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BIOCHEMICAL CHANGES INDUCED BY NANOSILICON IN MITIGATING DROUGHT STRESS IN GROUNDNUTS (*ARACHIS HYPOGAEA* L.)

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ABSTRACT

Drought is a significant abiotic stressor for plants, leading to oxidative stress, cell death, and reduced global crop yields. This study investigates the effects of drought stress on relative water content (RWC), photosynthetic pigments, carotenoids, and proline levels to assess the efficacy of nanosilicon (N-Si) in alleviating drought stress in two groundnut genotypes: GG-6 (drought susceptible) and GJG-22 (drought tolerant). Application of exogenous nanosilicon under drought conditions markedly increased RWC, carotenoids, proline, and photosynthetic pigments (chlorophyll a, b, and total chlorophyll) in the GJG-22 genotype compared to GG-6. The findings suggest that N-Si treatment enhances drought tolerance in groundnuts, presenting a cost-effective and environmentally friendly approach to improving crop resilience.

Keywords: Groundnut, Drought, Nano-silicon, RWC, Chlorophyll, Proline

Introduction

The world population could increase to 6-9.3 billion people by 2050, whereas agricultural production has been declining significantly due to several deleterious environmental stress. Extreme weather events are expected to occur more frequently in tropical and subtropical regions, according to climate change forecasts. Abiotic stresses brought on by extreme weather conditions, including water stress, temperature stress, radiation stress, and salt stress, have significant effects on the production of crops like groundnut (*Arachis hypogaea* L.) (Kalarani *et al.,* 2023). Drought is one of the most important abiotic stresses and occurs for several reasons, including low rainfall, salinity, high and low temperatures, and high intensity of light, among others. Drought stress is a multidimensional stress and causes changes in the physiological, morphological, biochemical, and molecular traits in plants (Salehi & Bakhshayeshan, 2016; Seleiman *et al.,* 2021). Drought can negatively affect the germination or establishment of seedlings in the early stages of crop growth. Drought can limit the growing season of certain crops and create conditions that encourage the invasion of insects and diseases. Drought can also lead to a lack of crop yield, leading to rising food prices, shortages, and possibly malnutrition in vulnerable populations (Kunene *et al.,* 2022). Drought stress is one of the most important factors of physiological stress and the major constraint on crop productivity which limits plant growth and metabolism (Kokkanti *et al*., 2022). In drought stress, physiological indicators such as relative water content (RWC), photosynthetic rate, transpiration rate, stomatal conductance, a maximum efficiency of photosystem II decreased and intercellular carbon dioxide increased (Kokkanti *et al.,* 2019).

Groundnut or peanut (*Arachis hypogaea* L.) is a tropical legume grown as an oilseed or food crop is the fourth most important crop in the world (Ajay *et al.,* 2023). Groundnut (*Arachis hypogaea* L.) also known as peanut, monkey nut, goober, or earth nut because the seed develop underground, is in the division Papiolionaceae of the family Leguminosae (Sanders, 2003). Groundnut is the principal vegetable oil crop in India and occupies the top slot in terms of area as well as production of total oilseeds in the country. However, about 85 percent area under groundnut remains rainfed of which nearly 80 percent comes under dryland where irrigation facilities do not exist at all (Roy & Shiyani, 2000).

Peanuts provide food and nutritional security to millions of people across the globe because of its high nutritive values. Drought and heat stress alone or in combination cause substantial yield losses to peanut production. The stress, in addition, adversely impact nutritional quality. Peanuts exposed to drought stress at reproductive stage are prone to aflatoxin contamination, which imposes a restriction on use of peanuts as health food and also adversely impact peanut trade (Puppala *et al.*, 2023). If the drought stress persists for three weeks or more during the early vegetative stage, the impact of the length of the dry period at different groundnut growth stages on pod yield decreases. The impact will be more noticeable in the early stages of seed growth, pegging and pod yields dropped significantly (Ajay *et al.,* 2023). Under drought stress, numerous physiological and biochemical systems are negatively impacted that reduced not only yield but also product quality diminishes. From peg initiation through pod filling, drought stress can significantly diminish pod development phase and pod output (Nautiyal *et al.,* 1991; Songsri *et al.,* 2008). Water stress caused a significant decrease in number of seed per pod/plant, seed weight, pod yield, pod yield per hectare (Nassar *et al.,* 2018). The production, quality, and composition of pods are known to be negatively impacted by drought or moisture deficiency stress (Kandoliya *et al.,* 2015).

Recently, agricultural scientists have focused a lot of attention to nano-particles. Nanoparticles (NPs) as exogenous spraying and/or soil application have received great interest recently owing to their ability to mitigate the deleterious effects of various soil stresses, such as salinity and drought, which positively influence the morpho-physiological growth traits in plants (Tripathi *et al.,* 2015). Drought is a major abiotic stress that restricts plant growth and efficiency although some nutrients such as silicon improve drought tolerance by regulating the biosynthesis and accumulating some osmolytes (Hajizadeh *et al.,* 2022). Silicon nanoparticles (SiNPs) has been shown to have a positive impact on plants through the regulation of physiological and biochemical responses and the synthesis of specific metabolites (Verma *et al.,* 2022). One of the most crucial techniques for reducing drought stress in plants is the exogenous application of silicon (Si) (Ma *et al.,* 2015; Ning *et al.,* 2020).

Silica nanoparticles (SiNPs) are one of the well-studied inorganic nanoparticles for many applications. They offer the advantages of tunable size, biocompatibility, porous structure, and larger surface area (Alhadhrami *et al.,* 2022). Silicon nanoparticles are well known to improve plant growth under normal and stressful environments. Nanosilicon has been reported to enhance plant stress tolerance against various environmental stress and is considered a non-toxic and proficient alternative to control plant diseases (Mahawar *et al.,* 2023). SiNPs can increase the net photosynthetic rate by increasing total chlorophyll contents and regulate the growth of leaves and stems, partly by regulating the metabolisms of plant hormones and soluble sugar (Li *et al.,* 2023). According to Luyckx *et al.* (2017), silicon improves the water status of plants surviving a water deficit by lowering leaf transpiration by forming a double layer silica cuticle beneath the leaf epidermis. In plants, Si also controls the equilibrium of phytohormones and alters morphological and physiological processes in response to stress (Tripathi *et al.,* 2020). In Plants, silicon increased Chl *a* and Chl *b* contents in sugarcane and energy cane plants under severe water deficit. Si supply accentuated the increase in proline content in plants under water deficit (Teixeira *et al.*, 2022). Moreover, it has been reported that application of SiNPs lowered hydrogen peroxidase (H_2O_2) and lipid peroxidation (MDA) content and increased relative water content (RWC), antioxidant enzyme activities (APOX, CAT, and SOD), chlorophyll content, and proline content (Ali *et al.*, 2023; Boora *et al.,* 2023). When silicon used externally, particularly during the reproductive stage, it effectively mitigated the yield loss caused by the prolonged drought and improved in antioxidant activity and soluble sugar, and the reduction in the content of ROS, increased the leaf relative water content (LRWC), chlorophyll content, photosynthetic rate (Pn), stomatal conductance (Sc) and transpiration rate (Tr) in wheat (Ning *et al.,* 2023). SiO_2NPs treatment increased net photosynthetic rates, stomatal conductance, intercellular CO_2 concentration, and transpiration, also led to increase in light use efficiency, rubisco carboxylation rate, increased activities of antioxidant enzymes (superoxide dismutase, catase, and peroxidase), concentrations of antioxidants (ascorbate and glutathione) and decrease in water use efficiency, oxidative damage caused by drought stress in *Cunninghamia lanceolata* seedlings (Liu *et al.,* 2023).The beneficial role of Si under water deficit has been previously described in various plant species such as groundnut (Patel *et al*., 2021), pea (Sutulienė *et al*., 2022) , wheat (Raza *et al.,* 2023), potato (Seleiman *et al*., 2023), sorghum (Kaaria *et al*., 2023) and barley seedlings (He *et al*., 2023).

Groundnut genotypes show significant variation in drought tolerance traits such as RWC, chlorophyll content, proline content and root architecture. Drought leads to a decrease in leaf water potential, stomatal closure, relative water content (RWC) and reduced photosynthesis. It remarkably increased the content of osmolytes (proline, soluble sugar, soluble protein) and lipid peroxidation. This hampers nutrient uptake and limits biomass production. Pod yield is significantly reduced when drought occurs during the pod-filling stage. (Ning *et al.,* 2023; Abady *et al.,* 2024). Drought stress increases the accumulation of proline, which acts as an osmoprotectant, helping to maintain cell turgor and reduce water loss. The accumulation of reactive oxygen species (ROS) during drought causes oxidative damage to chloroplasts and inhibits nitrogen fixation, critical for groundnut productivity (Kunday *et al.,* 2023). The purpose of the current study is to identify the mechanisms behind drought tolerance and the mitigating effect of N-Si through investigating the physiological and biochemical responses of two distinct genotypes of groundnuts under 10% polyethylene glycol-mediated (PEG) drought stress.

Material and Methods

Experimental site

The green house experiment was conducted at Food testing Laboratory, Department of Biotechnology, Junagadh Agricultural University, Junagadh. All physiological and biochemical work was carried at the laboratory of Department of Biotechnology, College of Agriculture, Junagadh Agricultural University, Junagadh.

Plant Materials and methods

Groundnut (*Arachis hypogaea* L.) seeds of different genotypes were obtained from Main Pulse Research Station, Junagadh Agricultural University, Junagadh. Seeds were surface-sterilized by rinsing in 70% ethanol for 1 min followed by treatment with 0.1% (w/v) aqueous mercuric chloride for 10 min. Wash thoroughly four to six times with sterile-distilled water and soak the seeds in sterile water for 4 hrs before use. After surface sterilization, the seeds were germinated in sterile petri-dishes with filter paper moistened with sterile distilled water. After the emergence of radicles (~2.5–3 cm length), the germinated seedlings of both the genotypes were grown for 15 days in glass culture bottles (500 ml capacity) containing 350 ml of Hoagland's nutrient medium and the plants were continuously aerated to avoid hypoxia. The plants were maintained under a controlled environment (photoperiod of 14 h d^{-1} at temperature 25 ± 2 °C, and humidity 21% ±2) for the acclimatization. The Hoagland's nutrient media was replaced by every 7 days with freshly prepared nutrient media. Thereafter, the seedlings were treated with various concentrations of solitary drought (i.e. 10% PEG) and combination with nanosilicon (10% PEG + 2 mM NSi). The control plants were grown in Hoagland's nutrient media without PEG and nanosilicon (NSi). After sixth days of treatments, leaves samples were taken for various physiological and biochemical parameters.

Relative Water Content (RWC)

0.5 gm of fresh leaf of groundnut were taken and transferred in a petri dish, and 25 ml distilled water was added and kept for four hours. Then the leaves were taken out, dried by blotting paper and weighed (Turgid weight). The leaf was kept in oven at 84°C for 5 hrs and weighted until constant weight was obtained. After this RWC expressed as per cent relative water content were calculated according to Weatherley (1962).

Chlorophyll a and Chlorophyll b and Total Chlorophyll Content

The fresh groundnut leaf weighed to 0.1 g and was cut into small pieces and crushed into chilled 80 % acetone. The whole paste was filtered with whatman No. 1 filter paper. Filtrate collected and volume made to 10 ml with chilled 80 % acetone. Absorbance was measured in spectrophotometer at 645 nm and 663 nm for determination of chlorophyll a, b and total chlorophyll content were calculated according to Arnon (1949).

Total Carotenoids

Total Carotenoids content was determined using same extract (used for chlorophyll content) and absorbance was recorded at 480 nm. Total carotenoids content was calculated according to Chamovitz *et al.* (1993).

Quantification of free proline content

The free proline content of control and treated plants was determined using the method of Bates *et al.* (1973) with some modifications. Approximately 0.1 gm of leaf tissue were blended with 2 ml of aqueous solution of sulfosalicylic acid 3% (w/v) and then the homogenate was centrifuged for 20 min at 4000 rpm. Then 2 ml gacial acetic acid and 2 ml ninhydrin reagent (1.25 gm ninhydrine + 30 ml glacial acetic acid + 8 ml 6 M phosphoric acid in 12 ml distilled water) were added. Then tubes were kept in boiling water bath at 100 C for 60 min. After this period, the tubes were cooled in running water at room temperature after that 4 ml of toluene added. The samples were shaken vigorously and the chromophore containing toluene (upper layer) was transferred to a new test tube. Finally, the absorbance was read at 520 nm using a spectrophotometer and toluene as a blank. The concentration of free proline was calculated using proline standard and expressed as μmol g^{-1} FW.

Data analyses

For all the physiological and biochemical analysis, 4 replications were taken and analyzed statistically using factorial complete randomized design (FCRD). Data were subjected to two-way analysis of variance (ANOVA) and significant differences among means were calculated by Duncan's multiple range test ($p \leq 0.05$). Analysis of variance will be worked out using standard statistical procedure as described by Panse and Sukhatme, (1985).

Results

Effect of Nano-silicon on relative water content (RWC) under drought stress

Drought stress significantly reduced the relative water content (RWC) in groundnut leaves. When compared to the control plant after drought stress treatments, RWC decreased by 24% and 44% in the drought tolerance (GJG-22) and susceptible genotype (GG-6) respectively. Exposure to N-Si after drought stress intensities caused a significant increase in RWC by14% in drought tolerance and 32% in drought susceptible genotypes of groundnut leaves (Fig.1).

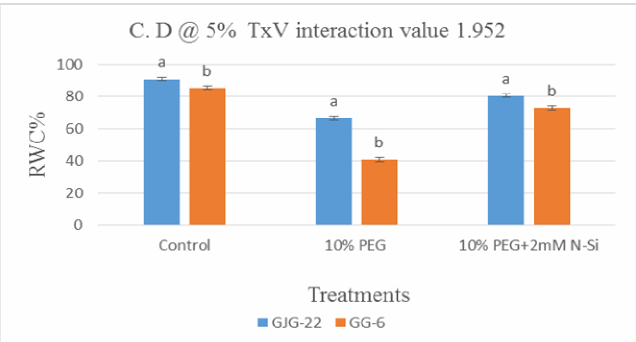

Fig. 1: Exogenous N-Si induced changes in the relative water content (RWC%) in leaf of two genotypes under drought stress. GJG-22: Drought tolerance genotype & GG-6: Drought susceptible genotype.

Impact of Nano-silicon on photosynthesis pigments under drought stress

Chlorophyll a, b, and total chlorophyll Content

Drought stress caused to reduce photosynthesis and PSII efficiency in the photosynthetic pigments including Chl *a*, Chl *b*, and total Chl in comparison with the control conditions, since they are an indicator of stress. The results indicated that all treatments under control conditions had the highest Chl *a* compared to the drought treatments which were the lowest value in both genotypes, although the amount of Chl *a* in GJG-22) was more than that in (GG-6). Chlorophyll a significantly decreased 40% in drought tolerance (GJG-22) and (47%) in susceptible genotypes (GG-6) under drought stress compared to the control. However, when compared to a 10% PEG drought stress plant, the exogenous administration of N-Si considerably increased chlorophyll a content by 25% and 32% in the GJG-22 and GG-6 respectively (Fig. 2A). In general, the application of drought caused a significant decrease in Chl *b* compared to the control condition in both genotypes. GJG-22 was more resistant to the 10% PEG drought stress as amount of chlorophyll b decreased 30% in GJG-22 and 39% in GG-6. However, application of N-Si significantly enhanced chlorophyll b content by 19% in GJG-22 and 30% in GG-6 as compared to 10% PEG drought stress plant (Fig. 2B). The same results were observed in total Chlorophyll as the total Chlorophyll value reduction by 37% in GJG-22 and 43% in GG-6 under 10% PEG drought stress compared to control. while treatment of the groundnut leaves N-Si significantly increased total chlorophyll content by 24% and 25% in the same genotypes under 10% PEG drought stress (Fig. 2C).

Total carotenoids

Drought stress led to a decrease in total carotenoids by 32% and 42% in drought tolerance and susceptible genotype respectively compared to the control. Results of treatment with N-Si under 10% PEG drought stress significantly increased total carotenoids content by 24% in drought tolerance and 25% in susceptible genotypes (Fig. 2D).

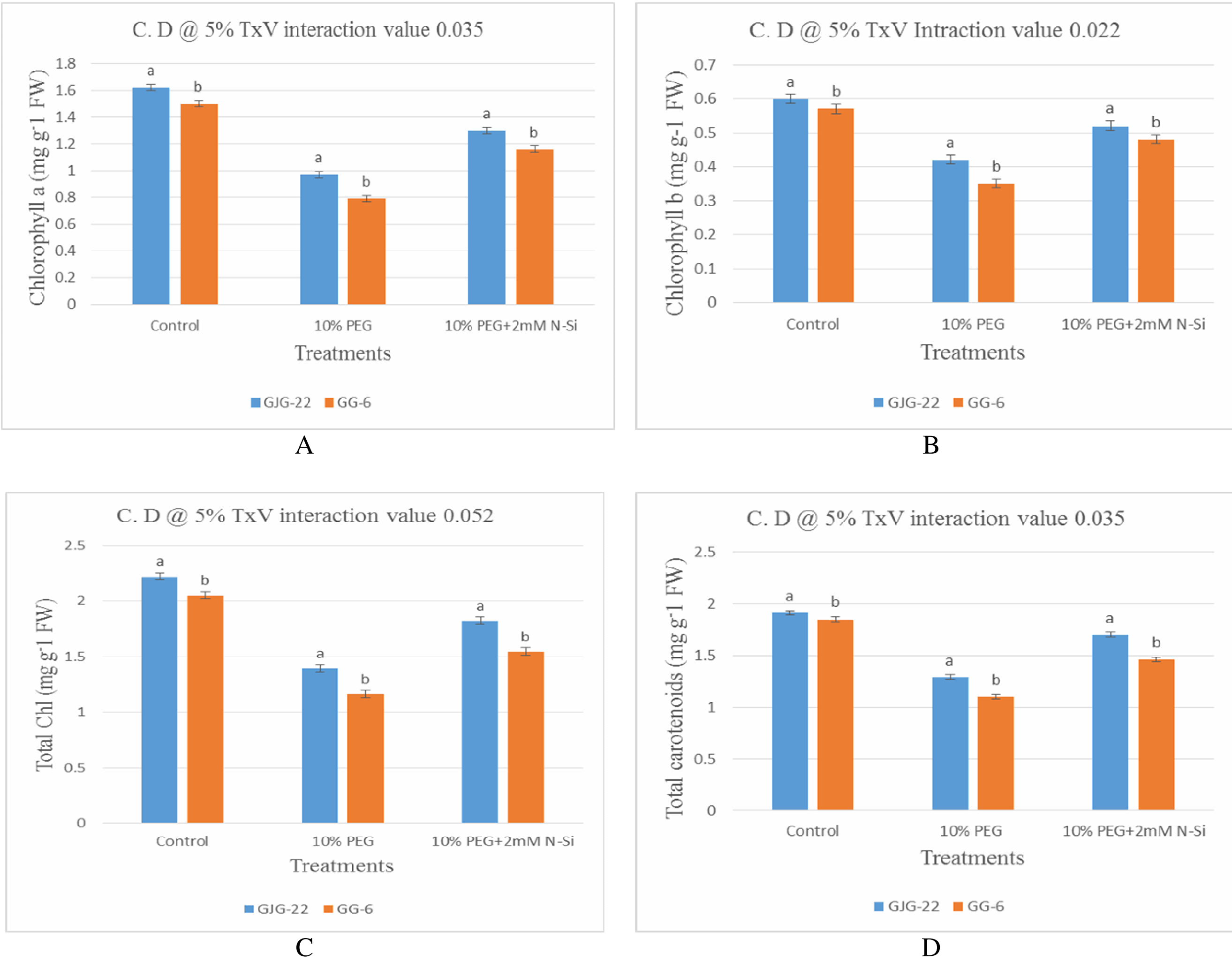

Fig. 2: Exogenous N-Si induced changes in photosynthetic pigments in leaf of two genotypes under drought stress. (A) Chlorophyll a; (B) Chlorophyll b; (C) Total Chlorophyll a; (D) Total Carotenoids. GJG-22: Drought tolerance genotype & GG-6: Drought susceptible genotype.

Effect of Nano-silicon on free proline content under drought stress

Based on Fig. 3, the maximum proline content was observed in GJG-22, followed by GG-6, under 10% PEG drought stress. Drought stress treatment resulted in accumulation in 4- and 5-fold higher free proline content as compared to the control in the drought tolerant and susceptible genotype, respectively. In contrast, the application of nano-silicon significantly decreased the free proline concentration in drought tolerance and susceptible genotypes as compared with 10% PEG drought stress (Fig. 3).

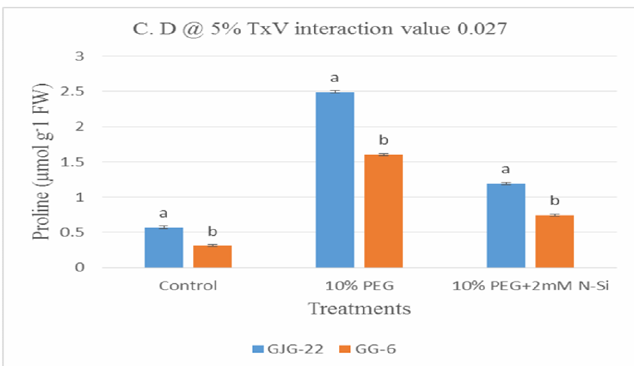

Fig. 3: Exogenous supplementation of N-Si induced changes osmolyte proline in leaf of two genotypes under drought stress. GJG-22: Drought tolerance genotype & GG-6: Drought susceptible genotype.

Discussion

Groundnut, a valuable oilseed crop in semi-arid and arid areas of developing countries, is particularly susceptible to drought stress, resulting reduced yield of groundnut but also poor quality of products. Drought stress is one of the most important factors of physiological stress and the major constraint on crop productivity which limits plant growth and metabolism and significantly influence of drought stress on the physiological and biochemical levels in all the groundnut genotypes (Kokkanti *et al.,* 2022). Peanut contains many other functional compounds like vitamins, proteins, minerals, fibers, polyphenols, antioxidants, and which can be added as a functional ingredient into many processed foods (Arya *et al.,* 2016). The present investigation was undertaken to generate information on drought tolerance in two groundnut varieties, and its effect on various characteristics. We evaluated the two varieties (GJG-22 and GG-6) under three treatments such as control, 10% PEG and 10% PEG+ 2mM N-Si. The parameters we evaluated were the physiological and biochemical, of the two varieties. Under physiological and biochemical parameters, we have taken relative water content (RWC), chlorophyll content (chlorophyll a, b and total chlorophyll), carotenoids and free proline content.

Physiological and Biochemical Parameters

In the present study, revealed that RWC of groundnut was strongly affected by nano-silicon treatments under drought stress and the silicon applied plants still maintain higher water potential and water content with compared to those without application of silicon under drought, which indicated that application of silicon improved the water status of stressed wheat plants. Application of silicon increased the relative water content, water potential and leaf transpiration rate under drought (Gong *et al.,* 2012). Supplementary application nSi effectively increased the leaf RWC of stressed plants compared to the drought stress (Hassan *et al.*, 2022). Si promotes the uptake and transport of mineral nutrients, which eventually promote plant growth and significantly increasing the relative water content (RWC), growth and biomass under drought stress in groundnut (Patel *et al.*, 2021). Additionally, exogenous application of silicon increased the higher retention of relative water content under drought stress conditions (El-Beltagi *et al.,* 2024). Our study exhibited that the Si application partly enhanced the RWC under drought condition in both genotypes. The positive effect of Si which significant increases in relative water contents could enhanced drought tolerance and increased production in drought conditions (Sattar *et al.,* 2023).

In the present investigation drought-induced significant reduction in the chlorophyll a, b and total Chl content in both the groundnut genotypes. However, Si application significantly alleviated this reduction in GJG-22 and GG-6 genotype under drought stress. Chlorophyll is a green pigment found in special cellular organelle called chloroplast present only in plant cells. Chlorophyll plays a pivotal role in photosynthesis and has a unique capacity to trap light energy that utilize in photolysis of water molecules to replenish the reducing power of the cells - which is needed in carbon assimilation in subsequent steps of photosynthesis play a crucial role in adaptation and survival of plants in undesired environmental condition (Mandal *et al.,* 2020). Drought stress triggers the production of reactive oxygen species (ROS), which in turn decompose chlorophyll molecules and eventually thylakoid structure disappears and substantially decreased all photosynthetic pigments (chl *a*, chl *b*, and carotenoids) were compared to well-watered conditions. However, nano-silicon substantially mitigated the deleterious effects of drought stress and markedly enhanced photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) compared to untreated stressed control (Abd-El-Aty *et al.*, 2024). The applying SiNPs increases 51% chlorophyll content as compared to untreated plants, thereby enhancing photosynthesis rate (Alsaeedi *et al.,* 2019). Application 2mM silicon increased the water potential, leaf water contents ratio and leaf photosynthesis pigments (for example, chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids), maximum quantum efficiency of PSII photochemistry (Fv/Fm), actual photochemical quantum efficiency of PSII photochemistry (Y) and chloroplast ultrastructure, under water deficit stress which indicated that the positive effect of Si on photosynthesis was partly associated with stomatal and non-stomatal factors. Si has been found more effective for alleviating the adverse effects of water deficit stress (Ju *et al.*, 2020; Bukhari *et al.*, 2021). Sattar *et al.* (2023) reported that exogenously applied Si significantly improved the activity of photosynthetic photosynthetic pigments (Chl a, Chl b & total Chl) involved in the Si-mediated regulation of photosynthesis in wheat under drought stress. Moreover, Si application alleviated the negative effect of drought by positively affected the growth and increased the Chl a, Chl b, total Chl, and decreased the oxidative damage by decreased electrolyte leakage and hydrogen peroxide, thus enhance drought tolerance (Khan *et al.*, 2024).

Carotenoids, pigment molecules found in plants, play a crucial role in protecting plant cells from photooxidative damage by actively scavenging reactive oxygen species (ROS) like singlet oxygen, and by quenching excited triplet chlorophyll molecules, which are primary sources of ROS generation within the photosynthetic apparatus (Swapnil *et al.,* 2021). Carotenoids play important roles in drought resistance in higher plants. Physiological drought can affect respiration, growth hormone levels, nutrient metabolism, and ultimately photosynthesis and related pigments such as chlorophyll and carotenoid content (Gaurana *et al.,* 2022). Groundnut plants in drought stress induced a significant decrease in carotenoids relative to the non-stressed plants (Bakhoum *et al.,* 2023). This adversely affects the functioning of the plant as it hinders the process of photosynthesis (Mibei *et al.*, 2016). Therefore, externally administered of silicon dioxide nanoparticles increased the content of carotenoids and decreased oxidative damage (Ashraf *et al.,* 2024; Amini *et al.,* 2024). The amount of total carotenoids greatly increased upon root exposure to Si-based NMs (Xu *et al.,* 2023). Additionally, Ghorbanpur *et al.* (2022) reported that applying Si-NPs to barley plants under drought stress significantly increased the amount of carotenoid present in the leaves.

Proline is a very important amino acid and considered as osmoregulators, playing an essential role in osmoregulation to mitigate the injurious impact of stresses such as drought (Abdelaal *et al.,* 2020). Proline accumulation generally improves osmotic stress tolerance whereas proline metabolism can have varying effects from ATP generation to the formation of reactive oxygen species, besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule (Hayat *et al.,* 2012; Furlan *et al.,* 2020). Like our result, under drought stress, the concentration of proline was found to be significantly higher in the drought tolerant variety compared to drought susceptible variety (Solanki *et al.*, 2014). However, N-Si application, affect the proline content under drought condition in both the genotypes. Si application significantly decreased proline content under drought stress in winter wheat (Ning *et al.*, 2023). Exogenous nSi at drought resulted in that proline decreased at irrigation regimes under drought also improved yield and fruit weight and reduced fruit drop percentage in olive leaf (Hassan *et al.*, 2022). On the contrary, the decrease in the proline content in response of applied nano-silica treatment under drought stress reported in barley and improving the nutrient status in plants (Hellala *et al.,* 2020). Proline, accumulates in response to stress conditions and accumulation of proline is an indicator of drought stress and it proves to be a better marker for selective improvement of drought tolerance (Gavhare *et al.,* 2020).

Conclusions

The current study clearly indicates that plants react to drought stress through decreasing their relative water content, photosynthetic pigments (such as chlorophyll a, b, and total chlorophyll content), carotenoids, and increasing the proline content in both groundnut genotypes. The adverse effects of drought stress are mitigated by the external application of N-Si. In both groundnut genotypes, the exogenous application of N-Si increased the relative water content under drought stress. Additionally, the drought stress with N-Si supplementation improved photosynthetic pigments and carotenoids. Under drought stress, plants treated with N-Si showed reduced proline levels in both genotypes. The present investigation indicates that GJG-22 more tolerant than GG-6 genotype under drought conditions in the presence of N-Si which enhanced RWC, photosynthetic pigment content, carotenoid, and proline content in the leaf tissue of GJG-22 compared to GG-6 genotype. As a result, in areas characterized by semi-arid and arid climates, the application of N-Si can play a vital role in promoting the development and productivity of groundnut crops. These results briefly highlight that the N-Si may provide greater tolerance to drought stress in groundnut.

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