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Synergistic Approaches for Wheat Productivity in Saline-Sodic Soils: Nano-Gypsum, Bacillus Inoculation and Nanomaterial Foliar Treatments

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ABSTRACT

Soil salinization and sodication adversely affect soil fertility and agricultural output, particularly in arid and semi-arid regions. This research sought to assess the effects of soil application of nano-gypsum (NG), inoculation with salt-tolerant plant growth-promoting bacteria (PGPB), and foliar application of nano-calcium oxide (NCaO), nano-zinc oxide (NZnO), and nano-silica ($NSiO_2$) on wheat cultivated in saline-sodic soil. The results demonstrated significant enhancements in various growth parameters of wheat, including pigments, proline acid (PA), total soluble sugars (TSS), ascorbic acid (ASA), biomass yield, and wheat grain production. The levels of essential nutrients in the wheat increased as a result of applying NG, PGPB, and the foliar application of nanoparticles, with foliar application of NZnO exhibiting the most significant effect, followed by $NSiO_2$. Additionally, the concentration of Na^+ in the wheat tissues was reduced. In conclusion, the combined application of NG, PGPB, and foliar nanoparticles markedly improved the growth and productivity of wheat in saline-sodic conditions, particularly through the foliar application of NZnO and $NSiO_2$, as well as soil application of NG and subsequent inoculation with PGPB.

Keywords: Nanoparticles; Nano-gypsum; Nano-calcium oxide; Nano-zinc oxide; Nano-silica; Plant growth promoting-bacteria (PGPB); Salt stress; Wheat.

1 Introduction

Salinity stress poses a significant environmental challenge that obstructs plant growth and development, as noted by various researchers (Ali F. et al., 2023; Ayman et al., 2024; Bouabdallah et al., 2022; Shahzadi et al., 2024; Shereen et al., 2022). On a global scale, soil salinization represents a critical issue, affecting approximately 836 million hectares (Ayman et al., 2024; Ud Din et al., 2023). Projections indicate that by the year 2050, salinity is expected to impact more than 50% of the world's agricultural land (Kumar et al., 2020; Zhou et al., 2024). The presence of elevated saline levels in soil or irrigation water disrupts the normal physiological functions of plants, leading to a variety of detrimental effects (Khan et al., 2021; Naz et al., 2019; Shahzadi et al., 2024). Salinity stress predominantly hinders plant growth (Hu et al., 2021; Omara et al., 2022). The presence of sodium (Na^+) and chloride (Cl^-) ions in plants disrupts osmotic balance and transpiration rates, thereby reducing crop yield (Javaid et al., 2019). High salt concentrations in the soil also obstruct root water uptake, resulting in water deficits within plant tissues (Ait-El-Mokhtar et al., 2020). This water deficiency, combined with osmotic stress due to excess salts, inhibits cell expansion and overall plant development (Kravchik and Bernstein, 2013; Naz et al., 2021). As a result, plants may exhibit suppressed growth, reduced shoot and root development, as well as diminished biomass accumulation (Ahmed et al., 2020; Farooq et al., 2022; Huang et al., 2023; Kaya et al., 2018; Zafar-Ul-Hye et al., 2022). Furthermore, salinity stress leads to oxidative damage within plants, as elevated salt levels trigger the production of reactive oxygen species (ROS) in plant cells. These reactive species, such as hydrogen peroxide (H_2O_2) and superoxide radicals (O_2^-), cause oxidative harm to essential cellular components, including lipids, proteins, and DNA. Consequently, oxidative stress disrupts normal cellular functions and may culminate in cellular mortality (Hasanuzzaman et al., 2021; Hossen et al., 2022). Elevated levels of salt have a detrimental effect on the synthesis of chlorophyll, leading to its instability. As a result, plants experiencing salt stress often show a reduction in chlorophyll levels, which in turn negatively affects their ability to carry out photosynthesis effectively (Ali F. et al., 2023). To counteract this challenge, both traditional and modern strategies have been employed to support plant growth in saline environments. These solutions include the use of nano-gypsum, along with plant growth-promoting bacteria (PGPB), and foliar treatments incorporating nanoparticles such as iron, selenium, titanium, silicon, calcium, magnesium, and zinc. Gypsum is particularly valuable because of its rich composition of calcium and sulfur, which can significantly enhance plant growth, emergence, and yield potentials in soils characterized by salinity and high sodium content (with electrical conductivity greater than 4 dS/m, exchangeable sodium percentage over 15, and pH less than 8.5) (Qayyum et al., 2017; Sparks, 2003). The application of gypsum effectively reduces the impacts of salt stress by raising calcium ion availability on the surfaces of clay particles, subsequently improving soil structure and lowering sodium levels in saline-sodic soils (Abdul Qadir et al., 2022). When gypsum is applied to salt-stressed plants, it contributes to increases in grain weight, overall crop yield, and the uptake of essential nutrients while simultaneously reducing sodium concentrations in leaves, largely due to its sulfur content (Ghafoor et al., 2008). Moreover, El-Henawy et al. (2024) demonstrated that utilizing gypsum in its nano form enhances its efficacy by increasing surface area and further improving soil conditions affected by salinity. In a complementary role, PGPB has emerged as an important factor in fostering plant growth under conditions of salt stress (Ayman et al., 2024). This has resulted in heightened interest among researchers regarding the application of PGPB (Poria et al., 2022; Ruiu, 2020; Stegelmeier et al., 2022). A range of studies have highlighted the advantages of using various species of PGPB, including Bacillus, Azospirillum, Pseudomonas, Halomonas, Azotobacter, Enterobacter, and Nitrosomonas, among others (Costa et al., 2018; Khan et al., 2023). Research endeavors are ongoing to explore how these beneficial microbes can provide protection for crops against a variety of abiotic stressors such as drought, salinity, and toxic heavy metals (Meng et al., 2023; Mishra et al., 2022). Additionally, recent investigations have focused on the application of bacteria as PGPB, as well as bio fungicides and bioinsecticides, reflecting a growing understanding of how these microorganisms can suppress the growth of harmful pathogens, supply vital nutrients to plants (Ali et al., 2021), regulate plant hormone levels (Orozco-Mosqueda et al., 2023), and detoxify hazardous substances present in the soil (Orozco-Mosqueda et al., 2020). The application of nanoparticles is increasingly recognized as a strategy to alleviate salt stress effects (Taqdees et al., 2022). Oxide nanoparticles like SeO_2, CaO, SiO_2, TiO_2, FeO, CeO_2, Al_2O_3, and ZnO are used for fertilization and enhancing plant growth under various stresses, including salt stress (Ayman et al., 2024; Carrasco-Correa et al., 2023; Herrera et al., 2024; Hussan et al., 2024; Mazhar et al., 2023). Their unique physicochemical properties make them effective for improving plant resilience to salt stress. One approach is precise delivery of essential nutrients and growth promoters (Azmat et al., 2023). Salinity stress disrupts nutrient absorption in plants, causing shortages (Shahzadi et al., 2024). Utilizing nanoparticles for nutrient administration can help counteract imbalances caused by salt stress and support optimal growth (Adhikari et al., 2022). Additionally, nanoparticles can carry beneficial substances like antioxidants and growth regulators, protecting them from degradation and ensuring their direct delivery to plant tissues (Kareem et al., 2022).

Following rice and maize, wheat ranks third in global production, supplying 35% of the world's food grain (Ghonaim et al., 2021). Its production reaches around 700 million tons from 200 million hectares (Shahzadi et al., 2024). As a predominant cereal from the Poaceae family, wheat accounts for about 50% of global commerce and 30% of grain output (Sharma et al., 2020). Wheat serves as a staple for nearly one-third of the population, valued for its affordability and nutrition (Teshager, 2023). It is a rich source of protein, carbs, vitamins, and calories, making it essential for a balanced diet compared to other cereals (Desoky et al., 2020). Wheat (*Triticum aestivum* L.) provides fatty acids, minerals, tocopherols, and phytosterols, with over one-third of the global population relying on it as a primary food crop (Shahzadi et al., 2024).

Despite advances in nanotechnology for enhancing plant tolerance to salt stress, research hasn't explored the combined effects of these materials with PGPB in soil alongside foliar treatments. This study investigates the synergistic application of NG, PGPB inoculation, and foliar nanomaterials NCaO, NZnO, and $NSiO_2$ on wheat in saline soil.

2. Materials and Methods

2.1 *Experimental design and treatments*

A pot experiment was undertaken to evaluate the influence of the application of nano-gypsum (NG), soil inoculation with plant growth-promoting bacteria (PGPB), and the foliar application of nano-calcium oxide (50 mg NCaO L^{-1}), nano-zinc oxide (50 mg NZnO L^{-1}), and nano-silica (300 mg NSiO2 L^{-1}) on the growth of wheat cultivated in saline-sodic soil. The experimental design employed a split-plot arrangement, wherein the main factor comprised three treatments: Soil, Soil+NG, and Soil inoculated with PGPB. The sub-factor consisted of four foliar treatments, namely distilled water (DW), 50 mg NCaO L^{-1}, 50 mg NZnO L^{-1}, and 300 mg SiO_2 L^{-1}. Thus, the total number of plots accounted for 36 pots, being a product of three soil treatment variations, four foliar application treatments, and three replicates. Soil samples were gathered from the surface layer (0.2 m) of salt-affected soil located in the San El Hagar area, Sharkia Governorate, Egypt (geographical coordinates: latitude 31◦02′ 16.8″ N, longitude 31◦51′ 30.1″ E). The wheat cultivar utilized in the study was Misr1 (Triticum aestivum), sourced from the Agricultural Research Center in Giza City, Egypt, and was grown in a pot experiment within the wirehouse of the Faculty of Technology & Development in Zagazig City, Egypt (geographical coordinates: latitude 30◦35′ 23.7″ N, longitude 31◦28′ 53.2″ E) commencing on November 25, 2022, coinciding with the 2022/2023 winter season. The wheat grains underwent soaking treatments with 0.05 M CaCl2 L^{-1} for 12 hours, 10 mg ZnO L^{-1} for 18 hours, and 300 mg $NSiO_2$ L^{-1} for 18 hours. One-third of the pots (12 pots) were filled with 7 kg of control soil, the second third contained soil augmented with 210 g (0.25 g NG hg^{-1} or 17.5 g pot^{-1}), and the final third included soil inoculated with PGPB (Bacillus_sp_Esmail_NVU) using 1 mL of inoculum equivalent to 10^7 CFU mL^{-1} from the tested bacterial strains. The characteristics of this strain are documented as per Abdelhafez et al. (2023), noting its tolerance to salinity levels of up to 27.5% NaCl. Each pot was sown with 15 grains at a uniform depth and spacing. Irrigation was conducted with fresh water (EC= 0.41 dSm^{-1}) whenever 75% of the available water was depleted (approximately 0.5 L every 1.5 to 2 weeks). Plants were harvested at intervals of 80 and 140 days post-sowing.

2.2 *Soil, water, and plant analysis methods*

The determination of particle size distribution was accomplished through the application of a hydrometer protocol. The assessment of bulk density (BD) and particle density (PD) was carried out utilizing core sampling and a pycnometer, respectively. The pH of soil and water were measured employing a pH-meter. The quantification of organic matter (OM) was executed through a wet oxidation method that utilized K_2CrO_7, with subsequent back titration performed using NaOH. Available nitrogen (N) was extracted utilizing potassium chloride (KCl) and distilled employing the Kjeldahl method. The electrical conductivity (EC) of both extracts and water was measured utilizing an EC-meter. Soluble potassium (K) and sodium (Na) were assessed with a flame photometer, while soluble calcium (Ca) and magnesium (Mg) underwent titration using 0.01M EDTA-Na_2. The titration of soluble bicarbonate (HCO3) and carbonate (CO_3) was conducted with 0.01M hydrochloric acid (HCl), and soluble chloride (Cl) was determined following the Mohr method. The cation exchange capacity (CEC) of the soil was evaluated using a 1M sodium acetate (NaOAc) solution. Extractable phosphorus (P) was obtained using 1M sodium bicarbonate ($NaHCO_3$) at a pH of 8.5 and subsequently analyzed using a colorimetric technique via spectrophotometry. The quantification of ammonium acetate potassium (NH4OAc-K) and sodium (Na) in the soil was performed using a flame photometer. Plant-available silicon (Si) was extracted with a 0.01M calcium chloride ($CaCl_2$) solution, with measurement conducted through spectrophotometry. The micronutrients iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) were extracted using diethylene triamine pentaacetic acid (DTPA) prepared in a solution of 0.005M DTPA, 0.1M triethanolamine, and 0.1M CaCl2, with quantification performed using atomic absorption spectroscopy (AAS-PerkinElmer). Analytical techniques were carried out in accordance with the guidelines established by Estefan (2013). A comprehensive summary of the various physical and chemical parameters of soil and water is presented in Table 1.

Wheat samples were subjected to oven drying, grinding, and wet digestion employing a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) at a temperature of 420 °C, following the methodology outlined by Parkinson and Allen (1975). The determinations of total nitrogen (N), phosphorus (P), and potassium (K) levels were executed using the Kjeldahl, colorimetric, and flame photometry techniques, respectively. The analysis of micronutrients (Fe, Zn, Mn, and Cu) was performed utilizing atomic absorption spectroscopy (AAS-PerkinElmer). Total silicon (Si) in wheat straw was subjected to digestion using a 50% sodium hydroxide (NaOH) and 50% hydrogen peroxide (H_2O_2) solution, with measurement conducted via spectrophotometry in accordance with Estefan (2013). Additionally, wheat samples were prepared for the analysis of various parameters including pigments (chlorophyll a, b, total chlorophyll, and carotenoids), non-enzymatic antioxidants (ascorbic acid), and organic osmolytes (free proline and total soluble sugars) utilizing a method of preparation and measurement specifically applied by Lalarukh et al. (2022).

2.3 Characterization of NG, NCaO, NZnO and NSiO$_2$

A detailed investigation of the physio-chemical properties of NG, NCaO, NZnO, and NSiO2 was conducted utilizing an X-ray diffractometer (X' Pert Pro, Netherlands). This analysis employed Cu Kα radiation (λ = 1.5406 Å) and covered a 2θ range of 10° to 80° at a temperature of 293°K, which facilitated the exploration of the crystalline structure of the silica samples. Additionally, the morphology and dimensions of NG, NCaO, NZnO, and $NSiO_2$ were scrutinized using transmission electron microscopy (TEM) tools (CM 200, Philips, USA). The mean diameter of the particles was effectively determined through the use of a particle size analyzer from Particle Sizing Systems, Inc. located in Santa Barbara, California, USA.

2.4 Statistical analysis

For statistical assessment, an analysis of variance (ANOVA) was conducted utilizing the R software package (version 4.3.3). Differences among means were determined using Duncan's test for significant differences, with a significance level set at 5% to assess the disparities between treatment averages. All graphical representations were generated using the OriginLab software package (version 9).

Table (1). Some physical and chemical analysis of soil and water

Analyses		Soil		Water
Physical chaacreization				
Bulk density (BD), g cm^{-3}		1.10		-
Particles density (PD), g cm^{-3}		2.39		
Water holding capacity, %		55		
Field Capacity, %		26		
Permanent wilting pointe, %		14		
Particle size distribution %	Sand	Silt	Clat	
	21.4	41.6	37	
Textural class		Clay loam		
Chemical characterization				
EC, dS m^{-1}		11.32		0.41
pH		7.80		7.81
ESP, %		16.18		1.71
$CaCO_3$, g kg^{-1}		50.70		-
Organic matter, %		0.83		
CEC, $cmol_{(+)}$ kg^{-1}		38.5		
KCl-N, mg kg^{-1}		28		
$NaHCO_3$-P, mg kg^{-1}		23		
NH_4OAc-K, g kg^{-1}		0.313		
NH_4OAc-Ca, mg kg^{-1}		6.90		
NH_4OAc-Mg, mg kg^{-1}		2.06		
$CaCl_2$ –Si, mg kg^{-1}		26.22		
DTPA-Zn, µg g^{-1}		1.39		
DTPA-Mn, µg g^{-1}		6.60		
DTPA-Fe, µg g^{-1}		6.30		
DTPA-Cu, µg g^{-1}		0.94		
Soulble ions				
K^+, meq L^{-1}		17.5		0.26
Ca^{2+}, meq L^{-1}		2.2		1.06
Mg^{2+}, meq L^{-1}		1.1		0.81
Na^+, meq L^{-1}		89		1.97
Cl^-, meq L^{-1}		70		1.73
HCO_3^-, meq L^{-1}		1.5		1.01
CO_3^{2-}, meq L^{-1}		0.00		0.00
SO_4^{2-}, %		39.6		1.36

3 RESULTS & DISCUSSION

3.1 Effect of applied NG, inoculation with PGPB and foliar application of NCaO, NZnO and $NSiO_2$ on growth attributes of wheat grown on a saline-sodic soil

The data presented in Figure 1. delineate the outcomes of the analysis conducted on the nanomaterials, incorporating transmission electron microscope imagery (Figures 1A-1D). Furthermore, Figures 1E-1H illustrate the X-ray diffraction patterns corresponding to each material. The analysis of the nanoparticles revealed average particle diameters of 40 nm, 35 nm, 8 nm, and 9 nm, respectively. The peaks identified in the X-ray diffraction patterns substantiate that the materials in question are nano gypsum (NG), nano-calcium oxide (NCaO), nano-zinc oxide (NZnO), and nano-silica oxide ($NSiO_2$). These findings confirm the purity of the employed nanomaterials, with particle diameters consistently measuring below 100 nm.

Conversely, the data presented in Table 2 substantiate the existence of statistically significant enhancements in the majority of wheat growth parameters when cultivated in saline-sodic soil, attributable to the soil application of NG and the inoculation with PGPB, alongside the foliar applications of the employed nanomaterials. The findings detailed in the aforementioned table reveal that soil application of NG yielded superior results in comparison to the subsequent inoculation with PGPB, relative to the control treatment concerning the principal study variable. Conversely, the foliar application of nanomaterials similarly resulted in advancements in most wheat growth characteristics within saline-sodic soils, when juxtaposed with the untreated control utilizing distilled water. Notably, the foliar application of NZnO and $NSiO_2$ surpassed the efficacy of the other treatments, particularly alongside the soil application of NG. Overall, the average values for the NG soil application treatment—specifically, wheat height, leaf area, spike length, shoot weight, spike weight, 1000-grain weight, grain weight per pot, and biomass yield—demonstrated increases of 3.71%, 29.10%, 8.33%, 11.45%, 22.76%, 2.79%, 7.67%, and 18.94%, respectively, in relation to the untreated control. In a similar vein, the foliar application of NZnO resulted in growth parameter enhancements of 4.40%, 56.48%, 26.00%, 31.12%, 25.69%, 10.60%, 42.03%, and 27.37% for the aforementioned wheat growth metrics. Table 2 further illustrates various growth characteristics of wheat cultivated in saline-sodic soils, corroborating that the soil application of NG or PGPB, along with the foliar application of the tested nanomaterials, contributed to significant improvements in the majority of growth characteristics for wheat in saline-sodic soil, compared to the untreated control. Additionally, the foliar application of nanomaterials exhibited marked enhancements in wheat growth parameters. The soil application utilizing the NG treatment exhibited the most favorable outcomes in comparison to the other treatments, particularly in conjunction with the foliar application of NZnO. This reinforces the conclusion that our applied treatments had a substantial effect in augmenting wheat growth under conditions of salt stress.

According to the findings presented in Table 3, the concentrations of various compounds in wheat, specifically chlorophyll A (Ch. A), chlorophyll B (Ch. B), chlorophyll T (Ch. T), carotenoids (CART), polyphenolic acid (PA), total soluble sugars (TSS), and ascorbic acid (ASA), exhibited a range of values. Ch. A ranged from 1.92 to 2.77 mg g^{-1}, Ch. B from 0.54 to 1.65 mg g^{-1}, Ch. T from 2.47 to 4.42 mg g^{-1}, CART from 0.70 to 1.05 mg g^{-1}, PA from 19.21 to 67.06 µg g^{-1}, TSS from 21.39 to 65.27 µg g^{-1}, and ASA from 0.13 to 0.19 µg g^{-1}. At both the primary and secondary factor levels, the application of nitrogen fertilizer (NG) and plant growth-promoting bacteria (PGPB) led to a notable enhancement in the concentrations of these compounds, with increases of 14.77% and 8.11% for Ch. A; 18.27% and 6.09% for Ch. B; 15.92% and 7.47% for Ch. T; 29.71% and 19.21% for PA; 66.44% and 23.62% for TSS; and 6.49% and 17.12% for ASA, when compared with untreated soil samples, as detailed in Table 3. Moreover, foliar applications of nanomaterials such as NCaO, NZnO, and $NSiO_2$ resulted in substantial increases as well, reported as 13.70%, 32.12%, and 24.08% for Ch. A; 41.39%, 95.08%, and 81.17% for Ch. B; 21.16%, 49.06%, and 39.44% for Ch. T; 38.41%, 135.69%, and 95.43% for PA; 14.57%, 101.87%, and 47.09% for TSS; and -1.80%, 0.00%, and 13.05% for ASA, respectively. Overall, the observed trends in some bio-physiological characteristics remained consistent, with the NG treatment yielding the highest values for Ch. A, Ch. B, Ch. T, PA, TSS, and ASA. In contrast, this same treatment coincidentally resulted in the lowest values for CART.

Salt stress poses a significant challenge to global agriculture, impacting plant growth and crop yields (Ayman et al., 2024; Duan et al., 2024). It negatively affects early growth stages and physiological functions, leading to ionic toxicity, nutrient imbalance, and oxidative damage from reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) (El-ramady et al., 2024). Additionally, osmolyte accumulation and limited cell division contribute to reduced growth (Kanwal et al., 2018). This study explored the mitigation of salt stress in wheat on saline-sodic soil using nano-gypsum (NG) and plant growth-promoting bacteria (PGPB) with nanoparticles (NCaO, NZnO, $NSiO_2$). Salt stress led to decreased growth metrics, but NG and PGPB improved these conditions, particularly with NZnO and $NSiO_2$ (Akhtar et al., 2015).

The application of NG and PGPB, along with foliar nanomaterial treatments, greatly improved wheat growth in saline-sodic soils. Statistical analysis indicated a 7.67% and 1.84% increase in grain yield from NG and PGPB respectively, while biomass yield rose by 18.94% and 13.26% over the control. Gypsum helps reduce sodium levels in affected soils, especially sodic ones, diminishing harmful plant effects. Nano gypsum is particularly effective due to its larger surface area and smaller particle size. Studies confirm that NG improves soil properties and plant growth in saline conditions (Abd El-Halim et al., 2023; Amer et al., 2023; El-Henawy et al., 2024). Specific PGPB strains, particularly Bacillus, promote plant growth under salt stress. Bacillus_sp_Esmail_NVU can tolerate up to 27.5% NaCl and enhances plant growth when combined with nanomaterials. Prior research shows that PGPB can accelerate seed germination and establish crops faster (Mehrabi et al., 2024). The benefits of PGPB arise from their ACC deaminase metabolism, phosphate mineral solubilization, and IAA production, which greatly aid wheat growth amid salt stress (Ali et al., 2024; Albdaiwi et al., 2024; Anees et al., 2020; Mehrabi et al., 2024). These findings collectively suggest that beneficial bacteria enhance plant growth and soil properties in saline conditions, improving the rhizosphere and soil biodiversity, representing a sustainable alternative to traditional reclamation methods.

Most wheat growth parameters improved, with grain yield increasing by 15.81%, 42.03%, and 27.89%, and biomass yield by 10.76%, 27.37%, and 17.76% for NCaO, NZnO, and $NSiO_2$, respectively. This improvement stems from Ca and Zn being vital nutrients, while silicon boosts tolerance to abiotic stress like salinity. Studies show NCaO, NZnO, and NSiO2 enhance plant tolerance to salt. Research indicates NCaO promotes growth under salt stress, aligning with findings that foliar nanoparticles enhance salinity tolerance. Overall, these studies confirm that foliar applications of NCaO, NZnO, and $NSiO_2$ improve wheat's salt stress tolerance, consistent with our research.

The fresh wheat tissue contents of certain pigments, including Ch. A, Ch. B, Ch. T, CART, PA, TSS, and ASA, were reduced in the control treatment, except for CART, which had the highest values. This aligns with Akhtar et al. (2015), indicating that increased chlorophyllase activity and ROS production led to inhibited photosynthesis and unstable pigment protein compositions. Treatments with NG, PGPB inoculation, and nanomaterials enhanced levels of chlorophyll, PA, TSS, and ASA in wheat tissues when compared to controls. Salt treatment notably increased chlorophyll in some plants, particularly wheat, while decreased chlorophyllase activity may explain the reduction in chlorophyll content (Akhtar et al., 2015). Numerous studies document the adverse effects of salt stress, emphasizing decreased chlorophyll due to saline conditions. Research by Shahzadi et al. (2024) showed that salt stress elevated PA, TSS, and ASA, indicating plant resilience against salinity, with PA and TSS being crucial compatible solutes that accumulate to aid growth under stress. Proline acid is vital for protein synthesis, metabolic activities, and immune responses during stress (Kanwal et al., 2018). Clearly, This evidence underscores the advantageous contributions of NG and PGPB soil inoculation, alongside the application of specific foliar nanomaterials like NCaO, NZnO, and $NSiO_2$.

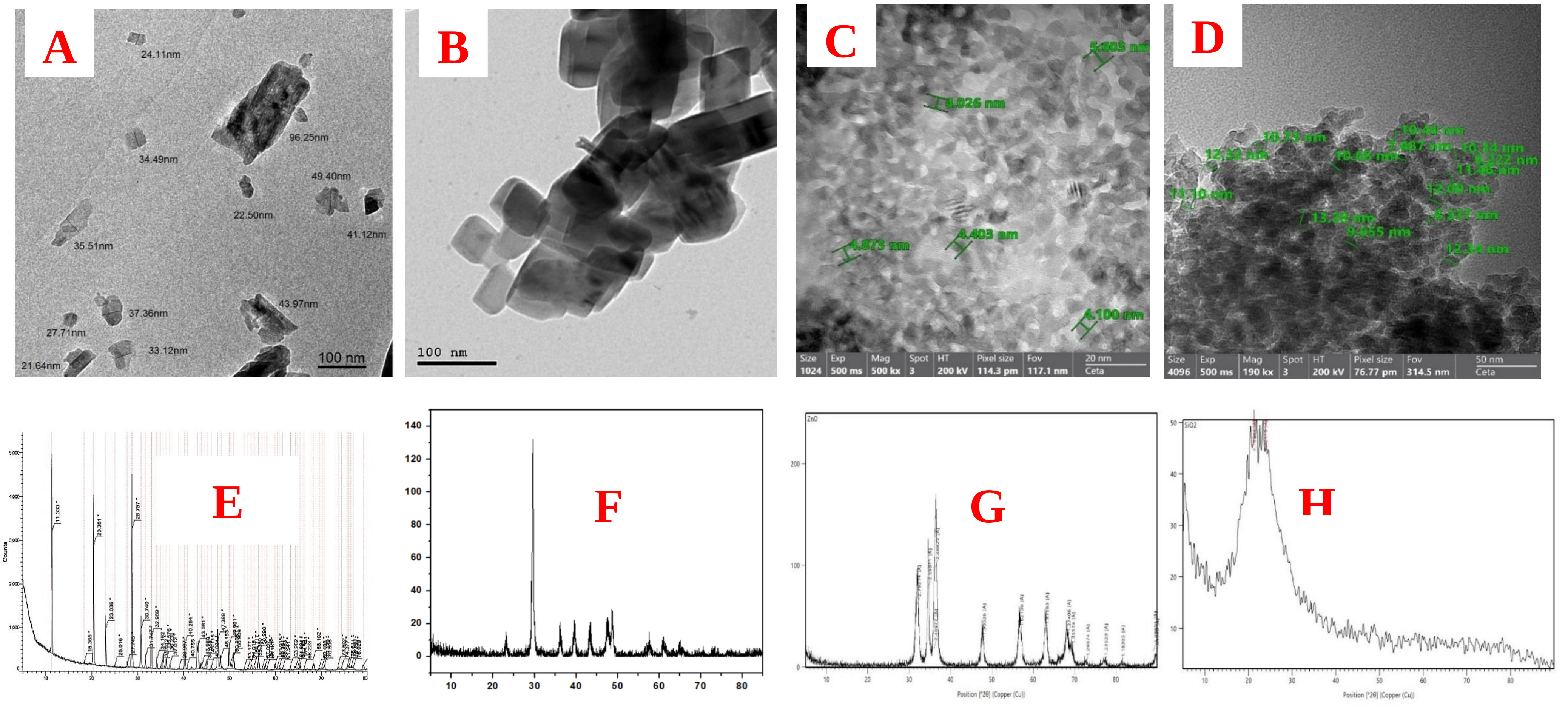

Fig. 1. Transmission electronic microscope (TEM) images of NG (Fig. 1A), NCaO (Fig. 1B), NZnO (Fig. 1C), and nano-SiO_2 (Fig. 1D), as well as, x-ray diffraction patters of NG (Fig. 1E), NCaO (Fig. 1F), NZnO (Fig. 1G), and $NSiO_2$ (Fig. 1H).

Table 2. Effects of applied NG, inoculation of soil with plant growth promoting bacteria (PGPB) and foliar application of NCaO, NZnO, and $NSiO_2$ on some growth and yield parameters of wheat grown on a saline-sodic soil.

Treatments	Stem length (cm)	Leaf area (cm^2)	Spike length (cm)	Shoot weight (g pot^{-1})	Spike weight (g pot^{-1})	1000-grain weight (g)	Grain yield (g pot^{-1})	Biomass yield (g pot^{-1})
				Soil application (SA)				
Without	69.67±1.23 b	21.73±1.70 b	9.00±0.36 c	11.01±0.35 b	21.58±0.61 b	44.72±0.68 b	17.75±0.81 b	32.59±0.87 c
NG	72.25±0.69 a	28.05±1.38 a	9.75±0.28 a	12.27±0.43 a	26.49±0.68 a	45.97±0.41 a	19.11±0.80 a	38.76±1.07 a
PGPB	70.33±0.53 ab	24.81±1.23 b	9.38±0.23 b	11.77±0.37 ab	25.14±0.72 a	45.39±0.56 ab	18.08±0.70 b	36.91±1.03 b
				Foliar application (FA)				
Without	68.22±0.76 c	20.40±1.16 c	8.33±0.32 c	9.81±0.18 c	21.85±0.74 d	42.79±0.43 c	15.08±0.38 d	31.66±0.89 d
NCaO	74.56±0.60 a	22.93±1.09 b	9.28±0.15 b	11.76±0.23 b	23.31±0.72 c	45.25±0.23 b	17.47±0.36 c	35.07±0.81 c
NZnO	71.22±0.70 b	31.92±1.33 a	10.50±0.22 a	12.86±0.39 a	27.47±0.84 a	47.33±0.34 a	21.42±0.52 a	40.33±1.12 a
$NSiO_2$	69.00±0.53 c	24.20±1.16 b	9.39±0.25 b	12.31±0.23 ab	24.98±0.84 b	46.06±0.37 b	19.29±0.44 b	37.29±1.04 b
SA X FA	**Sig.**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Table 3. Effects of applied NG, inoculation of soil with plant growth promoting bacteria (PGPB) and foliar application of NCaO, NZnO, and $NSiO_2$ on levels of chlorophyll A (Ch. A), B (Ch. B), total (Ch. T), carotenoids (CART), proline acid (PA), total soluble sugars (TSS) and ascorbic acid (ASA) in wheat plant.

Treatments	Ch. A (mg g^{-1})	Ch. B (mg g^{-1})	Ch. T (mg g^{-1})	CART (mg g^{-1})	PA (µg g^{-1})	TSS (µg g^{-1})	ASA (µg g^{-1})
				Soil application (SA)			
Without	2.18±0.07 c	1.05±0.10 b	3.22±0.15 c	0.89±0.03 a	36.75±5.18 b	26.54±2.88 b	0.15±0.01 a
NG	2.50±0.08 a	1.24±0.10 a	3.73±0.16 a	0.79±0.03 c	47.67±3.94 b	44.17±4.63 a	0.16±0.01 a
PGPB	2.35±0.09 b	1.11±0.09 b	3.46±0.16 b	0.84±0.02 b	43.81±4.2 8 ab	32.81±3.93 b	0.18±0.01 a
				Foliar application (FA)			
Without	1.99±0.05 d	0.73±0.07 c	2.73±0.09 d	0.97±0.02 a	25.54±1.98 d	24.49±1.11 c	0.16±0.01 a
NCaO	2.27±0.07 c	1.03±0.05 b	3.30±0.06 c	0.87±0.02 b	35.35±2.50 c	28.06±1.30 c	0.16±0.01 a
NZnO	2.64±0.05 a	1.43±0.07 a	4.06±0.10 a	0.73±0.01 d	60.19±2.86 a	49.44±6.10 a	0.16±0.01 a
$NSiO_2$	2.47±0.07 b	1.33±0.07 a	3.80±0.08 b	0.78±0.02 c	49.91±3.85 b	36.03±4.84 b	0.18±0.01 a
SA X FA	**Sig.**	**NS**	**NS**	**NS**	**NS**	**Sig.**	**NS**

All values =means ± standard errors of triplicate values of each treatment are presented. Different small letters in column indicate significant differences (at $p \leq 0.05$) among treatments.

3.2 Effect of applied NG, inoculation with PGPB and foliar application of NCaO, NZnO and $NSiO_2$ on some macro and micro-nutrients of wheat tissues grown on a saline-sodic soil

The levels of macro and micro-nutrients in wheat significantly increased with soil application of NG and PGPB inoculation, combined with foliar NZnO application. Nutrient content in wheat straw ranged from 0.37 to 0.57g N hg^{-1}, 0.02 to 0.24g P hg^{-1}, and 0.68 to 2.01g K hg^{-1}. In grain, nitrogen ranged from 2.05 to 2.50g N hg-1, phosphorus from 0.48 to 0.72g P hg^{-1}, and potassium from 0.36 to 0.49g K hg^{-1} (Table 4). Trace elements in straw included Zn (38.46 to 79.43μg g^{-1}), Mn (6.41 to 8.97μg g^{-1}), Cu (2.35 to 6.27μg g^{-1}), Si (3.05 to 7.42g hg^{-1}), and Na (0.06 to 0.75g hg^{-1}). Grain levels of Zn ranged from 26.16 to 40.08μg g^{-1}, Mn from 5.13 to 9.23μg g^{-1}, and Na from 0.003 to 0.05g hg^{-1}. Higher Zn and Si concentrations were observed with NZnO and NSiO2 (Table 5). Na content significantly decreased due to treatments with NZnO alongside NG. NG treatments improved most nutrient levels and Si, except for Fe in straw, while NZn showed the best levels aside from Mn, Cu, Si, and Na. Si levels increased with NG and PGPB, and the nutrient/Na ratio improved, particularly with foliar NZnO under NG treatment. This study illustrates that interventions effectively enhanced wheat growth in saline-sodic conditions; control treatments had the highest Na absorption, which decreased with applied treatments, confirming increased nutrient uptake and reduced Na absorption. The ratios of K/Na, P/Na, Zn/Na, and Fe/Na increased with soil and foliar treatments. Foliar NZnO yielded the highest K, P, Zn, or Si/Na ratios, while $NSiO_2$ and NCaO demonstrated comparable ratios (Fig. 2). The application of NG or PGPB, along with foliar nanoparticles, reduced sodium absorption in wheat tissues, mitigating sodium's adverse effects. Furthermore, Pearson correlation coefficients among examined parameters indicated strong positive correlations in most studied parameters, with varying significance in carotenoids, manganese, and sodium, particularly in foliar applications with NG compared to PGPB. This indicates the beneficial effects of applying NG or PGPB in enhancing wheat growth and productivity, as well as improving soil fertility in salt-impacted soils (Fig. 3).

Nutrient absorption in wheat straw and grains for N, P, K, Fe, Zn, and Si increased, while Cu, Mn, and Na decreased with applied treatments. Soil application with NG showed significant advantages, especially with foliar NZnO compared to others. P and Zn content in wheat significantly increased with all treatments, enhancing salt stress tolerance and growth parameters under saline conditions. Benefits of soil and foliar nanoparticle applications are outlined in the discussion.

Numerous studies show the significant impact of treatments on plant growth and nutrient absorption amid soil salinity, effectively mitigating salinity's negative effects and promoting wheat growth in saline-sodic soil (Abd El-Halim et al. 2023, Amer et al. 2023, and Ayman et al. 2024).

Na^+ levels significantly decreased with NG soil application, PGPB inoculation, and nanoparticle foliar application, while control showed an increase. These treatments proved superior to untreated options. The K/Na, P/Na, Si/Na, and Zn/Na ratios increased, indicating that NG and PGPB inoculation reduced Na+ uptake in wheat, especially with NZnO application, attributed to NG replacing Na^+ ions in the soil.

All characters and parameters showed generally positive correlations, except for CART, Mn, and Na in wheat tissues, which had negative correlations with other traits. Pearson's coefficient was clearly correlated with NG soil application and PGPB inoculation compared to control. Overall, NG application, PGPB inoculation, and NCaO, NZnO, and $NSiO_2$ foliar application improve soil fertility and boost wheat growth and yield in salt stress.

Table 4. Effects of applied NG, inoculation of soil with plant growth promoting bacteria (PGPB) and foliar application of NCaO, NZnO, and $NSiO_2$ on some macro and micronutrients of wheat shoots and grains.

Treatments	N ($g\ hg^{-1}$)		P ($g\ hg^{-1}$)		K ($g\ hg^{-1}$)		Fe ($\mu g\ g^{-1}$)	
	Shoots	Grains	Shoots	Grains	Shoots	Grains	Shoots	Grains
Soil application (SA)								
Without	0.46±0.01 b	2.13±0.02 b	0.07±0.01 c	0.57±0.01 a	1.06±0.09 c	0.39±0.01 b	269.33±10.26 b	106.86±9.34 ab
NG	0.51±0.01 a	2.44±0.01 a	0.16±0.02 a	0.62±0.03 a	1.59±0.09 a	0.45±0.01 a	256.72±16.45 b	117.37±10.41 a
PGPB	0.45±0.03 ab	2.39±0.02 a	0.11±0.01 b	0.62±0.02 a	1.31±0.09 b	0.43±0.01 a	312.04±25.86 a	99.51±9.40 b
Foliar application (FA)								
Without	0.45±0.01 c	2.26±0.06 d	0.07±0.01 d	0.53±0.03 c	0.96±0.10 d	0.40±0.01 b	239.22±19.44 b	69.28±5.02 d
NCaO	0.43±0.03 bc	2.29±0.05 c	0.10±0.01 c	0.59±0.01 b	1.24±0.09 c	0.42±0.01 b	290.10±30.02 a	96.83±6.57 c
NZnO	0.52±0.01 a	2.40±0.05 a	0.18±0.02 a	0.67±0.02 a	1.66±0.10 a	0.45±0.01 a	294.30±16.05 a	144.91±7.02 a
$NSiO_2$	0.48±0.01 ab	2.33±0.05 b	0.12±0.01 b	0.62±0.01 b	1.41±0.08 b	0.42±0.01 b	293.84±19.71 a	120.63±6.04 b
SA X FA	NS	Sig.	NS	NS	NS	NS	Sig.	NS

Table 5. Effects of applied NG, inoculation of soil with plant growth promoting bacteria (PGPB) and foliar application of NCaO, NZnO, and $NSiO_2$ on some micronutrients, Si and Na of wheat shoots and grains.

Treatments	Zn, ($\mu g\ g^{-1}$)		Mn, ($\mu g\ g^{-1}$)		Cu, ($\mu g\ g^{-1}$)		Na, ($g\ hg^{-1}$)		Si, ($g\ hg^{-1}$)
	Shoots	Grains	Shoots	Grains	Shoots	Grains	Shoots	Grains	Shoots
Soil application (SA)									
Without	53.95±3.71 b	32.1±1.34 b	7.8±0.28 a	6.35±0.32 a	6.27±0.00 a	10.20±0.00 a	0.50±0.06 a	0.03±0.00 a	4.34±0.47 a
NG	58.68±4.15 a	34.0±1.29 a	7.4±0.43 a	6.15±0.39 a	2.35±0.00 c	10.20±0.00 a	0.30±0.05 b	0.01±0.00 b	4.40±0.43 a
PGPB	55.27±3.67 ab	32.5±1.58 b	6.9±0.38 a	7.24±0.51 a	3.66±0.56 b	10.20±0.00 a	0.55±0.04 a	0.02±0.00 a	4.52±0.54 a
Foliar application (FA)									
Without	40.91±1.26 c	27.8±0.75 c	6.7±0.21 a	5.98±0.42 c	4.53±0.69 a	10.20±0.00 a	0.64±0.05 a	0.03±0.01 a	3.31±0.26 b
NCaO	56.32±1.39 b	31.8±0.65 b	7.2±0.53 a	5.73b±0.29c	4.53±0.69 a	10.20±0.00 a	0.39±0.06 c	0.02±0.01 c	3.74±0.11 b
NZnO	74.18±1.66 a	39.4±0.26 a	7.7±0.51 a	7.09±0.40 a	3.66±.65 a	10.20±0.00 a	0.24±0.05 d	0.01±0.00 d	3.65±0.21 a
$NSiO_2$	52.46±2.41 b	32.5±1.25 b	7. 8±0.37 a	7.52±0.58 ab	3.66±0.65 a	10.20±0.00 a	0.52±0.04 b	0.02±0.00 b	6.99±0.21 b
SA X FA	NS	NS	NS	NS	NS	NS	NS	NS	Sig.

All values =means ± standard errors of triplicate values of each treatment are presented. Different small letters in column indicate significant differences (at $p \leq 0.05$) among treatments.

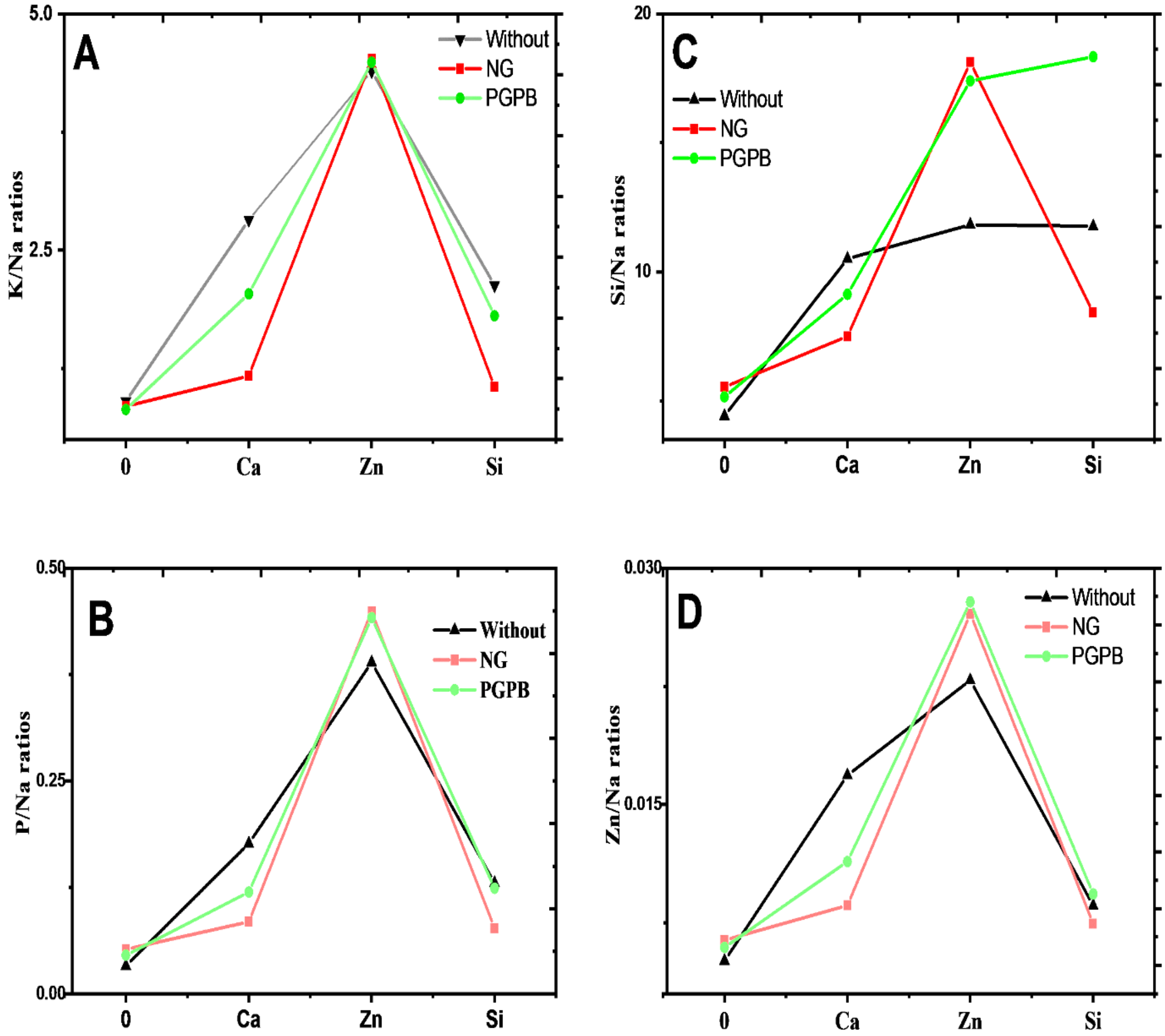

Fig. 2. Effects of soil applied NG, inoculation of soil with plant growth promoting bacteria (PGPB), and foliar application of Nano-Ca (Ca), Nano-Zn (Zn), and Nano-Si (Si) on K/Na (A), P/Na (B), Si/Na (C) and Zn/Na (D) ratios of wheat grown on a saline-sodic soil.

4 Conclusion

The results of this investigation indicate that the synergistic implementation of nano-gypsum (NG) applied to the soil, coupled with soil inoculation utilizing salt-tolerant plant growth-promoting bacteria (PGPB), and the foliar application of nano-calcium oxide (NCaO), nano-zinc oxide (NZnO), and nano-silica (NSiO2), significantly promotes the growth and productivity of wheat under saline-sodic soil conditions. These integrated treatments have collectively enhanced critical growth parameters, elevated essential nutrient levels, and minimized sodium absorption in wheat tissues, thereby alleviating the detrimental impacts of salinity stress. Among the various treatments applied, the foliar application of NZnO exhibited the most significant influence, followed closely by NSiO2, underscoring the effectiveness of nanomaterial-based strategies in mitigating stress conditions.

The findings highlight the possibility of merging nanotechnology with biological inoculants to foster sustainable agricultural practices in soils affected by salinity. This pioneering strategy presents a feasible solution to improve crop resilience and productivity, thereby serving as a promising pathway to tackle the worldwide issue of soil salinization.

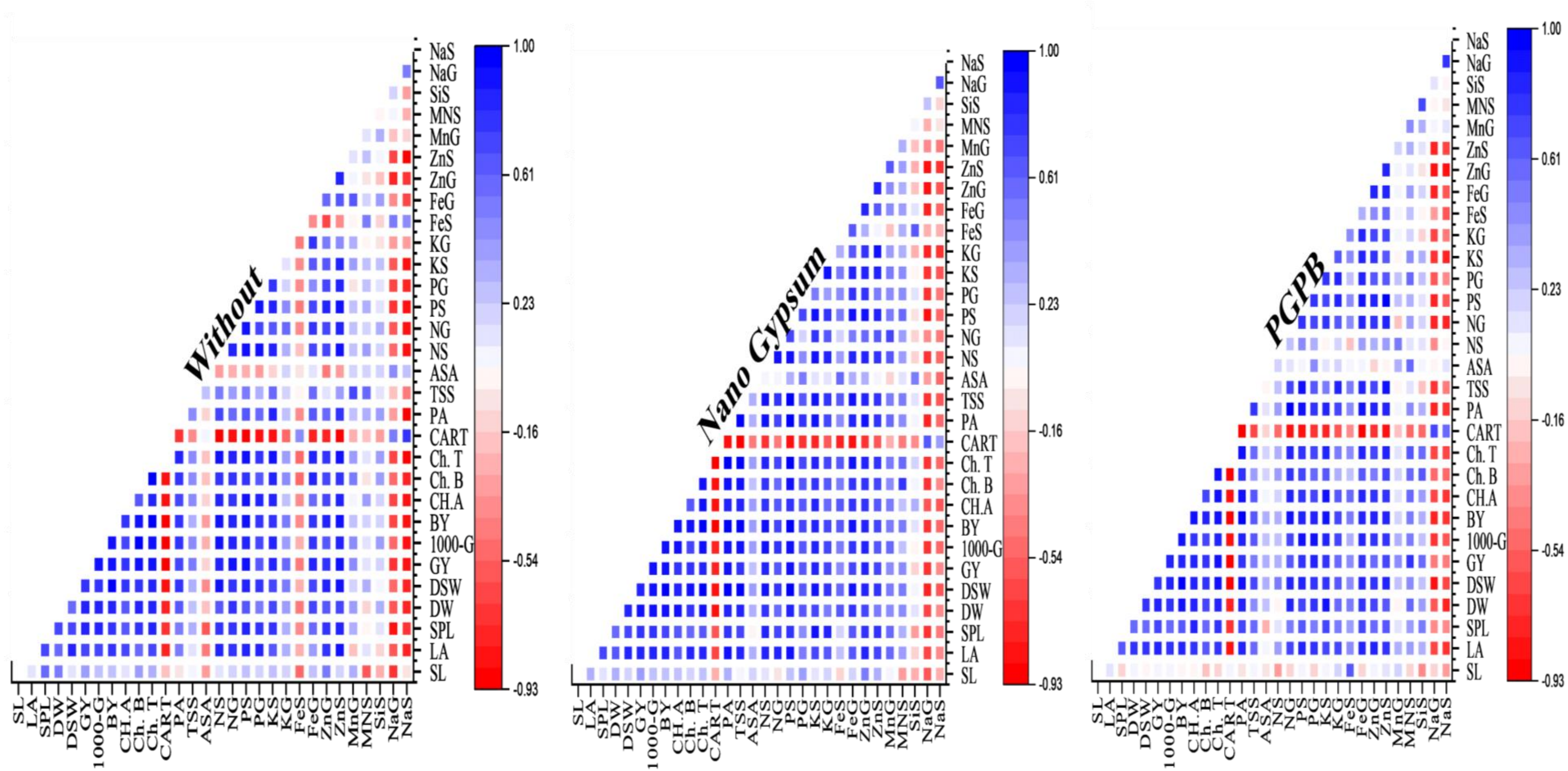

Fig. 3. Pearson's correlation coefficients of control treatment (A), NG (B) and PGPB (C) of plant growth attributes of wheat plant grown on a saline-sodic soil.

Author Contributions

Conceptualization, and supervision Sh.M.M, and M.A; methodology, M.A.S, A.M.A.; software, Sh.M.M.; validation, Sh.M.M and M.A.; formal analysis, investigation, resources, data curation, writing -initial draft preparation, writing, reviewing and editing, M.A, Sh.M.M, A.M.A., and M.A.S. The final paper has been reviewed and approved by all authors.

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Conflicts of Interest

The authors declare no conflict of interest.

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