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Effects of ZnO and hBN Nanopriming on Germination Characteristics of Two Leek Cultivars under Salt Stress

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ABSTRACT

Vigorous and fast germination of seeds are critical in vegetable production. This study was performed to investigate the genotypic effects of two leek cultivars' germination properties in response to different nanoparticles (NPs) seed priming and salt treatments. Seeds of the two leek cultivars (cv. Yaprak and cv. Porro) were primed with different concentrations (0,10,20,40,80 mg L^{-1}) of zinc oxide (ZnO) and hexagonal boron nitride (hBN) nanoparticles in the dark at 20±2 °C and the primed seeds were exposed to salinity stress (200 mM of NaCl) along with control treatment with distilled water. Our results showed that in terms of all germination characteristics and antioxidant responses there are strong two-way, three-way and four-way interactions among genotypes, NPs, their concentrations and salt treatment ($p<0.05$). Therefore, when selecting leek genotypes better responding to nanopriming, different NPs with varying concentrations should be considered for better germination especially under stress conditions. The results will also be helpful for homogeneous, rapid germination and stand establishment of vegetable crops which have highly priced seed.

Keywords Germination, Leek, Nanoparticles, Nanopriming, Salinity, Seed priming, Stress.

INTRODUCTION

Leek (*Allium ampeloprasum* L.), is an herbaceous, cool season, biennial vegetable with thick roots and sparsely branched, in comparison with those of most species. One of the most widely grown vegetables worldwide is the leek, which is a member of the family Amaryllidaceae/Alliaceae. Seed dormancy and germination are crucially regulated by the environment, which serves as the foundation for plant reproduction. Similarly, stress exposure can alter cellular memory in response to subsequent stressors, which may be mirrored by seed priming and have pleiotropic effects on germination (Khan *et al.* 2023). In recent decades, researchers have developed numerous strategies for managing stress in plants. Global warming, industrialization, urbanization along with increased world population lead many scientists to search ways for low input agriculture (Sarkar *et al.* 2020). One of the emerging strategies that have been anticipated to increase crop productivity is nanotechnology. However,

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most of the research on nanoparticles (NPs) currently focuses on their toxicity. The role of NPs in crop protection, particularly under various abiotic stress conditions, is the subject of relatively fewer publications (Rajput *et al*. 2021). In general, a nanoparticle is defined as a particle of a matter which have less than 100 nm in one dimension (Vert *et al.* 2012). However, over 500 nm rod-like ZnO NPs were successfully used as nanofertilizers in soybean to improve crop yield and quality (Yusefi-Tanha *et al*. 2020). Seed priming and other technological advancements such as nanoparticles have improved seed performance. Utilizing NPs in agriculture and natural ecosystems aims to reduce inputs while increasing plant and soil performance and sustainability (Abbasi Khalaki *et al.* 2021). It was reported that onion plants sprayed with ZnO nanoparticles 30 $\mu g\ ml^{-1}$ resulted in early flowering and improved seed yield (Laware and Raskar 2014). Similarly, Acharya *et al.* (2019) showed that green-synthesized Au nanoparticle priming enhanced seedling growth, yield, and quality of onion. However, numerous studies have shown that crops can be poisoned by large quantities of NPs (Gebeyaw 2020, Nile *et al.* 2022). Although ZnO-NPs have beneficial effects at low concentrations (about 50 ppm), effects can be harmful at concentrations above 500 ppm on plant growth and development (Reddy-Pullagurala *et al.* 2018). Raskar and Laware (2014) revealed that lower concentration of ZnO nanoparticles alleviated seed germination, however as the concentration was increased, mitotic index decreased and chromosomal abnormalities occurred in onion. Therefore, assessing right NPs and their doses is also critical. Due to their low toxicity priming with nanoparticles of micronutrients, especially ZnO, have gain great attention since Zn is closely involved in many biochemical and physiological processes (Li *et al.* 2013).

Boron (B) is also one of the most important micronutrients for crop plants affecting plant growth and yield. It is an essential nutrient for improving growth, development, productivity and quality of crops (Pereira *et al.* 2021). It regulates the balance between sugar and starch, and pollination and seed development (Goldbach *et al.* 2001). The nano boron seed treatment enhanced sunflower seed yield by 42.3% and photosynthetic rate, stomatal conductance and transpiration rates were maximum in nano boron treatment (Biradar *et al*. 2023).

So far, no reports have been published for germination effects of seed priming with ZnO and hBN with varying concentrations and using different genotypes in onion family under salt stress. To our best knowledge, genotypic differences with respect to different NPs with varying concentrations were rarely known for leek seed germination and growth parameters under salt stress. Therefore, the objective of this study is mainly to determine how different genetic backgrounds respond to NPs and their concentrations under salt stress along with untreated controls. The results will likely assist breeders for selection of genotypes better germination response with appropriate NP treatments and doses even under abiotic stress conditions such as salinity. The results will also be helpful for vegetable growers who desire rapid and homogeneous germination of expensive vegetable seed such as leek seed.

MATERIALS AND METHODS

Plant material: The experiment was conducted in the laboratory of Cyprus International University (CIU). Seeds of two leek cultivars- *Allium ampeloprasum* L. cv Bulgarian giant (Porro) and cv İnegöl-92 (Yaprak) were obtained from Sgaravatti N and C. S.p.A, Italy and Toprak Bahçe Tarim ve Zirai Urunleri San Tic. Ltd. Sti. Turkey, respectively. Thousand seed weight of cv Porro was 3.1 ± 0.03g and of cv Yaprak was 3.5 ± 0.05g.

Nanoparticles synthesis: To make synthetic zinc oxide nanoparticles (ZnO NPs) in a typical experiment, a 0.45 M aqueous solution of zinc nitrate ($Zn(NO_3)_2, 4H_2O$) and a 0.9 M aqueous sodium hydroxide (NaOH) solution were prepared in distilled water. The NaOH solution was then heated at about 55 ºC. The $Zn(NO_3)_2$ solution was added dropwise (slowly for 40 minutes) under high-speed stirring to the above-warmed solution after two hours it was turned off in this state. Afterwards, the precipitated ZnO NPs were cleaned with deionized water and ethanol, they were dried in air atmosphere at about 200 ºC. The crystal structure of the sample was examined using X-ray diffraction (XRD) method. This technique was used to determine the average size and nature of mycosynthesized ZnO NPs. To analyze crystallographic characteristics, X' Pert High Score software was used and the sizes of ZnO NPs were calculated by the formula of $D = k\lambda/\beta \cos\theta$ where D is the average crystalline size perpendicular to the reflecting planes, β is the full width at the half maximum (FWHM), K represents the shape factor, λ is the X-ray wavelength, and θ is the diffraction angle (Munis *et al*. 2022). Synthetic hexagonal boron nitride (hBN) nanoparticle was purchased from Merck AG (Germany). Physical Properties of hBN nanoparticles are described as follows; 99.50% purity, average diameter particle size 70 nm, Density 2.5 g/cm^3, white colored powder appearance, hexagonal morphology, thermal conductivity 27.

Nanoparticles concentrations preparation: Including control with distilled water, the five different concentrations of 0, 10, 20, 40 and 80 mg L^{-1} each ZnO NPs and hBN NPs were prepared by dissolving the NPs in distilled water using vortex mixer at 3200 rpm for 45 min.

Sodium chloride solution: A total of 200 mM of sodium chloride (NaCl) solution was prepared by dissolving 11.688 g of the solute in distilled water and stirred for 5 min using a magnetic stirrer. After that, the solution was made up to 1000 ml. Electricity conductivity value (EC) of the NaCl solution was 19.69 mS cm^{-1}, while distilled water had pH 7.32 and EC 17.02 µS cm^{-1} which is about thousand-fold lower than salt solution.

Seed treatment: Seeds of each of the leek cultivars were surface sterilized with 70% of ethanol for one min, followed by 20% commercial bleach (5.25% sodium hypochlorite) for 10 min and rinsed with distilled water three times.

Priming: A total 300 seeds of each cultivar were soaked in 25 ml each of different NP concentrations (0, 10, 20, 40, 80 mg L^{-1}) of ZnO nanoparticles and hBN nanoparticles which were imbibed at 20±2 °C in the dark for 24 hrs and were gently shaken at six hours interval. The temperature for NPs imbibition was kept relatively low compared to earlier studies since planting time of leek seed corresponds to low temperature (Adhikary *et al.* 2022). After treatment, seeds were washed three times with distilled water and kept on filter papers to dry overnight (Hanci 2019).

Planting: A total of 50 seeds treated with each concentration of NPs were placed on a double layered filter paper in a 9 cm petri dish, evenly distributed at about one cm apart and moistened with distilled water while another 50 seeds were moistened with 200 mM of sodium chloride solution. These were done in three replications, amounting to 108 petri dishes. The petri dishes were then placed in the incubator in darkness at 21±1°C for 21 days (Hanci *et al.* 2019). Germinated seeds were recorded daily until the end of the experiment. Seeds were considered as germinated when the emerging shoots elongated to two mm. Germination experiments were set according to Completely Randomized Design (CRD) with four factors in three replications. Those factors involve two genotypes, two salt levels, two NP types and five NP concentrations.

In this study, the following equations were used to determine the effects of priming with ZnO and hBN NPs on the germination of leek seeds under salt stress:

$$\text{Germination rate percentage, GP} = \frac{\text{Seeds germinated}}{\text{Total seeds}} \times 100$$

$$\text{Mean germination time, MGT} = \frac{\Sigma(n \times di)}{\text{Number of seed sown}}$$

Where n = number of seeds newly germinated on ith day, di = number of the day.

Germination index, GI = (10×n1) + (9×n2) + • • • + (1×n10) n1, n2 . . . n21.

Germinating index (GI) was calculated as number of germinated seeds on the first, second and subsequent days until the 21th day; 21, 20 . . . and 1 are weights given to the number of germinated seeds on the first, the second and subsequent days, respectively (Benech Arnold *et al.* 1991).

Germination Rate Index (GRI) (%/day) reflects the percentage of germination on each day of the germination period and was calculated as:

GRI=G1/1 + G2/2 +•••+ Gx/x G1=Germination percentage × 100 at the first day after sowing, G2=ermination percentage × 100 at the second day after sowing

The time to reach 50% germination (T50) was calculated according to the following formula of Coolbear *et al*. (1984).

T50 = ti + [(N / 2 - ni) (ti - tj)] / ni - nj

Where, N is the final number of emergence and ni, nj cumulative number of seeds germinated by adjacent counts at times ti and tj, respectively when ni < N / 2 < nj (Coolbear *et al*. 1984). Shoot length (SLT) was measured with ruler in centimeters. Shoot fresh weight (SFW) was measured with precision scale in milligrams.

Antioxidant activity: For DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical analysis, 0.04 g of DPPH reagent was dissolved in 100 ml of methanol. A total of 0.05 g of seedlings was placed in 24-well microtiter plate and one ml of the methanolic solution of DPPH reagent was pipetted into them and was allowed to incubate in the dark for 30 min. Afterwards the seedlings were removed from the sample wells and the absorbance of the reagent solution was recorded in the microplate reader (Kasote *et al.* 2019). Antioxidant analyses were performed using two replications.

Statistical analysis: The statistical SPPS software was used to analyse the experimental data (ver. 21). The data in tables and figures are the averages of three replicates (petri dishes) except antioxidant analysis with two replicates. An analysis of variance (ANOVA) was used for further analysis. A post hoc test with Tukey's Honestly Significant Differences (HSD) at 0.05 significance level was used to determine the significance of the difference among means. At a p-value less than 0.05 (≤0.05), the difference was considered significant. For the sake of simplicity in statistical analysis, genotypes, salt treatment, nanoparticles and their concentrations were denoted as GEN, SALT, NP and CONC, respectively.

RESULTS

Nanoparticle synthesis and properties: The nanoparticles were subjected to X-ray diffraction spectra (XRD) to examine the crystalline nature of the particles. The XRD pattern revealed seven diffraction peaks of corresponding to 100, 002, 101, 102, 110, 103, 112 pure ZnO NPs planes confirming the crystalline nature of the particle as shown in Figs. 1A- 1B. The broad peaks indicate the decrease in crystallinity which inwards suggest the formation of small particles. It is understood that the particles obtained from the peak sizes analyzed by Scherer's method are in the range of 172.8 to 484.3 nm, thus ZnO nanoparticles are obtained (Fig. 1C). The second nanoparticle, hBN (Merck, Germany) was purchased commercially and priming of leek seed with it resulted in significantly variable results depending on genotype, salt, NPs and

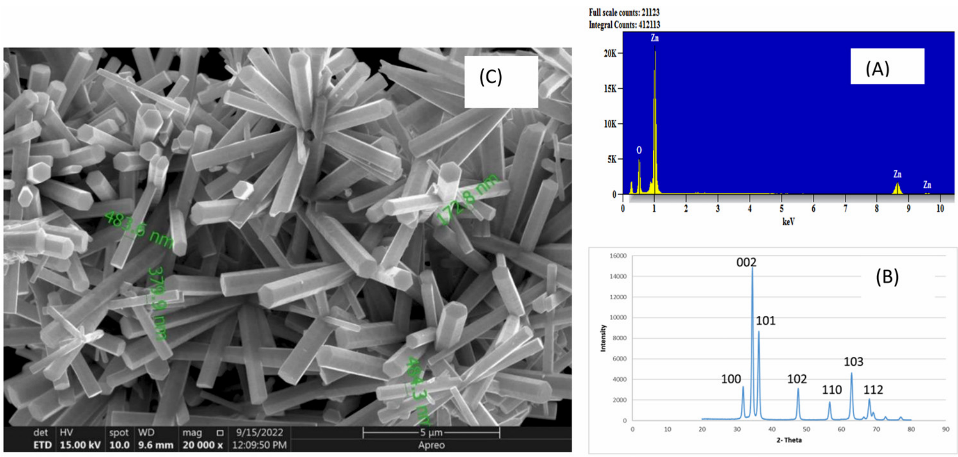

Fig. 1. (A) Energy dispersive X-ray spectrometer (EDPS) data view of ZnO nanoparticles in XRD. (B) The graph showing the peak size of ZnO drawn according to the results of Scherer's method. (C) Scanning electron microscope image (x 20000) and dimensions of ZnO nanoparticle structure supporting the XRD results.

NP concentrations.

Variance analysis of germination parameters: All main effects are influenced by at least one other factor. Significant multiple interaction effects suggest that negative impact of salt stress after nano-priming can be amended by different genotypes, NPs and their concentrations (Table 1). Strong interactions of factors with genotypes imply that NPs treatments with varying concentrations act on genes differently with respect to cultivars' genetic backgrounds even under salt stress. Each germination parameters had at least one different significant variation source when compared to others suggesting that each parameter is genetically characterized differently.

Germination rate (GR): The significant interactions ($p<0.05$) between GEN and SALT as well as among SALT, NP and CONC show that GR response of the two genotypes was significantly changed by salt treatment, on the other hand, irrespective of genotypes, GR was influenced by different NPs and their concentrations under salt treatment ($p<0.05$). Figure 2A shows that regardless of NP treatments, cv. Yaprak had about 10% and 17% higher GR than did cv Porro in control or salt treatments, respectively, indicating that cv Yaprak has more vigorous seed and is more resistant to salt stress than is cv. Porro. Figure 2B shows that the seeds treated with hBN and no SALT, there was a slight initial decrease in GR but compared to control it significantly increased at 80 mg L^{-1} of NP concentration. For those subjected to hBN and SALT, there was a significant decrease in GR at 40 mg L^{-1} but it increased at 80 mg L^{-1}. The seeds primed with ZnO without SALT treatment, there was a very little decrease in GR at 20 mg L^{-1} and seemed to remain at that level even at increasing NP concentrations. However, for those subjected to ZnO with SALT treatments, there was an initial drop in GR at 10 mg L^{-1} but increased at 20 and 40 mg L^{-1}. It can be said that germination for this treatment responded better to higher concentrations of ZnO NPs, 40 mg L^{-1} most especially. Different GR response under different NP treatments with or without salt regardless of genotypes and 40 mg L^{-1} hBN resulted in the lowest GR while 80 mg L^{-1} hBN without salt gave the highest GR.

Germination index (GI): The significant interaction ($p<0.05$) between SALT, NP and CONC shows different reaction to SALT and NPs concentrations for both NPs in GI (Table 1). Leek cv Yaprak had a significantly higher GI (832 which about 22% more) than did cv. Porro (654). In Fig. 3, the seeds treated with ZnO without SALT had not very significant GI change, but GI significantly increased at the highest hBN concentration (80 mg L^{-1}). In ZnO and SALT treated, there was a significant decrease in GI at 10 mg L^{-1} but it increased at higher concentrations even more than control, with 20 mg L^{-1} being the highest. However, with hBN and SALT, GI had more like an opposite reaction with the least GI at 40 mg L^{-1}.

Germination rate index (GRI): Since four-way interaction is significant for GRI ($p<0.05$, Table 1), main effects and lower interactions are not addressed.

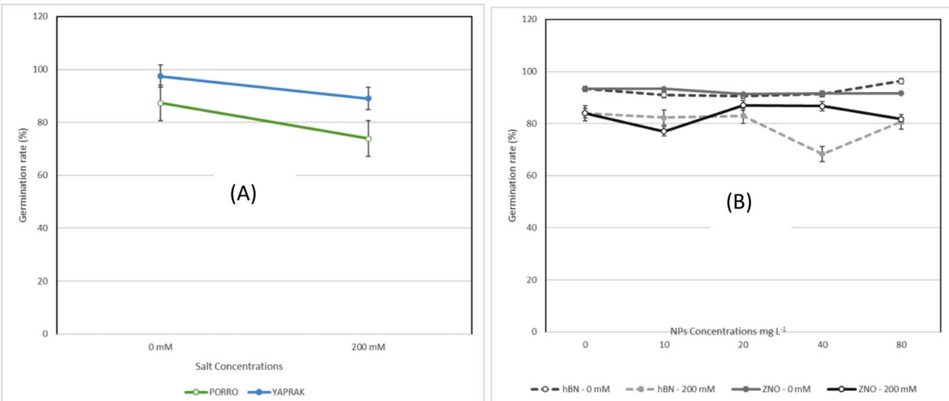

Fig. 2. (A) Germination rate based on GEN and SALT interaction, Tukey's $HSD_{0.05}$ for GEN*SALT = 4.022. (B) Germination rate based on SALT (mM), NPs and NP concentrations. Bars show standard error for the means, Tukey's $HSD_{0.05}$ for SALT*NP*CONC = 12.55.

Table 1. Variance analysis and corresponding p-value significance levels of germination variables.

Source	df	GR	GI	GRI	T50	MGT	SL	SFW	AO
GEN	1	**0.000	**0.000	**0.000	**0.000	**0.000	**0.000	**0.000	*0.014
SALT	1	**0.000	**0.000	**0.000	**0.000	**0.000	**0.000	**0.000	**0.000
NP	1	0.130	0.406	0.858	0.182	*0.021	**0.000	0.179	0.128
CONC	4	0.112	*0.048	**0.000	**0.000	*0.019	**0.000	0.438	*0.034
GEN x SALT	1	*0.023	0.051	**0.000	**0.000	*0.026	**0.000	**0.000	*0.013
GEN x NP	1	0.249	0.522	0.122	0.688	0.080	0.966	0.849	**0.000
GEN x CONC	4	0.085	0.077	**0.000	0.470	0.396	**0.000	**0.001	0.609
SALT x NP	1	0.080	0.241	0.287	0.253	*0.022	0.898	**0.000	*0.012
SALT x CONC	4	0.140	*0.037	**0.000	*0.013	0.210	**0.000	**0.000	0.525
NP x CONC	4	*0.010	**0.008	0.181	0.107	0.056	**0.000	**0.000	0.377
GEN x SALT x NP	1	0.249	0.517	0.416	0.246	0.082	0.408	0.427	0.781
GEN x SALT x CONC	4	0.557	0.079	**0.000	**0.000	*0.006	**0.000	**0.002	**0.001
GEN x NP x CONC	4	0.390	0.115	**0.000	**0.000	0.517	**0.000	**0.000	0.971
SALT x NP x CONC	4	*0.008	**0.007	**0.000	0.985	0.128	**0.000	**0.000	0.517
GEN x SALT x NP x CONC	4	0.211	0.201	**0.000	0.764	0.225	**0.000	**0.002	0.903
Error	80								
Corrected total	119								
Total	120								

GR: Germination rate, GI: Germination index, GRI: Germination rate index,T50: The time to reach 50% Germination,MGT: Mean germination time, SL: Shoot length,SFW: Shoot fresh weight (g), AO: Percent antioxidant, df: Degrees of freedom, * and ** denote significance at $p<0.05$ and 0.01, respectively. § this experiment replicated twice with error df= 40.

Apparently, salt stress largely affected GRI values as the control treatments including 0 mg L^{-1} NPs had higher GRI values. The highest GRI values were obtained from control treatments of cv Yaprak while the lowest value obtained from cv Porro in 200 mM SALT with 40 mg L^{-1} hBN treatments. Interestingly, GRI values in control and SALT treatments tend to significantly increase after 40 mg L^{-1} in hBN concentration (Fig. 4).

Time for 50% germination (T50): The significant interaction ($p<0.05$) of GEN*NP*CONC shows that the two leek genotypes reacted to NPs and their concentrations differently (Table 1). Interestingly reaction of genotypes to NP types did not differentiate in salt stress, however, GEN*SALT*CONC interaction suggests that concentration changes the salt stress response of genotypes for T50. The three- way GEN*NP*CONC interaction resulted in that cv Porro

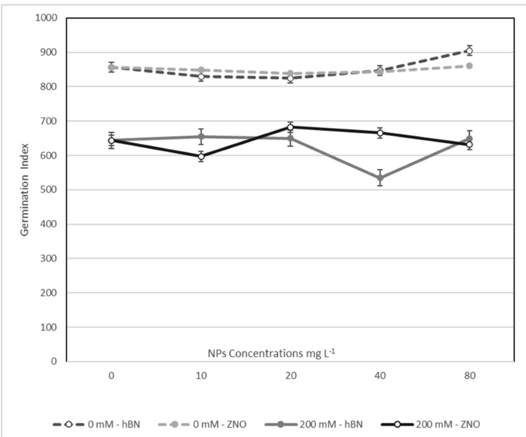

Fig. 3. Germination index based on NPs, NP concentrations and SALT (mM). Bars show standard error for the means, Tukey's $HSD_{0.05}$ for SALT*NP*CONC = 100.2.

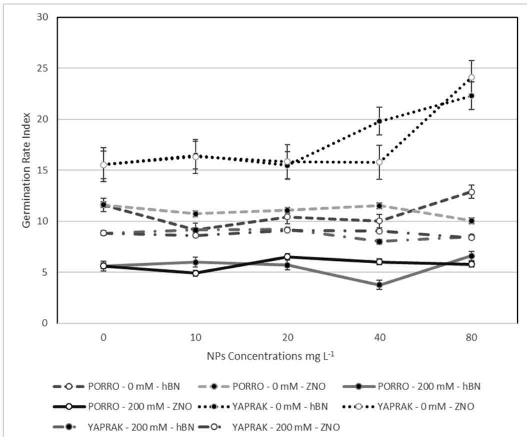

Fig. 4. Interaction effects of GEN*SALT*NPs*CONC on GRI. Salt treatments are denoted as mM. Bars show standard error for the means, Tukey's $HSD_{0.05}$ for GEN*SALT*NP*CONC=2.094.

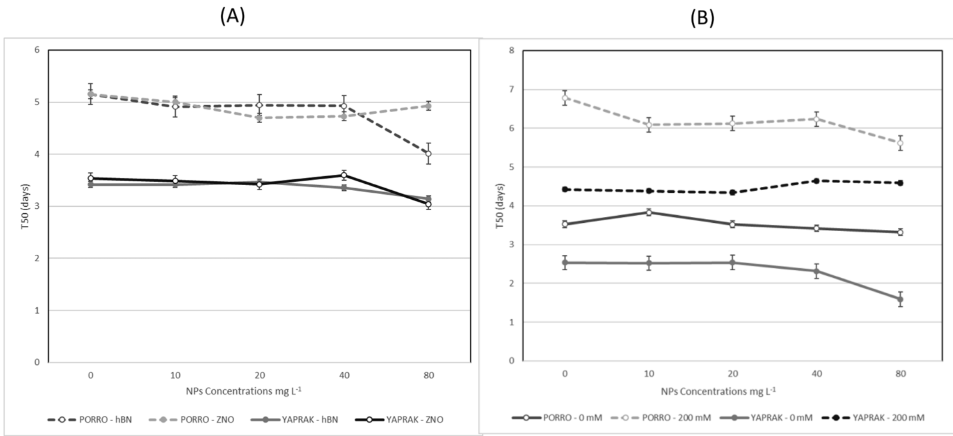

Fig. 5. (A) Time for 50% germination based on genotype, NPs and NP concentrations. Bars show standard error for the means. (B) Time for 50% germination based on genotypes, salt treatments (mM) and NP concentrations. Bars show standard error for the means, Tukey's $HSD_{0.05}$ for 3-way interactions= 0.734.

had the longest T50 values except for those treated with 80 mg L^{-1} hBN. The highest concentration of hBN reduced the 50% germination time of that cultivar (Fig. 5A). However, in Fig. 5B, it appears that cv Yaprak germinated earliest at 80 mg L^{-1} and cv. Porro treated with SALT took the longest time at the highest concentration.

Mean germination time (MGT): The variance analysis of MGT and GEN*SALT*CONC showed that genotypes are significantly affected by SALT and NP concentrations, however, different NP types significantly prolonged MGT values under salt stress regardless of genotypes ($p<0.05$, Table 1). Figure. 6A shows that the MGT of leeks for both NPs were the same at 0 mM salt treatment but hBN treatment resulted in a significantly one-day shorter MGT at 200 mM of salt treatment than did ZnO treatment ($p<0.05$). Additionally, Fig. 6B shows that at 80 mg L^{-1} of both NPs, cv Yaprak had about 6 days shorter MGT than did cv Porro. Remarkably, MGT time of cv Yaprak at the highest concentration of NPs was as early as that of cv Porro without salt stress. Under the salt treatment MGT of cv Porro was significantly shortened about more than two days compared to its control, suggesting that higher concentrations of NPs

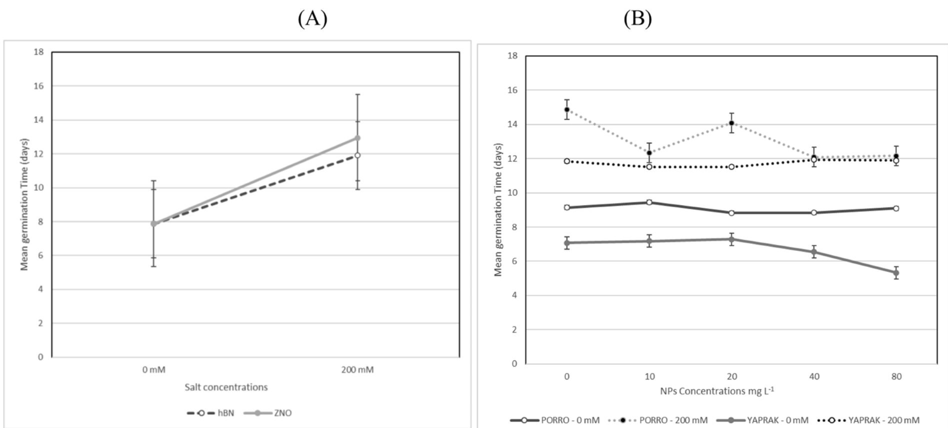

Fig. 6. (A) Mean germination time (MGT) based on salt treatment and NPs. Bars show standard error for the means, Tukey's $HSD_{0.05}$ for SALT*NP = 0.825. (B) Mean germination time (MGT) based on genotype, salt (mM) and NP concentrations. Bars show standard error for the means, Tukey's $HSD_{0.05}$ for GEN*SALT*CONC = 0.452.

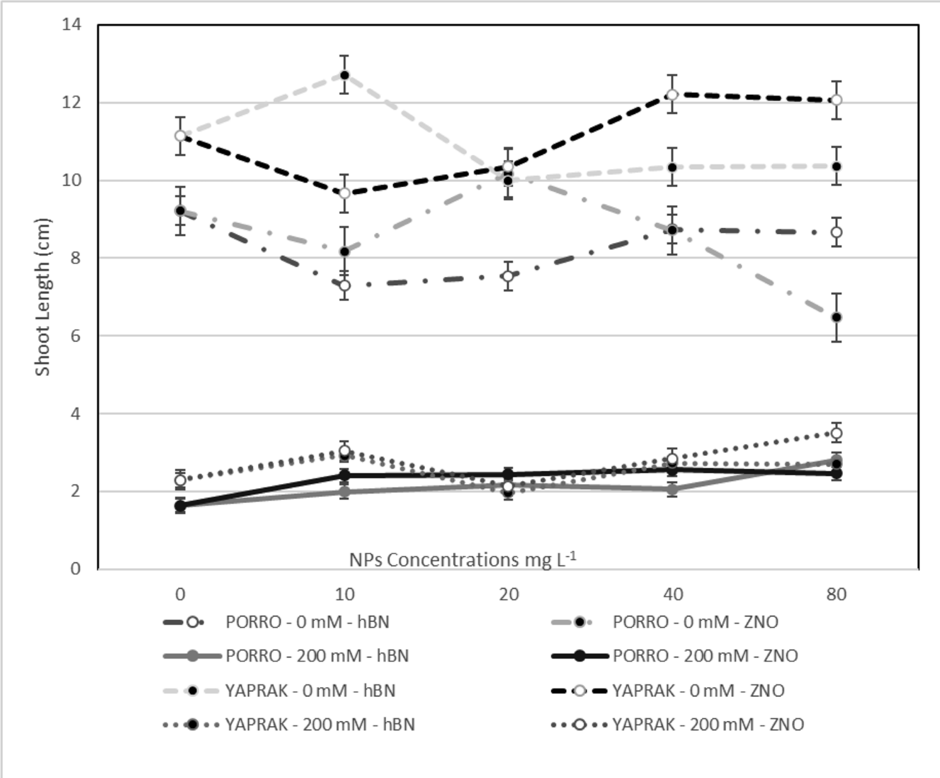

Fig. 7. Shoot length (SLT) based on genotype, salt (mM), NP types and NP concentrations. Bars show standard error for the means, Tukey's $HSD_{0.05}$ for GEN*SALT*NP*CONC= 0.810.

will accelerate MGT under salt stress.

Shoot length (SLT): The significant four-way interaction ($p<0.05$) of GEN*SALT*NP*CONC shows that the two SLTs of genotypes were significantly influenced by different treatments of SALT, NP type and NP concentrations (Table 1). Figure 7 shows that cv. Yaprak treated with hBN at 10 mg L^{-1} without salinity had the seedlings with the longest shoots, but at higher concentrations, the SLT was reduced. However, in those of the same cv Yaprak, treated with ZnO, there was an initial decrease in SLT at 10 mg L^{-1}. Then at higher concentrations, the SLT was improved. It can be suggested that cv Yaprak responded differently to the different NPs. Conversely for cv. Porro, ZnO caused a decrease in SLT at 10 mg L^{-1} while there was a little spike at 20 mg L^{-1} concentrations after that there was dramatic decrease in SLT at higher concentrations with no salt stress suggesting that higher concentration of ZnO NPs may be toxic to leek cultivars under no salt stress while under salt stress ZnO NPs seemed to enhance SLT values more than 30% right after the 10 mg L^{-1} of concentration compared to control treatment without NPs. On the other hand, SLT values under no salt stress with hBN treatment tend to decrease in lower concentrations but were improved at 40 mg L^{-1}. The SLT of both cultivars treated with salt were greatly affected with fluctuating responses at different concentrations of NPs. Under salt stress condition cv Yaprak treated with both ZnO and hBN increased SLT about 22-25% at the highest concentration.

Shoot fresh weight (SFW): The significant interaction ($p<0.01$) among GEN*SALT*NP*CONC shows that the two genotypes reacted to salt, NP type and NP concentrations differently (Table 1). Therefore, reaction of leek cultivars to salt stress may be affect-

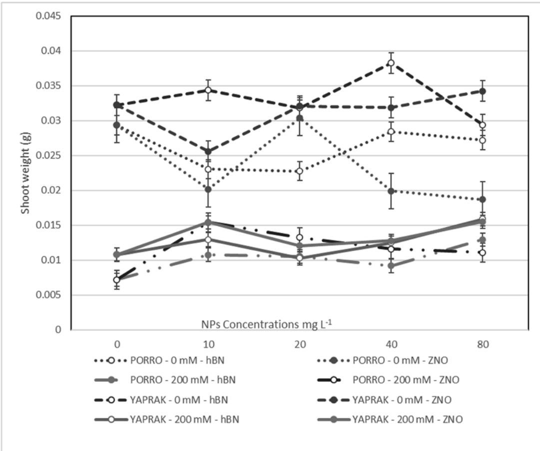

Fig. 8. Effect of genotype, salt (mM), NP types and NP concentrations on SFW of leek. Bars show standard error for the means, Tukey's $HSD_{0.05}$ for GEN*SALT*NP*CONC= 0.651.

ed by NPs and their concentrations. Figure 8 shows that 40 mg L^{-1} hBN priming resulted in the highest SFW in cv Yaprak without salt treatment, however as the concentration increased to 80 mg L^{-1} the SFW decreased about 25%. On the other hand, cv Porro had the highest SFW at 20 mg L^{-1} after which dramatical decrease was observed as the NP concentrations increased. Except cv Yaprak primed with hBN, 10 mg L^{-1} concentration of both NPs caused significant decrease in SFW in both leek cultivars. ZnO NP without SALT treatment caused cv Yaprak to increase in SFW at the highest concentration (80 mg L^{-1}), however, its reaction at 10 mg L^{-1} either NPs was opposite. On the other hand, cv. Porro had the highest SFW at 20 mg L^{-1} ZnO and it decreased dramatically at higher concentrations while the hBN caused increase in SFW after 20 mg L^{-1} showing completely opposite effect. In salt treatment, SWF increased in the lowest NP concentration of ZnO regardless of genotypes, however at the highest ZnO concentration cv Yaprak had significantly higher SFW than that of cv Porro. In hBN and salt treatments, cv Yaprak performed better SFW than did cv. Porro in all concentrations. Extraordinarily, the SFWs of cv Yaprak under salt treatment with the highest ZnO and hBN concentrations were not significantly different from those without salt treatment at 80 mg L^{-1} indicating at that at higher concentrations NPs may cause another source of stress.

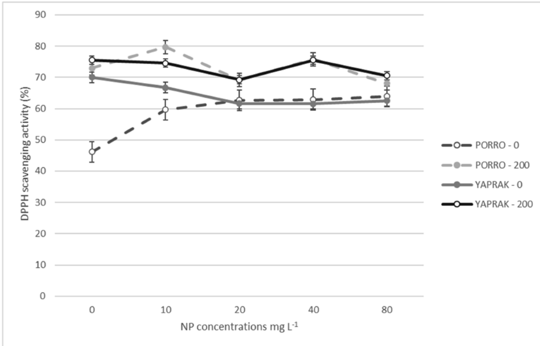

Fig. 9. Antioxidant activities of cvs. Porro and Yaprak shoot tissues at 630 nm absorbance in different NP concentrations. Bars show standard error, Tukey ($HSD_{0.05}$) for GEN*SALT*CONC=12.23.

Antioxidant activity: The variance analysis of percent antioxidant activities showed that there are significant differences ($p<0.05$) between genotypes, salt treatments and NP concentrations while type of NPs did not render any significant differences ($p>0.05$, Table 1). Since antioxidant activities significantly different in terms of the three-way interaction of GEN* SALT* CONC, significant main effects and lower order interactions were not addressed. The significant interaction ($p<0.05$) of GEN*SALT*CONC shows that the two genotypes reacted to salt treatment and NP concentrations differently. Types of NPs did not significantly influence the antioxidant activities. Figure 9 shows that the antioxidant activities of the two cultivars have similar trends in the salt treatments. Interestingly, in control treatment antioxidant activity of cv. Yaprak tended to decline as the concentration of NPs increased on the contrary antioxidant activity in cv. Porro significantly increased as the NP concentration increased to 80 mg L^{-1} in 200 mM salt solution.

DISCUSSION

In this experiment ZnO and hBN nanoparticles showed proof of enhancing germination properties and seedling growth of leek even under salt stress. The germination rate (GR) and mean germination time (MGT) of the two cultivars of leek used were found to improve at certain high concentrations of nano-priming. However, it was observed that one cultivar, cv Yaprak performed better than cv Porro in all treatments they were exposed to. This shows that the response of leek seeds to nanoparticles is dependent on genotypes, indicating that a genetic variation existed for it among genotypes.

The positive effect of seed priming with nanoparticles was found with a higher percentage of germination and germination speed in primed seeds at higher concentrations (40 and 80 mg L^{-1}) as compared to non-nanoprimed seeds. The cultivar recorded to have better response to nanopriming was cv Yaprak, which is one the most grown in Turkey (Kiremit and Arslan 2016). There are limited studies that have investigated the effect of nanoparticles on leeks, but onion, which both belong to the same genus Allium, have received the most attention.

Effect of nanoparticles on germination characteristics (GR, GI, T50, MGT): In this study, it was observed that nanoparticles positively impacted the germination rate of leeks, even though the level of impact was observed differently between the two cultivars and the different nanoparticles also. As represented in Fig. 4, the best germination rate was observed in the leek seeds primed with 80 mg L^{-1} hBN without salt treatment. Ebrahim Pour Mokhtari and Kizilgeci (2021), reported that soybean germination and germination rate increased in response to high boron concentrations. As seen in the effect of nanoparticles on wheat seed germination and seedling growth, seeds treated with 1000 ppm ZnO recorded significantly higher germination and seedling vigour index than control (Kumar and Pandey 2018). The germination index (GI) of leeks exposed to salinity were found to be lower than those not under salinity stress. Those primed with ZnO at 20 and 40 mg L^{-1} had better GI than the ones primed with hBN, both exposed to salinity. The leek cultivars had higher GI under hBN at 80 mg L^{-1}. At 20 and 40 mg L^{-1}, those primed with ZnO had a better GI. Promptness index, as it was referred to by Raskar and Laware (2013), was seen to be significantly increased by the effects of the different concentrations of TiO_2 NPs on seed germination indices in onion. It is possible to assert that NPs increase the germination of seeds in a dose-dependent manner in comparison to the control. This was also observed in the study of the effect of ZnO nanoparticles on germination of *Triticum aestivum* seeds (Awasthi *et al.* 2017). It is also on record that the different genotypes of tomatoes' genetic background, the kind, size, and concentration of the nanomaterial all had different effects on the germination index (Karami Mehrian *et al.* 2016). As seen in results above, T50 and MGT happen to be the similar. The seeds not primed with nanoparticles required more days to attain 50% germination. However, this was cultivar dependent. This is similar to the report of Acharya *et al*. (2020), contained in, nanoparticle-mediated seed priming improves germination, growth, yield, and quality of watermelons (*Citrullus lanatus*) at multi-locations in Texas, which they stated that when compared to the AgNP treatment, the control treatment required significantly more days for 50% germination of the Riverside and Maxima cultivars in the first and second years, respectively. On the other hand, when compared to the other treatments, seeds of the "Riverside" variety treated with AgNPs had the lowest mean germination times (MGT) and the highest germination rates (GR), demonstrating significant differences in MGT and GR. The physiology of various plant species varies, resulting in variations in their uptake of nanoparticles into nanopriming and in the rate and manner of species growth (Abbasi Khalaki *et al.* 2021).

Effect of nanoparticles on SLT and SFW: As observed, both cvs Yaprak and Porro had different responses to the different NPs. The outcome of cv Yaprak were more impressive than cv. Porro under the various treatments they were subjected. While both NPs caused the increase of SLT and SFW for cv. Yaprak, there was no significant positive effect of hBN on cv. Porro. Although both NPs at different concentrations affected SLT and SFW of both cultivars, ZnO NP was recorded to have better impact than hBN. Interestingly, despite cv Yaprak primed at 10 mg L^{-1} of hBN producing seedlings with the highest SLT, those of 40 mg L^{-1} of same NP with lower SLT had higher SFW. Similarly, seedlings from 80 mg L^{-1} of ZnO weighs higher than those of 40 mg L^{-1} of same NP, despite the later having higher SLT. As seen in this study, NPs caused increase in SLT and SFW. Ebrahim Pour Mokhtari and Kizilgeci (2021), reported also that boron increased shoot length and shoot fresh weight among other growth parameters of soybean. In a separate study, it was observed that silica nanoparticles caused the increase of SLT and SFW in wheat even better under salinity stress (Mushtaq *et* *al.* 2017). Several nanoparticles have been reported to have caused the increase in shoot lengths and shoot fresh weights of crops at different concentrations not only by nanopriming but also by foliar application (Thiruvengadam *et al.* 2021).

Effect of nanoparticles on antioxidant activities: The antioxidant activity of cv Yaprak was seen to reduce at concentrations above the control, while the antioxidant activity cv Porro increased. Under salinity conditions, the antioxidant activity of both cultivars was seen to rise beyond their various control counterparts. This conforms to the report of Abdel Latef *et al.* (2017) where the antioxidant activity of lupine (*Lupinus termis*) was mostly stimulated when treated with zinc nanoparticles (Zn NP) and 150 mM of NaCl. Also, there was an acceleration of antioxidant activity in an onion cultivar (Fepagro 27) with an increase in salt via irrigation water (Silveira Corrêa *et al.* 2013). Similarly, the antioxidant activity of *Capsicum annuum* was seen to be increased under ZnO NP treatment (García-López *et al.* 2018).

Effect of salinity: It is on record that variations in the salinity levels have varying effects on the plant growth parameters of leek. The relative stem fresh weight threshold and slope values when calculated was 1.21 mS cm^{-1} and 9.622%, respectively, indicating that leeks are moderately sensitive to salinity (Kiremit and Arslan 2016). As observed in this study, increased salt level had negative effects on germination characteristic of the leek cultivars under investigation. Note that the electricity Conductivity of salt solutions used in this study are 17.02 µS/cm and 19.69 mS/cm for control (0 mM of NaCl) and salinity stress (200 mM of NaCl) respectively. The EC of the salinity solution used in this study is over 1500% higher than the threshold stated above, which suggests the high level of negative impact on the leek cultivars. Abdel Latef *et al.* (2017) reported that priming with zinc oxide nanoparticle especially at 60 mg L^{-1} concentration, the harmful effect of NaCl at 150mM on lupine plants was reduced. (Saranya *et al.* 2017) reported that onion seeds priming with 0.5% ZnSO4 for 10 h could be able to withstand NaCl salt stress up to 0.75% concentration when compared to unprime seeds. El-Badri *et al.* (2021), reported also for two rapeseed cultivars, Yang You 9 and Zhong Shuang 11, that under salinity stress, seed priming only with ZnO NPs, particularly 100 mg L^{-1}, enhanced germination as well as early seedling growth while also reducing toxicity of sodium ion (Na^+) on the seeds. Mechanisms that enable osmotic adjustment and effective mechanisms for coping with salinity-induced stress must complement this ability. The present study, therefore, is among the very few showing the genotype dependency for NPs treatments. Another interesting finding in this report is the relatively low imbibition temperature. Imbibition temperatures for nanoparticles generally range from 25-30 °C (Adhikary *et al*. 2022, Rai-Kalal *et al.* 2021), however planting time of leek generally commence during fall in relatively lower temperatures. Our study also shows that even in low temperatures seed imbibition with nanoparticles was successfully performed and enhanced germination and seedling growth even under salt stress.

CONCLUSION

This study concludes that salt stress led to the delayed germination and seedling development of both cultivars of leek. Different cultivars of leek respond differently to nanoparticles and salinity. The nanoparticles improved the germination and growth parameters compared to control treatment even under the salt stress. Apparently, cv Yaprak responded better to the treatments than cv. Porro. Therefore, seed nanopriming (ZnO & hBN NPs) might be effective in influencing early germination as well as uniformity in germination in the cultivations of leeks. Therefore, selecting leek cultivars which responds to better to certain NPs will have great advantage for fast and better germination of seeds and establishment even under stress conditions.

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