ABRATES

Journal of Seed Science

ISSN 2317-1545
www.abrates.org.br/revista

ARTICLE

Journal of Seed Science, v.45, e202345029, 2023

http://dx.doi.org/10.1590/2317-1545v45274866

Influence of SiO_2, Al_2O_3 and TiO_2 nanoparticles on okra seed germination under PEG-6000 simulated water deficit stress

Jiyue Wang[1,2,3], Jiajun Xu[1,2], Mingli Yang[1,2], Mingzhi Miao[1,2*]

Abstract: A comparative study for evaluating the influence of metal oxide nanoparticles (MONPs) (Al_2O_3, TiO_2, and SiO_2) on seed germination and seedling growth in okra was implemented under water deficit stress stimulated by PEG-6000. The results revealed that additive of SiO_2 nanoparticles (SNPs) and Al_2O_3 (ANPs) have significant positive effect on seeds germination, while a reduction of that were observed by using TiO_2 nanoparticles (TNPs) at 50 $mg.L^{-1}$ concentration under 5% PEG-6000 stimulated drought stress as compared to that without MONPs. Further, relative to non-MONPs treatment, all the tested MONPs dramatically enhanced the drought stress tolerance in okra seedlings due to the improvement of morphological growth despite a few traits were inhibited under certain water deficiency to some degree. Improvement of drought resistance induced by TNPs and ANPs were found to be higher than that by SNPs. Our finding provides a promising approach to cope with water scarcity, as application of metal oxide nanoparticles to be a potential option to protect okra plants against drought stress.

Index terms: nanoparticles, okra, seed germination, seedlings.

Resumo: Um estudo comparativo para avaliar a influência de nanopartículas de óxido metálico (MONPs) (Al_2O_3, TiO_2 e SiO_2) na germinação de sementes e no crescimento de plântulas de quiabo foi implementado sob estresse de déficit hídrico estimulado por PEG-6000. Os resultados revelaram que a adição de nanopartículas de SiO_2 (SNPs) e Al_2O_3 (ANPs) tem efeito positivo significativo na germinação das sementes, enquanto uma redução foi observada com o uso de nanopartículas de TiO_2 (TNPs) na concentração de 50 $mg.L^{-1}$ sob estresse hídrico estimulado com 5% de PEG-6000 em comparação com ausência de MONPs. Além disso, em relação ao tratamento sem MONPs, todos os MONPs testados aumentaram siginificativamente a tolerância ao estresse hídrico em plântulas de quiabo devido à melhoria do crescimento morfológico, apesar de algumas características terem sido inibidas até certo ponto sob déficit hídrico. A melhoria da resistência à seca induzida por TNPs e ANPs foi maior do que por SNPs. Os resultados fornecem uma abordagem promissora para lidar com a escassez de água, já que a aplicação de nanopartículas de óxido metálico é uma opção potencial para proteger as plantas de quiabo contra a seca.

Termos para indexação: nanopartículas, quiabo, germinação de sementes, plântulas.

***Corresponding author**
miaomz74@163.com

Received: 05/17/2023.
Accepted: 08/21/2023.

[1]Guiyang University, School of Biological and Environmental Engineering, 550005-Guiyang, China.

[2] Guiyang University, Platform for Exploitation and Utilization of Characteristic Plant Resources, 550005-Guiyang, China.

[3]Key Laboratory of Surveillance and Management of Invasive Alien Species in Guizhou Education Department, 550005-Guiyang, China.

INTRODUCTION

Generally, nanoparticles (NPs) are regarded as entities with a size range between 1 and 100 nm (Hamuda, 2015). The use of NPs in agriculture is beneficial to crops, such as the control of plant diseases (Sarlak et al., 2014), improvement of seeds germination and seedlings growth (García-López et al., 2018), yield (Acharya et al., 2020) and alleviation of damage induced by drought stress (Rai-Kalal et al., 2021; Sutulienĕ et al., 2022). Although the great efficacy of NPs in agricultural area have been reported continually, they also have raised concerns about the potential toxicity in pant. Metal oxide NPs (MONPs), such as TiO_2, Al_2O_3, CuO and ZnO, have been broadly showed on ecotoxicity in *Daphnia similis*, zebrafifish, bacteria, nematodes (*Caenorhabditis elegan*s), soil earthworm (*Eisenia fetida*), microalgae (*Scenedesmus* sp.) and green algae (Karunakaran et al., 2016).

Al_2O_3 NPs (ANPs) have been widely applied in biosensors, textiles, electronics, drug delivery systems and tissue engineering (Lanone et al., 2009). The increasing application of ANPs will lead to theirs accumulation in the ecosystem and food chain, thus researches requires more attention on assessing the biological impacts. A study conducted by Hayes et al. (2020) investigated the effect of ANPs on lettuce seed germination. They reported that ANPs was found to positively influence seeds germination and seedlings growth at low concentrations (10 and 25 $mg.L^{-1}$), while decline of that was observed at higher concentrations (50 and 100 $mg.L^{-1}$) as compared to control.

As an unusual MONPs, silicon dioxide (SiO_2) NPs (SNPs) has been widely used in many fields, such as photocatalysis, cosmetics, cancer therapy and bio-imaging (Cheng et al., 2010), fertilizers (Rastogi et al., 2019), pesticides (Ahmed et al., 2019), since they are easy to obtain and process. The study of Siddiqui et al. (2014) has reported the positive effect of SiO_2 NPs on tomato seeds germination. However, the increase of SiO_2 in the environment may result in phytotoxicity. The influence of SNPs on wheat seed germination have been investigated by Chourasiya et al. (2021), who reported that SNPs promoted seed germination at lower concentrations (10 and 100 $mg.L^{-1}$), while that was inhibited at higher concentrations (500 and 1000 $mg.L^{-1}$). The positive effect of SNPs on seed germination could be attributed to the role of NPs on enhancing seed water uptake and activate enzymes involved in germination.

TiO_2 nanoparticles (TNPs) is used as a catalyst in water, electronic devices, energy conversion equipment, and energy storage as its high photocatalytic activity (Khalaki et al., 2021). A study of Hojjat (2020) have investigated the impact of TNPs on Grass Pea (*Lathyrus sativus* L.) seed germination under drought stress. They found that TNPs enhanced Grass Pea to tolerance to drought by increasing morphological growth. However, it seemed that TNPs at 100 and 200 $mg.L^{-1}$ concentrations had a positive negative influence on seed germination in soybean, which could be attributed to their ability to induce ROS generation and oxidative stress in cells, thus leading to cell damage and inhibition of germination (Xiao et al., 2021).

Overall, the effect of MONPs on seed germination seems to depend on their concentration and the type of plant species to some extent. In practice, they have a positive effect on seed germination and growth of early seedling at certain concentrations, other concentrations may inhibit germination on account of their potential to induce oxidative stress and damage cells. Since MONPs have definite advantage on pharmacy, chemical, agriculture, and other industry, their effect on human health and ecosystems is still not clear. The focus of this work is to investigate the influence of Al_2O_3, SiO_2 and TiO_2 NPs at different concentrations on seed germination in okra (*Abelmoschus esculentus* L.) subjected to PEG-6000 simulated water deficit stress.

MATERIAL AND METHODS

Three types of nanoparticles (NPs) were used in this study, including Al_2O_3, SiO_2 and TiO_2, which were purchased from Beijing Dk nano S&T Ltd, (China) whose morphology are all nearly spherical with an average particle size of 40 nm.

At first, seeds of okra were sterilized by 75% ethanol for 60 min. and washed absolutely through deionized water. And then, the sterilized seeds were randomly dipped into the SNPs/ANPs/ solution in the concentrations of 0, 25 $mg.L^{-1}$,

50 mg.L^{-1}, 100 mg.L^{-1}, 200 mg.L^{-1} with the same volume, and put them in constant temperature incubator without light at 28 °C for 20 h. Afterward, okra seeds were rinsed several times with deionized water for germination. The treated seeds were placed on culture dishes with moist filter paper which was supplemented with different concentrations (5%, 10%, 15%) of PEG-6000 solution for simulating drought stress, and then germinated in a constant temperature cultivator in dark at 28 °C for one week. Similar treatment with deionized water was conducted as the non-stress condition (control). All treatments were taken in this experiment with three biological replicates. Germination attributes and phenotypic traits of early seedlings were investigated as our previous study (Wang et al., 2018). The moisture content (MC) in okra seedlings were also detected as method of Wang et al. (2022).

Data analysis

All the data were represented as mean ± standard deviation of triplicate samples, which were analyzed one-way analysis of variance (ANOVA) and Duncan's test using SPSS 16.0. The significance of treatment was considered at the level of P value < 0.05. The method of subordinate function value was adopted to comprehensively evaluate drought resistance of okra plant.

RESULTS

Germination percentage (GP), germination Energy (GE), germination rate (GR), vitality index (VI) of okra seeds exposed to SNPs at 25 mg.L^{-1} were significantly increased under water deficit stress induced by 10% PEG-6000 solutions compared with that Non-SNPs (Table1). Application of 100 mg.L^{-1} SNPs resulted in remarkable increment of GP, GE, GR and VI under water deficiency condition induced by 5% PEG solutions compared to without SNPs,

Table 1. Germination of seeds in okra exposed to SNPs under water stress simulated by PEG-6000.

Properties	PEG-6000 (%)	Concentration of SNPs (mg.L^{-1})				
		0	25	50	100	200
GP (%)	0	0.98±0.04a(a)	0.93±0.00b(a)	0.98±0.04ab(a)	0.87±0.00c(b)	0.96±0.04a(a)
	5	0.87±0.00c(bc)	0.91±0.04b(b)	0.93±0.00ab(ab)	0.98±0.04a(a)	0.82±0.04b(c)
	10	0.89±0.04b(b)	1.00±0.00a(a)	0.91±0.08b(b)	0.91±0.04bc(b)	0.89±0.04ab(b)
	15	0.93±0.00ab(ab)	0.84±0.17c(b)	1.00±0.00a(a)	0.93±0.00ab(ab)	0.87±0.07ab(ab)
GE (%)	0	0.98±0.04a(a)	0.93±0.00b(a)	0.98±0.04ab(a)	0.87±0.00c(b)	0.96±0.04a(a)
	5	0.87±0.00c(b)	0.91±0.04b(b)	0.93±0.00ab(ab)	0.98±0.04a(a)	0.82±0.04b(c)
	10	0.89±0.04bc(b)	1.00±0.00a(a)	0.91±0.08b(b)	0.91±0.04bc(b)	0.89±0.04ab(b)
	15	0.93±0.00ab(ab)	0.84±0.17c(b)	1.00±0.00a(a)	0.93±0.00ab(ab)	0.87±0.07ab(ab)
GR	0	36.86±1.38a(a)	34.97±1.26a(ab)	37.2±1.80ab(a)	33.71±0.00c(b)	37.16±1.50a(a)
	5	33.21±0.87b(bc)	36.5±1.31a(ab)	34.1±2.03b(bc)	38.03±1.50a(a)	31.31±1.30b(c)
	10	34.57±1.5b(bc)	37.89±1.73a(a)	37.16±2.99ab(ab)	35.44±1.5bc(bc)	33.4±0.27b(c)
	15	34.44±0.63b(a)	32.34±7.33a(a)	38.56±0.58a(a)	36.3±0.00ab(a)	33.71±2.25b(a)
VI	0	10.97±1.17a(b)	9.88±2.77b(bc)	11.32±1.96a(ab)	8.54±2.16b(c)	13.61±2.60a(a)
	5	9.66±0.44b(a)	11.4±0.14a(a)	10.35±2.94ab(a)	11.56±0.97a(a)	11.08±2.07b(a)
	10	4.39±0.2d(b)	9.84±1.09ab(a)	4.33±0.47c(b)	10.04±1.47ab(a)	10.09±1.44b(a)
	15	7.40±0.26c(ab)	7.54±1.01b(ab)	7.12±1.07bc(ab)	8.42±0.22b(a)	6.64±1.53c(b)

GP: Germination percentage; GE: Germination energy; GR: Germination rate; VI: Vitality index. Different lowercase letters without parentheses showed the significant differences among PEG-6000 treatments in the same SNPs level at $p \leq 0.05$. Different lowercase letters within parentheses showed the significant differences among SNPs treatments in the same PEG-6000 level at $p \leq 0.05$.

whereas a remarkable reduction of this properties above-mentioned was found in Non-water deficit stressed seeds (control). Interestingly, under water deficit stress simulated by 5% PEG-6000 solution, adding 200 mg.L^{-1} SNPs significantly inhibited GE of seeds, while it dramatically increased VI with 1.30 folds in comparison with Non-SNPs seeds. Similarly, VI of seed exposed to 200 mg.L^{-1} SNPs was also increased up to 24.07% in control when compared to that without SNPs. Application of 50 mg.L^{-1} SNPs have no significantly influence on seed germination both in control and water deficit stress.

The growth characteristics of one-week-old seedlings exposed to SNPs and water deficit stress were reported in Table 2. It appeared that shoot length (SL), stem diameter (SD) and fresh weight each plant (FW) of seeds were notably increased by using 25 mg.L^{-1} SNPs under drought stress induced by 10% PEG-6000 solution, while dry weigh each plant (DW) was reduced in 15% drought stressed seeds as compared to Non-SNPs. Application of SNPs at 50 mg.L^{-1} dramatically reduced the Length of main root (LMR) and MC in seeds subjected to 10% drought stress, and DW in 5%

Table 2. Growth characteristics of one-week-old seedlings in okra germinated from seeds treated with SNPs under water stress simulated by PEG-6000.

Parameters	PEG-6000 (%)	Concentration of SNPs (mg.L^{-1})				
		0	25	50	100	200
SL (mm)	0	46.67±5.66a(a)	55.92±6.74a(a)	54.86±8.86a(a)	45.66±3.75a(a)	51.89±16.58ab(a)
	5	37.23±2.13b(b)	47.38±4.47b(ab)	43.29±5.44b(ab)	39.44±4.02a(b)	53.83±11.25a(a)
	10	25.65±0.51c(b)	32.02±3.15c(a)	21.59±0.25c(b)	31.66±3.70a(a)	34.02±4.89bc(a)
	15	20.18±1.01c(a)	22.4±1.45d(a)	22.76±2.32c(a)	19.04±0.52b(a)	21.26±3.64c(a)
LMR (mm)	0	26.52±0.75b(a)	29.03±3.32a(a)	29.57±6.88a(a)	27.37±7.65ab(a)	21.17±4.71a(a)
	5	31.25±2.33a(a)	31.67±1.18a(a)	24.68±7.48a(ab)	21.81±5.90b(b)	20.11±5.49a(b)
	10	26.1±2.36b(ab)	27.98±3.44a(ab)	21.14±3.58a(b)	34.11±2.04a(a)	26.8±9.25a(ab)
	15	25.52±1.82b(bc)	31.27±9.81a(ab)	29.05±0.23a(ab)	36.69±5.18a(a)	18.27±2.78a(c)
SD (mm)	0	1.65±0.04a(a)	1.47±0.22a(a)	1.55±0.02a(a)	1.48±0.15a(a)	1.54±0.08a(a)
	5	1.44±0.02bc(a)	1.51±0.11a(a)	1.52±0.05a(a)	1.43±0.09a(a)	1.51±0.07ab(a)
	10	1.31±0.11c(b)	1.49±0.04a(a)	1.23±0.05b(b)	1.34±0.04a(b)	1.35±0.11b(b)
	15	1.48±0.12b(a)	1.42±0.03a(a)	1.51±0.05a(a)	1.5±0.02a(a)	1.43±0.12ab(a)
FW (g)	0	0.30±0.02a(ab)	0.28±0.10ab(ab)	0.32±0.02a(ab)	0.25±0.06ac(b)	0.39±0.08a(a)
	5	0.28±0.02a(ab)	0.33±0.02a(a)	0.25±0.06b(b)	0.30±0.02a(ab)	0.32±0.04ac(a)
	10	0.12±0.00c(b)	0.28±0.00ab(a)	0.12±0.02c(b)	0.28±0.04ab(a)	0.25±0.04bc(a)
	15	0.21±0.01b(a)	0.22±0.01b(a)	0.20±0.02b(a)	0.23±0.00bc(a)	0.20±0.04b(a)
DW (g)	0	0.02±0.00b(b)	0.03±0.00b(ab)	0.02±0.00b(b)	0.03±0.00ab(a)	0.03±0.01a(ab)
	5	0.03±0.00b(a)	0.03±0.01a(a)	0.02±0.00b(b)	0.02±0.00b(b)	0.02±0.00a(b)
	10	0.02±0.01b(a)	0.03±0.00a(a)	0.03±0.01a(a)	0.03±0.00ab(a)	0.02±0.01a(a)
	15	0.05±0.02a(a)	0.03±0.00a(b)	0.03±0.00ab(b)	0.03±0.00a(b)	0.04±0.02a(ab)
MC	0	0.93±0.01a(a)	0.90±0.04a(a)	0.92±0.01a(a)	0.91±0.00a(a)	0.93±0.01a(a)
	5	0.91±0.01a(ab)	0.90±0.02a(b)	0.93±0.00a(a)	0.92±0.01a(ab)	0.93±0.01a(a)
	10	0.84±0.06ab(b)	0.89±0.01a(ab)	0.74±0.02c(c)	0.92±0.04a(a)	0.90±0.04ab(ab)
	15	0.77±0.09b(a)	0.87±0.01a(a)	0.85±0.02b(a)	0.87±0.01bc(a)	0.80±0.09b(a)

SL: Shoot length; LMR: Length of main root; SD: Stem diameter; FW: Fresh weight each plant; DW: Dry weigh each plant; MC: Moisture content. Different lowercase letters without parentheses showed the significant differences among PEG-6000 treatments in the same SNPs level at $p \leq 0.05$. Different lowercase letters within parentheses showed the significant differences among SNPs treatments in the same PEG-6000 level at $P \leq 0.05$.

drought stressed seeds, respectively, when compared to that without SNPs. Under 10% water shortage condition, application of SNPs at 100 mg.L^{-1} dramatically increased SL 22.55%, FW 133.33% and MC 9.52% comparison with Non-SNPs. And LMR in seeds exposed to 100 mg.L^{-1} SNPs was increased up to 43.77% in 10% water shortage condition in comparison with SNPs-free seeds. But, LMR and DW were markedly decreased up to 30.20% and 33.33% by additive of SNPs at 100 mg.L^{-1} in seeds subjected to water deficit stress stimulated by 5% PEG-6000 solutions as compared to that without SNPs. Besides, SL in seeds exposed to 200 was increased up to 44.59% and 32.63% under 5% and 10% drought stress conditions, respectively, in comparison with Non-SNPs seeds. However, using SNPs at 25 mg.L^{-1} reduced LMR 1.34% under 5% PEG solution simulated as compared to seeds unexposed to SNPs.

In Table 3, compared to Non-ANPs seeds, GP, GE, GR and VI of seeds were all significantly increased up to 14.94%,14.94%,14.47% and 14.49% by ANPs at 25 mg.L^{-1} in 5% water deficit stress conditions. In 10% water deficit stress conditions, using 25 mg.L^{-1} ANPs markedly showed a 2.82% and 2.82% increase in GP and GE, respectively, whereas it decreased GR and VI up to 19.47% and 6.26%, respectively, when compared to that without ANPs. And VI of seeds exposed to ANPs at 25 mg.L^{-1} also significantly decreased up to 35.91% in control. GR and GE of seeds were improved up to 9.8% and 30.98% in 10% water deficit stress conditions, while GP, GE and GR were reduced up to 9.37%,9.37% and 12.31% in 15% water deficit stress by applying ANPs at 50 mg.L^{-1} compared to that Non-ANPs. Application of ANPs at 100 mg.L^{-1} augmented GP and GE up to 22.54% and 22.54% in 10% water deficit stress, respectively, as compared with that Non-ANPs. GP and GE of seeds exposed to 200 mg.L^{-1} ANPs were increased up to 18.31% and 18.31% in control and 10% water deficit stress, whereas they were reduced up to 14.58% and 14.58% in 15% water deficit stress compared to that without ANPs. Moreover, additive of ANPs at 200 mg.L^{-1} also decreased GR up to 20.84% comparing that Non-ANPs.

Table 3. Germination of seeds in okra treated with ANPs under water stress simulated by PEG-6000.

Properties	PEG-6000 (%)	Concentration of ANPs (mg.L^{-1})				
		0	25	50	100	200
GP (%)	0	0.91±0.04a(b)	0.89±0.04ab(b)	0.93±0.00ab(ab)	0.91±0.04ab(b)	0.98±0.04a(a)
	5	0.87±0.00a(b)	1.00±0.00a(a)	0.93±0.07a(ab)	0.87±0.07b(b)	0.91±0.04ab(b)
	10	0.71±0.1b(c)	0.73±0.12c(b)	0.93±0.00ab(a)	0.87±0.00b(a)	0.84±0.04bc(ab)
	15	0.96±0.04a(a)	0.82±0.04bc(b)	0.87±0.00b(b)	0.98±0.04a(a)	0.82±0.04c(b)
GE (%)	0	0.91±0.04a(b)	0.89±0.04b(b)	0.93±0.00ab(ab)	0.91±0.04ab(b)	0.98±0.04a(a)
	5	0.87±0.00a(b)	1.00±0.00a(a)	0.93±0.07a(ab)	0.87±0.07b(b)	0.91±0.04ab(b)
	10	0.71±0.10b(c)	0.73±0.12c(b)	0.93±0.00ab(a)	0.87±0.00b(a)	0.84±0.04bc(ab)
	15	0.96±0.04a(a)	0.82±0.04bc(b)	0.87±0.00b(b)	0.98±0.04a(a)	0.82±0.04c(b)
GR	0	34.6±1.47ab(ab)	32.71±1.39ab(b)	36.3±0.00a(a)	36.16±1.30a(a)	37.2±2.13a(a)
	5	33.1±1.06ab(b)	37.89±1.73a(a)	37.03±1.63a(ab)	34.71±3.12ab(ab)	33.71±2.59bc(b)
	10	32.15±3.75b(a)	26.91±3.41b(b)	35.3±0.87a(a)	32.26±1.42b(a)	34.07±0.63ab(a)
	15	36.7±1.06a(a)	31.15±2.23b(b)	32.18±1.36b(b)	38.03±1.50a(a)	30.37±1.30c(b)
VI	0	11.43±2.51a(ab)	8.41±1.23b(c)	12.68±1.99a(a)	12.59±0.30a(a)	9.40±0.67b(bc)
	5	11.04±7.51a(a)	12.64±9.9a(a)	13.7111.98a(a)	7.15±5.35b(a)	13.65±4.95a(a)
	10	8.76±1.07a(ab)	4.97±0.99b(c)	6.37±2.27a(bc)	9.7±1.55ab(a)	6.17±2.03b(bc)
	15	6.87±4.14a(a)	5.81±1.20b(a)	7.98±0.95a(a)	7.31±1.27ab(a)	7.36±2.01b(a)

GP: Germination percentage; GE: Germination energy; GR: Germination rate; VI: Vitality index. Different lowercase letters without parentheses showed the significant differences among PEG-6000 treatments in the same ANPs level at $p \leq 0.05$. Different lowercase letters within parentheses showed the significant differences among ANPs treatments in the same PEG-6000 level at $P \leq 0.05$.

As Table 4 reported, SL of seedlings was observably improved in the treatment of 25 mg.L^{-1} ANPs and 5%, and 15% drought stress, that of 100 mg.L^{-1} ANPs and 5%, 10% drought stress, and non-stressed condition, and that of 200 mg.L^{-1} ANPs and 5% drought stress, respectively, compared to that without ANPs. Application of ANPs at 50 and 100 mg.L^{-1} were both significantly promoted MC of seedlings in 15% drought stress in comparison with Non-ANPs. Nevertheless, LMR of seedlings was observably decreased in the treatment of 50 mg.L^{-1} ANPs and non-stressed condition, and 5% drought stress, that of 100 mg.L^{-1} ANPs and 5% drought stress, and that of 200 mg.L^{-1} ANPs and non-stressed condition, and 10% drought stress, respectively, compared with Non-ANPs. Unique positive effect on LMR was found in seedlings exposed to 50 mg.L^{-1} ANPs in 15% drought stress conditions. SD of seedlings exposed to ANPs at 50, 100 and 200 mg.L^{-1} were all significantly reduced up to 8.43%,11.24% and 13.85% in control in comparison with Non-ANPs. Analogously, FW of seedlings was significantly decreased in the treatment of 25 mg.L^{-1} ANPs and 10% drought stress, that of 50 mg.L^{-1} ANPs and 10% drought stress, that of 100 mg.L^{-1} ANPs and 5% drought stress, and that of 200 mg.L^{-1} ANPs and non-stressed condition, and 10% drought stress, respectively, when compared to without ANPs. Only using 25 mg.L^{-1} ANPs

Table 4. Growth characteristics of one-week-old seedlings in okra germinated from seeds treated with ANPs under water stress simulated by PEG-6000.

Parameters	PEG-6000 (%)	Concentration of ANPs (mg.L^{-1})				
		0	25	50	100	200
SL (mm)	0	52.68±11.39a(b)	48.11±5.43a(b)	48.99±5.64a(b)	66.2±3.64a(a)	48.81±5.28a(b)
	5	25.95±5.99bc(c)	38.90±10.56ab(ab)	22.57±9.56b(c)	35.09±11.85ab(b)	44.93±12.06a(a)
	10	34.05±1.56b(b)	23.94±7.90b(c)	29.27±16.62ab(bc)	44.08±4.49ab(a)	24.51±4.73a(c)
	15	20.12±4.87c(b)	36.64±6.11ab(a)	28.02±3.56ab(ab)	23.28±7.55b(b)	26.11±7.05a(b)
LMR (mm)	0	35.12±2.75a(a)	29.24±1.50a(ab)	27.41±1.74b(b)	31.77±5.23ab(ab)	26.00±5.80a(b)
	5	26.19±6.1b(a)	26.28±2.78a(a)	16.94±5.25b(b)	14.79±4.83b(b)	23.52±1.43a(a)
	10	33.34±5.23ab(a)	29.26±8.14a(a)	29.44±6.44ab(a)	33.60±4.36a(a)	24.10±2.66a(b)
	15	25.48±2.23b(b)	32.55±5.74a(ab)	45.89±5.78b(a)	23.84±8.66ab(b)	29.23±11.96a(b)
SD (mm)	0	1.66±0.05a(ab)	1.69±0.17a(a)	1.52±0.03a(bc)	1.47±0.02a(c)	1.43±0.03bc(c)
	5	1.69±0.20a(a)	1.53±0.08a(a)	0.99±0.86a(a)	1.01±0.87a(a)	1.58±0.13b(a)
	10	1.47±0.02a(ab)	1.70±0.10a(a)	1.55±0.27a(a)	1.48±0.07a(ab)	1.24±0.13b(b)
	15	1.62±0.24a(ab)	1.66±0.06a(ab)	1.57±0.14a(ab)	1.41±0.13a(b)	1.79±0.11a(a)
FW (g)	0	0.33±0.07a(ab)	0.26±0.03b(bc)	0.35±0.05a(a)	0.35±0.01a(a)	0.25±0.01b(c)
	5	0.33±0.02a(b)	0.55±0.08a(a)	0.37±0.03a(ab)	0.18±0.06b(c)	0.52±0.11a(a)
	10	0.27±0.01a(a)	0.18±0.02b(b)	0.18±0.06a(b)	0.30±0.05ab(a)	0.18±0.06b(b)
	15	0.19±0.12a(a)	0.19±0.04b(a)	0.25±0.02a(a)	0.19±0.03ab(a)	0.24±0.05b(a)
DW (g)	0	0.02±0.00c(ab)	0.02±0.00b(ab)	0.02±0.00a(ab)	0.03±0.01a(a)	0.02±0.00a(b)
	5	0.02±0.00c(a)	0.04±0.02a(a)	0.03±0.03a(a)	0.02±0.02a(a)	0.04±0.03a(a)
	10	0.04±0.00a(a)	0.02±0.00b(b)	0.03±0.01a(a)	0.04±0.01a(a)	0.03±0.00a(ab)
	15	0.03±0.00b(a)	0.03±0.00ab(a)	0.03±0.00a(a)	0.03±0.00a(a)	0.02±0.01a(b)
MC	0	0.93±0.02a(a)	0.91±0.02a(a)	0.92±0.01a(a)	0.92±0.02a(a)	0.91±0.04a(a)
	5	0.88±0.03ab(a)	0.92±0.00a(a)	0.61±0.53a(a)	0.59±0.51a(a)	0.94±0.04a(a)
	10	0.87±0.00ab(a)	0.88±0.02a(a)	0.79±0.13a(a)	0.89±0.01a(a)	0.85±0.04b(a)
	15	0.81±0.08b(bc)	0.81±0.04b(c)	0.89±0.01a(a)	0.91±0.02a(a)	0.85±0.10a(ab)

SL: Shoot length; LMR: Length of main root; SD: Stem diameter; FW: Fresh weight each plant; DW: Dry weigh each plant; MC: Moisture content. Different lowercase letters without parentheses showed the significant differences among PEG-6000 treatments in the same ANPs level at $p \leq 0.05$. Different lowercase letters within parentheses showed the significant differences among ANPs treatments in the same PEG-6000 level at $P \leq 0.05$.

showed a 40% increase on FW in 5% drought stress compared with Non-ANPs. And DW of seedlings exposed to ANPs at 25 and 200 mg.L^{-1} were also markedly reduced up to 50% and 33.33% in 10% and 15% drought stress conditions, respectively, when compared to that without ANPs.

Interestingly, using 50 mg.L^{-1} TNPs decreased GP, GE and GR up to 6.52%, 6.52% and 8.11%, while VI of seeds exposed to TNPs at 100 mg.L^{-1} was increased up to 20.16% in 5% drought stress conditions compared to that without TNPs. Application of TNPs at other concentration have no noticeable impact on seeds germination both in control and water deficit conditions (Table 5).

As reported in Table 6, DW of seedlings was observably improved up to 4.56% by using 25 mg.L^{-1} TNPs in control compared to that without TNPs. Application of TNPs at 100 mg.L^{-1} dramatically increased LMR (27.10%) in 15% water deficit conditions, FW (19.44%) and DW (33.33%) in 5% water deficit stressed conditions, respectively, while markedly decreased FW (11.11%) in 10% water deficit stressed conditions in comparison with that Non-TNPs. Besides, SL in seeds exposed to 200 mg.L^{-1} TNPs was improved by 15.95% in 5% water deficit conditions as compared to that without TNPs. No remarkable influence was observed in 50 TNPs mg.L^{-1} treated seedling both in control and water deficit stress compared with that Non-TNPs.

For SNPs treatment, there was a significant correlation among GP, GE, and GR ($P<0.01$). VI showed a positive correlation with GP and GE ($P<0.05$). And VI also have a significant positive correlation with SL, SD, FW, and MC ($P<0.01$), indicating the augment of VI may be related to the growth of one-week-old seedlings. Furthermore, MC exhibited a significant positive correlation with SL and FW ($P<0.01$), implying that the improvement of SL and FW in seeds exposed to SNPs were attributed to increase of MC. For seeds exposed to ANPs, there was a significant correlation among GP,

Table 5. Germination of seeds in okra exposed to TNPs under water stress simulated by PEG-6000.

Properties	PEG-6000 (%)	Concentration of TNPs (mg.L^{-1})				
		0	25	50	100	200
GP (%)	0	0.95±0.10a(a)	0.92±0.10a(a)	0.93±0.05a(a)	0.93±0.05a(a)	0.92±0.06ab(a)
	5	0.98±0.03a(ab)	0.93±0.05a(bc)	0.92±0.03a(c)	1.00±0.00a(a)	0.98±0.03a(ab)
	10	0.93±0.05a(a)	0.95±0.06a(a)	0.95±0.03a(a)	0.95±0.03a(a)	0.92±0.03ab(a)
	15	0.90±0.09a(a)	0.93±0.08a(a)	0.90±0.04a(a)	0.90±0.12a(a)	0.87±0.09b(a)
GE (%)	0	0.95±0.10a(a)	0.92±0.10a(a)	0.93±0.05a(a)	0.93±0.05a(a)	0.92±0.06ab(a)
	5	0.98±0.03a(ab)	0.93±0.05a(bc)	0.92±0.03a(c)	1.00±0.00a(a)	0.98±0.03a(ab)
	10	0.93±0.05a(a)	0.93±0.05a(a)	0.95±0.03a(a)	0.95±0.03a(a)	0.92±0.03ab(a)
	15	0.90±0.09a(a)	0.93±0.08a(a)	0.90±0.04a(a)	0.88±0.10a(a)	0.87±0.09b(a)
GR	0	36.70±3.75a(a)	35.40±3.64a(a)	36.30±2.12a(a)	34.93±1.55ab(a)	35.40±2.06ab(a)
	5	37.87±2.05a(ab)	35.55±2.54a(bc)	35.03±1.94ab(c)	38.64±0.50a(a)	37.79±1.31a(ab)
	10	36.05±1.73a(a)	36.18±1.78a(a)	36.32±0.05a(a)	36.57±1.70a(a)	35.40±1.22ab(a)
	15	33.50±4.85a(a)	34.84±1.87a(a)	32.75±1.83b(a)	31.18±5.17b(a)	32.46±4.87b(a)
VI	0	12.69±1.02a(a)	15.42±6.34a(a)	13.89±1.76a(a)	14.57±2.08a(a)	13.03±1.21a(a)
	5	13.79±1.09a(bc)	12.70±0.84a(c)	12.95±0.99ab(c)	16.57±1.41a(a)	14.74±1.28a(b)
	10	10.84±0.98b(ab)	11.03±1.18ab(ab)	11.35±0.88b(a)	9.76±0.46b(b)	10.62±0.82b(ab)
	15	6.38±1.27c(a)	7.15±1.25b(a)	7.24±1.04c(a)	6.69±1.03c(a)	6.70±1.52c(a)

GP: Germination percentage; GE: Germination energy; GR: Germination rate; VI: Vitality index. Different lowercase letters without parentheses showed the significant differences among PEG-6000 treatments in the same TNPs level at $p \leq 0.05$. Different lowercase letters within parentheses showed the significant differences among TNPs treatments in the same PEG-6000 level at $p \leq 0.05$.

Table 6. Growth characteristics of one-week-old seedlings in okra germinated from seeds treated with TNPs under water stress simulated by PEG-6000.

Parameters	PEG-6000 (%)	Concentration of TNPs ($mg.L^{-1}$)				
		0	25	50	100	200
SL (mm)	0	34.30±1.75a(a)	31.88±5.64a(a)	31.32±3.17a(a)	32.90±1.86a(a)	33.36±2.27a(a)
	5	29.16±1.50b(b)	30.18±1.99a(b)	31.15±2.10a(ab)	30.80±1.50a(b)	33.81±1.86a(a)
	10	30.29±1.75b(a)	29.87±2.83a(a)	32.08±0.87(a)	30.90±2.54a(a)	29.43±1.81b(a)
	15	14.87±1.05c(ab)	16.15±2.39b(ab)	17.14±1.61b(a)	16.79±1.12b(ab)	14.81±0.87c(b)
LMR (mm)	0	44.87±8.44a(a)	51.00±10.08a(a)	42.94±3.55b(a)	46.78±1.92a(a)	49.18±3.29a(a)
	5	44.76±5.66a(a)	46.83±4.63a(a)	49.45±1.65ab(a)	46.48±2.91a(a)	45.49±5.40a(a)
	10	49.48±3.92a(a)	51.35±6.32a(a)	54.49±7.98a(a)	49.73±6.28a(a)	50.06±6.54a(a)
	15	35.54±1.98b(bc)	34.22±5.70b(c)	41.79±7.41b(ab)	45.17±4.97a(a)	33.54±2.24b(c)
SD (mm)	0	1.70±0.04a(a)	1.68±0.04a(a)	1.63±0.06a(a)	1.72±0.05a(a)	1.81±0.23a(a)
	5	1.57±0.05b(a)	1.63±0.02a(a)	1.63±0.04a(a)	1.61±0.09b(a)	1.59±0.04b(a)
	10	1.31±0.05c(a)	1.32±0.04b(a)	1.34±0.05b(a)	1.30±0.05c(a)	1.27±0.05c(a)
	15	1.29±0.04c(a)	1.24±0.06c(a)	1.27±0.05c(a)	1.23±0.06c(a)	1.29±0.07c(a)
FW (g)	0	0.35±0.03a(a)	0.43±0.04a(a)	0.38±0.03a(a)	0.42±0.06a(a)	0.37±0.01a(a)
	5	0.36±0.00a(b)	0.36±0.03ab(b)	0.37±0.01a(b)	0.43±0.03a(a)	0.39±0.02a(b)
	10	0.30±0.02b(a)	0.30±0.03bc(a)	0.31±0.03b(a)	0.27±0.02b(b)	0.30±0.02b(a)
	15	0.19±0.01c(a)	0.21±0.02c(a)	0.22±0.01c(a)	0.22±0.03b(a)	0.21±0.02c(a)
DW (g)	0	0.03±0.00c(b)	0.03±0.00a(a)	0.03±0.00b(ab)	0.03±0.01a(ab)	0.03±0.00b(ab)
	5	0.03±0.00b(b)	0.03±0.00a(b)	0.03±0.00b(b)	0.04±0.01a(a)	0.03±0.00ab(ab)
	10	0.03±0.00bc(ab)	0.03±0.00a(a)	0.03±0.00b(a)	0.03±0.00a(b)	0.03±0.00b(ab)
	15	0.04±0.00a(a)	0.04±0.00a(a)	0.04±0.00a(a)	0.04±0.02a(a)	0.04±0.00a(a)
MC	0	0.92±0.01a(a)	0.92±0.00a(a)	0.92±0.00a(a)	0.92±0.00a(a)	0.92±0.01a(a)
	5	0.91±0.01a(a)	0.92±0.00a(a)	0.91±0.01a(a)	0.91±0.01a(a)	0.91±0.01a(a)
	10	0.90±0.00a(a)	0.89±0.01b(a)	0.90±0.01b(a)	0.90±0.01a(a)	0.90±0.01b(a)
	15	0.80±0.04b(a)	0.83±0.01c(a)	0.83±0.02c(a)	0.80±0.11b(a)	0.82±0.01c(a)

SL: Shoot length; LMR: Length of main root; SD: Stem diameter; FW: Fresh weight each plant; DW: Dry weigh each plant; MC: Moisture content. Different lowercase letters without parentheses showed the significant differences among PEG-6000 treatments in the same TNPs level at $p \leq 0.05$. Different lowercase letters within parentheses showed the significant differences among TNPs treatments in the same PEG-6000 level at $P \leq 0.05$.

GE, GR ($P<0.01$) and VI ($P<0.05$). Like SNPs treatment, VI also have a significant positive correlation with SL, DW, FW, MC and SD ($P<0.01$). MC also showed a significant positive correlation with SL, LMR SD, FW, DW and VI ($P<0.01$). Besides, SL have a significant positive correlation with LMR, SD, FW, MC, VI ($P<0.01$) and DW ($P<0.05$), suggesting that increase of SL could relate to the improvement of MC. For TNPs treatment, there are a significant positive correlation among GP, GE, GR and VI ($P<0.01$). VI showed a significant positive correlation with LMR, SD, FW, and MC, implying that the increase of LMR and FW would be as a result of VI augment. SL exhibited a significant positive correlation with GP, GE, GR, VI, LMR, SD, FW, and MC ($P<0.01$), while have a negative correlation with DW. These further indicated that increase of SL might be connected to the improvement of VI, FW and LMR.

Furthermore, SNPs interacted with water deficit stress significantly on GR, GE, GP, LMR, SD ($P < 0.05$), and FW, MC and VI ($P < 0.01$). The interaction of ANPs with water deficiency had prominent effect on GR, GE, GP ($P < 0.01$), and VI and LMR ($P < 0.05$). However, there are no interactions influence of TNPs and drought stress.

In consideration of function values of all determined indicators, seeds exposed to 50 mg.L^{-1} SNPs exhibited the highest drought tolerance in control condition, followed by application of 200 mg.L^{-1} SNPs in control (Table 7). Moreover, in 5% drought stress condition, seeds exposed to ANPs at 25 mg.L^{-1} presented highest drought tolerance, followed by using 200 mg.L^{-1} ANPs (Table 8). Similarly, seeds exposed to TNPs at 100 mg.L^{-1} presented the highest drought tolerance, followed by using 200 mg.L^{-1} TNPs in 5% drought stress condition (Table 9). These data indicated that application of SNPs, ANPs and TNPs could significantly improved resistance to drought of early seedlings in okra, although TNPs have negative influences on GP, GE, and GR in seeds.

Table 7. The function values of indicators analysis of germination of seed treated with SNPs under water stress simulated by PEG-6000.

Concentration of SNPs (mg.L^{-1})	Concentration of PEG-6000 (%)	Function values of indicators										Sum	Order
		GP	GE	GR	VI	SL	LMR	SD	FW	DW	MC		
0	0	0.88	0.88	0.77	0.72	0.75	0.45	1.00	0.66	0.09	0.97	0.72	3
	5	0.25	0.25	0.26	0.57	0.49	0.70	0.50	0.61	0.23	0.88	0.47	10
	10	0.38	0.38	0.45	0.01	0.18	0.42	0.20	0.00	0.07	0.49	0.26	16
	15	0.62	0.62	0.43	0.33	0.03	0.39	0.59	0.33	1.00	0.17	0.45	11
25	0	0.62	0.62	0.50	0.60	1.00	0.58	0.58	0.60	0.19	0.82	0.61	7
	5	0.50	0.50	0.72	0.76	0.77	0.73	0.65	0.77	0.42	0.83	0.67	5
	10	1.00	1.00	0.91	0.59	0.35	0.53	0.61	0.59	0.45	0.76	0.68	4
	15	0.12	0.12	0.14	0.35	0.09	0.71	0.44	0.36	0.44	0.66	0.34	14
50	0	0.88	0.88	0.81	0.75	0.97	0.61	0.76	0.74	0.00	0.95	0.74	1
	5	0.62	0.62	0.39	0.65	0.66	0.35	0.68	0.47	0.09	0.98	0.55	9
	10	0.50	0.50	0.81	0.00	0.07	0.16	0.00	0.01	0.45	0.00	0.25	17
	15	1.00	1.00	1.00	0.30	0.10	0.59	0.67	0.29	0.34	0.58	0.59	8
100	0	0.25	0.25	0.33	0.45	0.72	0.49	0.58	0.50	0.23	0.87	0.47	10
	5	0.88	0.88	0.93	0.78	0.55	0.19	0.49	0.69	0.13	0.94	0.65	6
	10	0.50	0.50	0.57	0.61	0.34	0.86	0.26	0.61	0.32	0.90	0.55	9
	15	0.62	0.62	0.69	0.44	0.00	1.00	0.64	0.41	0.39	0.67	0.55	9
200	0	0.75	0.75	0.81	1.00	0.89	0.16	0.73	1.00	0.20	1.00	0.73	2
	5	0.00	0.00	0.00	0.73	0.94	0.10	0.66	0.74	0.02	0.99	0.42	13
	10	0.38	0.38	0.29	0.62	0.41	0.46	0.28	0.49	0.15	0.82	0.43	12
	15	0.25	0.25	0.33	0.25	0.06	0.00	0.48	0.28	0.66	0.33	0.29	15

GP: Germination percentage; GE: Germination energy; GR: Germination rate; VI: Vitality index; SL: Shoot length; LMR: Length of main root; SD: Stem diameter; FW: Fresh weight each plant; DW: Dry weigh each plant; MC: Moisture content.

Table 8. The function values of indicators analysis of germination of seed treated with ANPs under water stress simulated by PEG-6000.

Concentration of ANPs ($mg.L^{-1}$)	Concentration of PEG-6000 (%)	Function values of indicators										Sum	Order
		GP	GE	GR	VI	SL	LMR	SD	FW	DW	MC		
0	0	0.69	0.69	0.69	0.41	0.71	0.65	0.84	0.40	0.11	1.00	0.62	4
	5	0.54	0.54	0.56	0.39	0.13	0.37	0.88	0.41	0.10	0.87	0.48	12
	10	0.00	0.00	0.47	0.24	0.30	0.60	0.60	0.25	0.59	0.83	0.39	14
	15	0.85	0.85	0.88	0.12	0.00	0.34	0.79	0.02	0.36	0.65	0.49	11
25	0	0.62	0.62	0.52	0.22	0.61	0.46	0.88	0.21	0.08	0.96	0.52	8
	5	1.00	1.00	0.99	1.00	0.41	0.37	0.68	1.00	0.96	0.96	0.84	1
	10	0.08	0.08	0.00	0.00	0.08	0.47	0.89	0.01	0.11	0.84	0.26	16
	15	0.38	0.38	0.38	0.05	0.36	0.57	0.84	0.02	0.34	0.63	0.40	13
50	0	0.77	0.77	0.84	0.49	0.63	0.41	0.66	0.46	0.14	0.97	0.61	5
	5	0.77	0.77	0.91	0.56	0.05	0.34	0.00	0.51	1.00	0.05	0.50	10
	10	0.77	0.77	0.75	0.09	0.20	0.47	0.70	0.00	0.48	0.59	0.48	12
	15	0.54	0.54	0.47	0.19	0.17	1.00	0.73	0.18	0.42	0.89	0.51	9
100	0	0.69	0.69	0.83	0.49	1.00	0.55	0.60	0.45	0.31	0.98	0.66	3
	5	0.54	0.54	0.70	0.08	0.32	0.00	0.03	0.01	0.00	0.00	0.22	17
	10	0.54	0.54	0.48	0.30	0.52	0.60	0.61	0.32	0.67	0.89	0.55	7
	15	0.92	0.92	1.00	0.15	0.07	0.29	0.53	0.03	0.38	0.91	0.52	8
200	0	0.92	0.92	0.93	0.28	0.62	0.36	0.55	0.20	0.00	0.99	0.58	6
	5	0.69	0.69	0.61	0.81	0.54	0.28	0.74	0.91	0.66	0.97	0.69	2
	10	0.46	0.46	0.64	0.08	0.10	0.30	0.31	0.00	0.40	0.63	0.34	15
	15	0.38	0.38	0.31	0.15	0.13	0.46	1.00	0.16	0.03	0.93	0.39	14

GP: Germination percentage; GE: Germination energy; GR: Germination rate; VI: Vitality index; SL: Shoot length; LMR: Length of main root; SD: Stem diameter; FW: Fresh weight each plant; DW: Dry weigh each plant; MC: Moisture content.

Table 9. The function values of indicators analysis of germination of seed treated with TNPs under water stress simulated by PEG-6000.

Concentration of TNPs ($mg.L^{-1}$)	Concentration of PEG-6000 (%)	Function values of indicators										Sum	Order
		GP	GE	GR	VI	SL	LMR	SD	FW	DW	MC		
0	0	0.63	0.62	0.74	0.62	1.00	0.54	0.79	0.66	0.00	1.00	0.66	6
	5	0.88	0.87	0.90	0.73	0.74	0.54	0.57	0.73	0.32	0.93	0.72	3
	10	0.50	0.50	0.65	0.44	0.79	0.76	0.13	0.46	0.17	0.84	0.52	10
	15	0.25	0.25	0.31	0.00	0.00	0.10	0.10	0.00	0.82	0.00	0.18	15
25	0	0.38	0.38	0.57	0.89	0.88	0.83	0.76	1.00	0.55	0.97	0.72	3
	5	0.50	0.50	0.59	0.62	0.79	0.63	0.67	0.71	0.22	0.95	0.62	8
	10	0.63	0.50	0.67	0.46	0.77	0.85	0.15	0.48	0.39	0.77	0.57	9
	15	0.50	0.50	0.49	0.08	0.07	0.03	0.02	0.07	0.60	0.25	0.26	12

Continue...

Table 9. Continuation.

Concentration of TNPs ($mg.L^{-1}$)	Concentration of PEG-6000 (%)	Function values of indicators										Sum	Order
		GP	GE	GR	VI	SL	LMR	SD	FW	DW	MC		
50	0	0.50	0.50	0.69	0.74	0.85	0.45	0.67	0.80	0.27	0.98	0.65	7
	5	0.38	0.38	0.52	0.64	0.84	0.76	0.67	0.75	0.34	0.94	0.62	8
	10	0.63	0.62	0.69	0.49	0.89	1.00	0.18	0.51	0.41	0.79	0.62	8
	15	0.25	0.25	0.21	0.08	0.12	0.39	0.06	0.13	0.81	0.23	0.25	13
100	0	0.50	0.50	0.50	0.80	0.93	0.63	0.83	0.95	0.47	0.98	0.71	4
	5	1.00	1.00	1.00	1.00	0.82	0.62	0.63	1.00	0.84	0.91	0.88	1
	10	0.63	0.62	0.72	0.33	0.83	0.77	0.11	0.33	0.03	0.80	0.52	10
	15	0.25	0.12	0.00	0.03	0.10	0.56	0.00	0.11	1.00	0.07	0.22	14
200	0	0.38	0.38	0.57	0.65	0.95	0.75	1.00	0.75	0.29	0.95	0.67	5
	5	0.88	0.87	0.89	0.82	0.98	0.57	0.60	0.84	0.50	0.93	0.79	2
	10	0.38	0.38	0.57	0.42	0.75	0.79	0.06	0.46	0.28	0.80	0.49	11
	15	0.00	0.00	0.17	0.03	0.00	0.00	0.10	0.07	0.67	0.21	0.13	16

GP: Germination percentage; GE: Germination energy; GR: Germination rate; VI: Vitality index; SL: Shoot length; LMR: Length of main root; SD: Stem diameter; FW: Fresh weight each plant; DW: Dry weigh each plant; MC: Moisture content.

DISCUSSION

Given these results, metal oxide nanoparticles, SNPs and ANPs used in this work, showed a positive influence on parameters of seed germination in okra under PEG-stimulated drought stress. Similar findings were also reported in wheat (Rai-Kalal et al., 2021) exposed to SNPs, and seeds of legume plants germinated by ANPs (Hamuda et al., 2015). According to numerous studies, MONPs can allow seed to be in direct contact with nanoparticles in solution, which can be easily and adequately absorbed by plant due to their peculiar chemical and physical properties (Tripathi et al., 2017). Thus, penetrating seed coats into xylem vessels, promoting the water absorption and retention in cell to protect cell against water loss, thereby enhancing seed germination. Nevertheless, the study of Karunakaran et al. (2016) reported that the exposure of seeds to ANOPs have a significant negative affect on the GP in maize as compared to control. It indicated that MONPs act on seed germination depend on the type of each MONP and plant type. In addition, significant reduction of seed germination (GP, GR, GE and VI) by using 100 $mg.L^{-1}$ SNPs were also found in Non-drought stress, implying that MONPs would be used to elevate germination performance under adverse environmental conditions as seed priming agent. It speculates that MONPs may increase seed germination through improving metabolic, antioxidant enzymatic activities of seed, thus as a result of rapid and uniform seedling emergence, which leads to successful stand establishment to resist environmental stress (Rai-Kalal et al., 2021). Nonetheless, GP, GE and GR in seeds subjected to water deficit stress were all decrease by additive of TNPs at 50 $mg.L^{-1}$, suggesting that this concentration of TNPs have certain toxicity on seed germination under drought stress. The underlying mechanism of phenomenon may correlate well with chemical characteristics of TNPs.

The data on exposure of okra seeds to SNPs appeared that SNPs have various impacts on growth characteristics of seedlings. Under water deficit stress, SL, SD and FW of seedlings were improved by SNPs with different concentrations, especially for SL, which was increased by using 25, 100 and 200 $mg.L^{-1}$ SNPs. Inversely, LMR was reduced by using 200 $mg.L^{-1}$ SNPs expect for the seeds exposed to 100 $mg.L^{-1}$ in 15% drought stress conditions. And DW also reduced by using 100, 200 $mg.L^{-1}$ SNPs, whereas it was increased by additive of SNPs at100 $mg.L^{-1}$ in control. MC was increased by using 100 $mg.L^{-1}$ SNPs in 10% water deficiency, while it was decreased by using 50 $mg.L^{-1}$ SNPs in 5% water deficit

stress. SNPs is suggested to be beneficial for growth parameters in seedlings due to the essential role on preventing oxidative stress induced by drought stress, which can be explained by the enhancement of nutrients accumulation (Elsheery et al., 2020). However, using ANPs at different concentrations only promoted SL and MC of water stressed seedlings. The rest of the growth features were inhibited under most conditions (combined with ANPs and drought). ANPs were confirmed to be assimilated as particles, not ions, indicating that the possible toxic effects of ANPs on okra seedlings is not due to Al ions. There is speculation it may be related with the properties of high electrical conductivity and transmittance, and chemical stability and large bandgap (Rani et al., 2002). Further, the positive impact of TNPs on seedlings was found in present study, which agrees with findings in radish, corn, lettuce, cucumber (Lin and Xing, 2007), canola (Mahmoodzadeh et al., 2013) and wheat plant (Jaberzadeh et al., 2013) due to the improvement of antioxidant activities. And it was also reported that TNPs may help *Cuminum cyminum* L. against drought stress as increase of leaf prolin, which is in membrane structures and scavenges ROS under water deficiency (Salajegheh et al., 2020). As known as, the efficacy of MONPs is determined by their chemical composition, size, surface covering, reactivity, and the dose at which they are effective (Hamuda, 2015). Hence, to enhance MONPs utilization in extractive industry, more studies are required to investigate the interaction of metal nanoparticles and drought stress on plant growth and development.

Based on comprehensive evaluation of drought resistance (Tables 7-9), under most drought stress conditions, all the tested MONPAs can significantly improve drought stress tolerance of seedlings in okra as compared to that without NPs due to the increment of seeds germination parameters or seedlings attributes. Apparently, the improvement of drought stress tolerance via applying SNPs was lower than that observed by using ANPs and TNPs. It also confirmed that various MONPs have different influence on plant, as the investigation in maize seed germination (Karunakaran et al., 2016), and antioxidant enzymes activity of *Cuminum cyminum* L. (Salajegheh et al., 2020). Thus, identify the appropriate MONPs based on different crops is significant for real world applications.

CONCLUSIONS

Using various MONPs ($SiO_{2,}$ Al_2O_3 and TiO_2 nanoparticles) have different influence on okra seeds germination under PEG-6000 simulating water deficiency. SNPs and ANPs significantly improved seeds germination arguments and certain seedlings features, TNPs promoted shoot length, length of main root, fresh weight each plant, and dry weigh each plant of seedlings notwithstanding they have negative impacts on seed germination parameters except for VI. Furthermore, all MONPs used in present work enhanced the drought stress tolerance in okra seedlings. This finding in our work is all-important for use and disposal of nanoparticles in the agriculture sector, especially in solving the issue of water scarcity.

ACKNOWLEDGEMENTS

This work was supported by the Program for Natural Science Research in Guizhou Education Department (QJJ[2023]024), The Sixth Batch of Guizhou Province High-level Innovative Talent Training Program[2022], and Guiyang University graduate research fund project(2023-05).

REFERENCES

ACHARYA, P.; JAYAPRAKASHA, G.K.; CROSBY, K.M.; JIFON, J.L.; PATIL, B.S. Nanoparticle-mediated seed priming improves germination, growth, yield, and quality of watermelons (*Citrullus lanatus*) at multi-locations in Texas. *Scientific Reports*, v.10, n.1, p.50-37, 2020. http://dx.doi.org/10.1038/s41598-020-61696-7.

AHMED, B.; RIZVI, A.; ZAIDI, A.; KHAN, M. S.; MUSARRAT, J. Understanding the phyto-interaction of heavy metal oxide bulk and nanoparticles: evaluation of seed germination, growth, bioaccumulation, and metallothionein production. *RSC Advances*, v.9, n.8, p.4210-4225, 2019. http://dx.doi.org/10.1039/c8ra09305a

CHENG, S.H.; LEE, C.H.; CHEN, M.C. Tri-functionalization of mesoporous silica nanoparticles for comprehensive cancer theranostics -the trio of imaging,targeting and therapy. *Journal of Materials Chemistry Mater*, v.20, n.29, p.6149-6157, 2010. http://dx.doi.org/10.1039/c0jm00645a

CHOURASIYA, V.K.; NEHRA, A.; SHUKLA, P.S.; SINGK, K.P.; SINGH, P. Impact of Mesoporous nano-silica (SiO) on seed germination and seedling growth of wheat, pea and mustard seed. *Journal of Nanoscience and Nanotechnology,* v.21,n.6, p.3566–3572,2021. https://doi.org/10.1166/jnn.2021.19013

ELSHEERY, N.I.; HELALY, M.N.; EL-HOSEINY, H.M.; ALAM-ELDEIN, S.M. Zinc oxide and silicone nanoparticles to improve the resistance mechanism and annual productivity of salt-stressed mango trees. *Agronomy*, v.10, n.4, p.558, 2020. https://doi.org/10.3390/agronomy10040558

GARCÍA-LÓPEZ, F.; ZAVALA-GARCÍA, E.; OLIVARES-SÁENZ, R.; LIRA-SALDÍVAR, E.; DÍAZ, BARRIGA-CASTRO.; RUIZ-TORRES, N.; RAMOS-CORTEZ, E.; VÁZQUEZ-ALVARADO, R., NIÑO-MEDINA, G. Zinc oxide nanoparticles boosts phenolic compounds and antioxidant activity of *Capsicum annuum* L. during germination. *Agronomy*, v.8, n.10, p.215, 2018. https://doi.org/10.3390/agronomy8100215

HAMUDA, H.E.A.F.B. Influence of engineered metal oxide nanoparticles on seed germination, seedling development and chlorophyll content. *Óbuda University e-Bulleti*n, v.5, n1, p.79-92, 2015.

HAYES, K.L.; MUI, J.; SONG, B.; SANI, E.S.; EISENMAN, S.W.; SHEFFIELD, J.B.; KIM, B. Effects, uptake, and translocation of aluminum oxide nanoparticles in lettuce: a comparison study to phy totoxic aluminum ions. *The Science of the Total Environmen*t, n.719, p.137393, 2020. https://doi.org/10.1016/j.scitotenv.2020.137393

HOJJAT, S.S. Effects of TiO_2 nanoparticles on germination and growth characteristics of Grass Pea (*Lathyrus sativus* L.) seed under drought stress. *Nanotechnologies in Russia*, v.15, n.2, p.204-211, 2020. https://doi.org/10.1134/S199507802002010X

JABERZADEH, A.; MOAVENI, P.; REZA, H.; TOHIDI, M.; ZAHEDI, H. Influence of bulk and nanoparticles titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat subjected to water deficit stress. *Notulae Botanicae Horti Agrobotanici*, v.41, n.1, p.201-207, 2013. https://doi.org/10.15835/nbha4119093

KARUNAKARAN, G.; SURIYAPRABHA, R.; RAJENDRAN, V.; KANNAN, N. Influence of ZrO_2, ZiO_2, Al_2O_3 and Tio_2 nanoparticles on maize seed germination under different growth conditions. *IET Nanobiotechnology*, v.10, n.4, p.171-177, 2016. https://doi.org/10.1049/iet-nbt.2015.0007

KHALAKI, M.A.; MOAMERI, M.; LAJAYER, B.A.; ASTATKIE, T. Influence of nano-priming on seed germination and plant growth of forage and medicinal plants. *Plant Growth Regulation*, v.93, n.1, p.13-28, 2021. https://doi.org/10.1007/s10725-020-00670-9

LANONE, S.; ROGERIEUX, F.; GEYS, J.; DUPONT, A.; MAILLOT-MARECHAL, E.; BOCZKOWSKI, J.; LACROIX, G.; HOET, P. Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. *Particle & Fibre Toxicology*, v.6, n.1, p.14-14, 2009. https://doi.org/10.1186/1743-8977-6-14

LIN, D.; XING, B. Phytotoxicity of nanoparticles, inhibition of seed germination and root growth. *Environmental Pollution*, v.150, n.2, p.243-50, 2007. https://doi.org/10.1016/j.envpol.2007.01.016

MAHMOODZADEH, H.; NABAVI, M.; KASHEFI, H. Effect of nanoscale titanium dioxide particles on the germination and growth of canola (*Brassica napus*). *Journal of Ornamental and Horticultural Plants*, v.3, n.1, p.25-32, 2013.

RAI-KALAL, P.;TOMAR, R.S; JAJOO, A. Seed nanopriming by silicon oxide improves drought stress alleviation potential in wheat plants. *Functional Plant Biology*, v.48, p.905-915, 2021. https://doi.org/10.1071/FP21079

RANI, P.; KAUR, G.; RAO, K.V.; SINGH, J.; RAWAT, M. Impact of green synthesized metal oxide nanoparticles on seed germination and seedling growth of *Vigna radiata* (mung bean) and *Cajanus cajan* (red gram). *Journal of Inorganic and Organometallic Polymers and Materials*, v.30, p.4053-4062, 2020. https://doi.org/10.1007/s10904-020-01551-4

RASTOGI, A.; TRIPATHI, D.K.; YADAV, S.; CHAUHAN, D.K.; ZIVCAK, M.; GHORBANPOUR, M.; ELSHEERY, N.I.; BRESTIC, M. Application of silicon nanoparticles in agriculture. *Biotech*, v.9, n.3, p.1-11, 2019. https://doi.org/10.1007/s13205-019-1626-7

SALAJEGHEH, M.; YAVARZADEH, M.; PAYANDEH, A.; AKBARIAN, M.M. Effects of titanium and silicon nanoparticles on antioxidant enzymes activity and some biochemical properties of *Cuminum cyminum* L. under drought stress. *Banat s Journal of Biotechnology*, v.10, n.21, p.19-25, 2020. https://www.researchgate.net/profile/Mohammad-Mehdi-https://www.researchgate.net/profile/Mohammad-Mehdi-Akbarian-2/publication/351527973_Effects_of_titanium_and_silicon_nanoparticles_on_antioxidant_enzymes_activity_and_some_biochemical_properties_of_Cuminum_cyminum_L_under_drought_stress/links/609c285ba6fdcc9aa7dd10ee/Effects-of-titanium-and-silicon-nanoparticles-on-antioxidant-enzymes-activity-and-some-biochemical-properties-of-Cuminum-cyminum-L-under-drought-stress.pdf

SARLAK, N.; TAHERIFAR, A.; SALEHI, F. Synthesis of nanopesticides by encapsulating pesticide nanoparticles using functionalized carbon nanotubes and application of new nanocomposite for plant disease treatment. *Journal of Agricultural & Food Chemistry*, v.62, n.21, p.4833-8, 2014. https://doi.org/10.1021/jf404720d

SIDDIQUI, M.H.; AL-WHAIBI, M.H. Role of nano-SiO_2 in germination of tomato (*Lycopersicum esculentum* seeds Mill.). *Saudi Journal of Biological Sciences*, v.21, n.1, p.13-17, 2014. https://doi.org/10.1016/j.sjbs.2013.04.005

SUTULIENĖ, R.; RAGELIENĖ, L.; SAMUOLIENĖ, G., BRAZAITYTĖ, A.; URBUTIS, M.; MILIAUSKIENĖ, J. The response of antioxidant system of drought-stressed green pea (*Pisum sativum* L.) affected by watering and foliar spray with silica nanoparticles. *Horticulturae*, v.8, n.1, p.35, 2022. https://doi.org/10.3390/horticulturae8010035

TRIPATHI, D.K.; SINGH, S.; SINGH, V.P.; PRASAD, S.M.; DUBEY, N.K.; CHAUHAN, D.K. Silicon nanoparticles more effectively alleviated UV-B stressthan silicon in wheat (*Triticum aestivum*) seedlings. *Plant Physiology and Biochemistry*, v.110, p.70-81, 2017. https://doi.org/10.1016/j.plaphy.2016.06.026

XIAO, Y.; DU, Y.; XIAO, Y.; ZHANG, X.; WU, J.; YANG, G. Elucidating the effects of TiO_2 nanoparticles on the toxicity and accumulation of Cu in soybean plants (*Glycine max* L.). *Ecotoxicology and Environmental Safety*, v.219, p.112312, 2021. https://doi.org/10.1016/j.ecoenv.2021.112312

WANG, C.X.; JI, Y.H.; CAO, X.S.; YUE, L.; CHEN, F.R.; LI, J.; YANG, H.Y.; WANG, Z.Y.; XING, B.S. Carbon dots improve nitrogen bioavailability to promote the growth and nutritional quality of soybeans under drought stress. *ACS Nano*, v.16, n.8, p.12415-12424, 2022. https://doi.org/10.1021/acsnano.2c03591

WANG, J.Y.; SHI D.H.; YU, B.; YANG. D.; ZHANG. T.; LIU. Y. Effect of drought stress with PEG-6000 on seed germination and physiological properties in Abelmoschus esculentus. *Journal of Tropical and Subtropical Botany*, v.26, n.6, p.611-616, 2018. https://www.cabdirect.org/cabdirect/abstract/20193109924