of SNP loaded CSNP.

Germination percentage

Under the ambient environment (AC) PEG-imposed seeds (Control) showed significantly less germination percentage (41%) than the absolute control (98%) (Table 1). Among the different concentrations of SNP nano formulations seeds treated with 100 µM (84%) and 80 µM (71%) exhibited considerably greater germination rates (Fig 4). My results coincide with Mohamed *et al.* (2016) who concluded that SNP enhances the germination and plant growth of maize under stressful conditions. Similar findings reported by Zheng *et al.* (2009) exogenous application of SNP increased amylase, starch metabolism and promoting seed germination in wheat under abiotic stress.

Vigor index

Results indicated that vigor index of the maize seedlings were substantially decreased under PEG-induced drought stress. Among the SNP nanoformulation treatments, seeds pre-treated with 20 µM of SNP nano formulation had the lowest vigor index (701%) (Table 1). At higher concentration (100 µM) vigor index (1624%) increased to 87.9% as compared to control. Similar findings reported by Wu *et al.* (2013) mobilisation of α-amylase, β-amylase and protease in wheat seeds during early germination was significantly influenced by SNP, which is known to be in charge of embryo extension and reserve destruction and improve vigor index under normal and osmotic stress circumstances.

Root and shoot length of seedlings

Results indicated that shoot and root length of the maize seedlings were substantially decreased under PEG-induced drought stress. Among the SNP nanoformulation treatments, seeds pre-treated with 20 µM of SNP nano formulation had the lowest shoot length (5.4 cm) and root length (5.9 cm) (Table 1). At a higher concentration (100 µM) the shoot (8.4 cm) and root length (11 cm) increased by 77.5% and 80% as compared to PEG induced drought-stressed seedlings. My findings coincide with Mohamed *et al.* (2016) who find out SNP is important for regulating phytohormones like auxin, gibberellin and cytokinin, which are needed for cell division, elongation and tissue differentiation ultimately for increase the root and shoot lengths of maize. Similar reports found by Hu *et al*. (2016) observed that lower doses (50 and 100 µM) of SNP increased root length and seedling growth as well as increase biomass and promote hypocotyl elongation plants.

Promptness index and germination stress tolerance index

The promptness index of maize seedlings were observed to be higher in the SNP nanoformulation pre-treatment at 100 µM (82.3%) and 80 µM (72.2%) compared to control treatments. Similarly, the germination stress tolerance index (86.7%) was higher at 100 µM SNP nanoformulation treatments than PEG-induced drought-stressed (Control) seedlings (Table 1). similar reports found by Bethke *et al.* (2007) reported the sensing, synthesis and NO-mediated reactions are said to occur in the seed aleurone layer. According to Bethke *et al.* (2006)

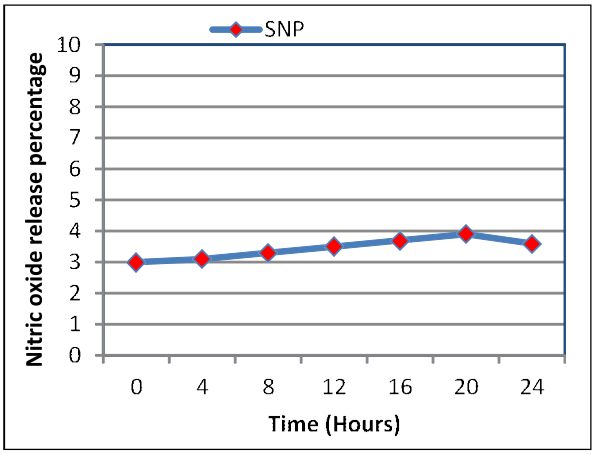


Fig 3: Kinetics of nitric oxide release from SNP Loaded CSNP.



Fig 4: Effect of SNP nano formulation on germination and early seedling growth maize seedlings under PEG-Induced drought stress levels.

Table 1: Effect of SNP nano formulation on germination and early seedling growth of maize seedlings under PEG-Induced drought stress levels (Mean comparison).

Treatments	G.P (%)	R.L (cm)	S.L (cm0	RFW (g)	SFW (g)	RDW (g)	SDW (g)	VI (%)	PI (%)	GSTI (%)	PHSI (%)	RLSI (%)
T_1: SNP20 µM concentration	61.5	5.9	5.4	35.0	81.6	17.3	40.3	701	55.0	57.9	56.0	47.7
T_2: SNP40 µM concentration	65.5	7.3	6.3	39.6	88.0	19.6	43.6	897	60.3	63.5	65.6	58.7
T_3: SNP60 µM concentration	71.0	8.0	7.4	43.3	96.0	21.6	47.6	1102	68.3	71.9	76.9	64.3
T_4: SNP80 µM concentration	71.7	10.0	8.3	45.6	115.0	23.0	57.3	1315	70.2	74.0	85.6	80.2
T_5: SNP100 µM concentration	83.3	11.0	8.4	52.6	123.6	25.0	61.6	1624	82.3	86.7	86.5	88.4
T_6: Control (Without SNP)	41.7	5.0	3.5	27.3	67.3	13.6	33.3	357	28.0	29.4	36.4	40.3
T_7: Absolute control	98.3	12.5	9.7	58.0	131.6	27.0	65.6	2184	94.9	100	100	100
SED	4.2	0.4	0.1	3.7	3.4	1.3	1.6	89	3.0	3.2	2.0	3.0
LSD (p<0.05)	9.0*	0.9*	0.4*	7.9*	7.4	2.9	3.5	190*	6.5*	6.9	4.3	6.4*

SNP: Sodium nitroprusside; G.P- Germination percentage; R.L- Root length; S.L- Shoot length; RFW- Root fresh weight; SFW- Shoot fresh weight; RDW- Root dry weight; SDW- Shoot dry weight; VI- Vigour index; P.I- Promptness index; GSTI- Growth stress tolerance index; PHSI- Plant height stress index; RLSI- Root length stress index. (Least significant difference test was used to compare the differences among group means and the critical difference was computed at (P≤0.05). *Significant at (P≤0.05).

with the start of GA-stimulated germination, NO reduce the ABA controlled dormancy by boosting the activity of ABA degrading enzymes and increases germination stress tolerance index.

Fresh and dry weights of shoot and root

The fresh and dry weight of maize seedlings varied significantly between the treatments. The absolute control showed a significant increase in fresh and dry weight over the control treatment. Among SNP treatments seeds pre-treated with 100 µM SNP recorded higher shoot fresh and dry weight (123.6 and 61.6 mg/20 seedlings) as well as higher root fresh and dry weight (52.6 and 25 mg/20 seedlings) over control seedlings (Table 1). The minimum fresh and dry weight of the seedlings was observed in 20 µM (81.6 and 40.3 mg/20 seedlings) SNP nanoformulation pre-treatment. Similar reports found by Cechin *et al*. (2015) reported growth-enhancing effect of SNP under drought stress in many crop species. Application of SNP improved wheat growth parameters such as fresh and dry weights were observed under oxidative stress (Tian *et al*., 2015).

Plant height stress index and root length stress index

Plant height stress index and root length stress index were significantly decreased in control (36.4% and 40.3%) compared with SNP nanoformulation treatments under drought stress. There was a significant variation among the pretreatment of SNP nanoformulation treatments (Table 1). Maximum plant height stress index and root length stress index were noticed in 100 µM (86.5% and 88.4%) and 80 µM (85.6% and 80.2%) of SNP nanoformulation treatments respectively. Similar results were found by Shabbir *et al*. (2016) reported plant height (measured in terms of PHSI in the present study) reduced under water deficit conditions due to dehydration of protoplasm or changes in cell-wall permeability due to lipid peroxidation (Cechin *et al*., 2015). The positive effects of SNP on PHSI and RLSI indicate that it can stimulate root growth and seedling establishment (Yu *et al*., 2014). NO-stimulated increase in RLSI and PHSI might be the consequence of increased NR activity.

CONCLUSION

The current study concludes that drought stress had a negative impact on seed germination and early seedling growth features. In maize seeds pre-treated with various concentrations of SNP nanoformuolulation (20, 40, 60, 80 and 100 µM) under drought conditions significantly improved the seed germination and other growth characteristics. Among the SNP nanoformuolulation treatments seed treated with 100 µM recorded the highest percentage of seed germination and seedling vigor under PEG-induced drought stress conditions.

Conflict of interest

All authors declare that they have no conflict of interest.

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