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Effect of ZnO, Cu and SiO_2 Nanoparticles on Drought Tolerance in Two Wheat Varieties: Kalar1 and Kalar2 during Seedling Stage

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ABSTRACT

Drought is abiotic stress that directly influences crop growth performance, including wheat. In this study, the nanotechnology method was applied to decrease the impact of drought on wheat growth. For this purpose, three types of drought resistance nanoparticles (Silicon dioxide (SiO2), Zinc oxide (ZnO), and Copper (Cu)) were used with two wheat varieties (kalar1 and kalar2) in the Garmian district. The results showed that nanoparticles increased specific leaf area, chlorophyll, soluble carbohydrate, catalase enzyme activity, phosphor, and potassium under drought stress compared with the control. SiO2 and ZnO nanoparticles had better impacts on some morphological and biochemical parameters than Cu. Different drought-resistance nanoparticles could be used to cope with drought impact in the Garmain district and improve wheat growth.

Keywords: wheat (Triticum aestivum), drought, nanoparticles, kalar1, kalar2.

1. Introduction

The growth performance of agricultural crops is highly subjected to the effects of abiotic (Environmental conditions) and biotic (species interactions) factors[1, 2]. The crops in the tropical and sub-tropical regions are highly suffering from the changes in environmental conditions[3]. Many studies showed that the productivity of agricultural crops have been declined due to environmental stresses[4]. For example, high temperature and low precipitation rates caused significant damage to agricultural productions[5]. Furthermore, inter and intraspecific competition among coexisting species, invasion of weeds, bacterial, viral, and fungal pathogens are the main biotic factors that have adverse impacts on plant crop productions[6, 7].

Drought is considered the most harmful factor that has a direct impact on the productivity of cereals[8]. It is defined as a shortage in the amount of water needed by plants to survive[9]. The main reason beyond this phenomenon is the elevation of the atmospheric temperature and declining/ un organizing in the precipitation rates[10, 11]. This stress is multidimensional and causes changes in plants' physiological, morphological, biochemical, and molecular traits[12]. Many plants have improved their resistance mechanisms to tolerate drought stress, but these mechanisms are varied based on plant species[13, 14]. Studies showed that wheat is more drought tolerant than maize, sorghum or millet[15].

Poaceae family is one of the important crops in the world, and it is the second most important staple food crop with high economic value and an important food source[16]. Due to the increase in population rates, it is necessary to use modern agricultural technologies to increase the drought tolerance of crops and ensure food requirements[17]. Technologies have been applied in the agricultural field to solve problems. Nanotechnology is one of those technologies which has been recently involved in agriculture for different purposes, such as fungicides, herbicides, nano-fertilizers and reducing drought impacts[18]. Nanotechnology is promising for agriculture and food products which deals with atomic or molecular aggregates of 1 to 100 nm in size[19]. Nanoparticles (NPs) are organic or inorganic materials which is used worldwide [20]**.** The different chemical and physical properties of nanoparticles such as chemical composition, size, surface covering and reactivity (positive and negative), help

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plants to overcome the undesirable environmental conditions that affect plant growth and development[21].

Many studies showed the importance of the application of different nanoparticles, such as Nanoparticles of gold (Au), silver (Ag), copper (Cu), zinc (Zn), aluminium (Al), silica (Si), zinc oxide (ZnO), caesium oxide (Ce_2O_3), titanium dioxide (TiO_2) and magnetized iron (Fe) in agricultural managements[19].

Furthermore, studies showed that applying nanoparticles increases the drought tolerance in plants via increasing leaf water contents and above-ground biomas[22, 23]. The physiological and chemical characteristics of plants have different response to nanoparticles. For example, the regulation of plant growth, such as seed production and chlorophyll formation, is mediated by Cu-nanoparticles[22].

Copper nanoparticles (Cu) mediate many physiological and biochemical regulations in plants[22]. For example, Nano-Cu have positive impacts on the activity of superoxide dismutase (SOD) ascorbate peroxidase (APX), and catalase (CAT) enzymes[24]. These enzymes regulate many biochemical reactions in plants[22]. It is also required in photosynthesis, which is essential for plant respiration and assists in the metabolism of carbohydrates and proteins[25]. Nanoparticle-Cu is responsible for drought stress in controlling maize growth and development[22,26].

Furthermore, zinc oxide (ZnO) nanoparticles in agriculture are contributed mainly in the regulation of crop growth, quality enhancement, and inducting stress tolerance, while the underlying mechanisms remain elusive[27]. Nano-ZnO-induced drought tolerance was investigated in crops[27, 28].

Silicon is the most abundant chemical element in The Earth's crust[29] It is found in soils and can be taken up by plant roots in large quantities. Silicon is known to have beneficial effects when added to the soil[30]. Silicon rich fertilization generally resists drought and improve salt stress tolerance, but its effects are inconsistent[31]. Nanoparticles may have negative or positive effects on agricultural crops, depending on the crop and its growth stage, nutrition, tillage, and applied nanoparticles[32]. In response to drought stress, crop grains show various morphological, biochemical, physiological and molecular responses[33]. Numerous physiological changes occur in plants due to insufficient water sources in plant development's vegetative and reproductive phases[34, 35].

The overall objective of this study was to test the efficiency of some drought resistance nanoparticles on two wheat varieties (K1 and K2). Therefore, we hypothesized that nanoparticles will improve growth performance in both varieties under drought stress and increase drought resistance enzymes in both varieties.

2. Materials and Methods

This experiment was conducted in plastic house at Kalar city (34°38'54.5" N and 45°19'19.4" E), under the following conditions temperature (20-40 C°), pH 7.5, moisture 4-7 and light (1500-2000 lux). The design of the experiment was randomized complete block design (RCBD) with two blocks (replicates) and 48 pots/ block. Two newly released wheat varieties in the region were used: kalar1 and kalar2[36] . Seed treatments were carried out using the colloidal solution of Cu, ZnO and SiO_2 nanoparticles (NPs). Variants of the experiment were as follows: K1/Unstressed/Control, K1/Unstressed/cu, K1/Unstressed/SiO_2, K1/Unstressed/ZnO, K1/Stressed/Control, K1/Stressed/cu, K1/Stressed/SiO_2, K1/Stressed/ZnO, K2/Unstressed/Control, K2/Unstressed/Cu, K2/Unstressed/SiO_2, K2/Unstressed/ZnO, K2/Stressed/Control, K2/Stressed/cu, K2/stressed/SiO_2, K2/Stressed/ZnO. Nanoparticles were purchased from (3302 Twig Leaf Lane, Houston, TX77084, USA). The purity and the diameter of nanoparticles were as follows, Zinc oxide nanoparticles (ZnO, 99%, 10-30nm). Colloidal Copper nanoparticles (Cu, 1000ppm in H_2O, 40nm), Silicon dioxide nanoparticles powder (SiO_2, 98+%, 20-30nm, amorphous). Most of nanoparticles were spherical or cubic shapes. Zinc oxide (ZnO) and silicone oxide (SiO_2) powders were dissolved in distilled water while copper (Cu) was used as a solution, a concentration of 50 mg\l was prepared from each nanoparticle. The solutions were homogenized using (ultrasonic homogenizer processor) to shake nanoparticle and distil water for 30 minutes. The seeds (10g) were soaked for 24 hours in the homogenized nanoparticle solution[37] The soaked seeds put on filter paper for 30 minutes then planted in the pots using sandy clay loam soil under controlled temperature and humidity in plastic house. The treatments were assigned randomly to the pots within each block (3 pots for each treatment). The seeds were cultivated in pots in November, 2021. After seed germination, water stress was applied on drought treatments based on field capacity. Each pot was irrigated every 5-6 days for water stressed treatments, and 2-3 days for unstressed treatments. The physiological and morphometric measurements were then taken using seedling leaves for all treatments. The enzymatic activity of catalase (CAT) and peroxidase (POD) were determined according to Aebi[38] and Bergmeyer[39], respectively. Soluble carbohydrate was determined according to a method by Willenbrink,[40]. The specific leaf area (SLA) was determined according to Chanda and Singh,[41]. Chlorophyll meter (SPAD 502) was used to determine the chlorophyll content index according to Ling et al,[42]. For elements assay, samples were digested according to Pequerul et al[43], potassium estimated by flame photometry and phosphor estimated using spectroscopy.

3. Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) R software, and Tukey test was used to tease out the differences among control, drought and nanoparticle treatments[44].

3. Results

Our results revealed significant effects of nanoparticle treatments on the growth performance of both wheat varieties under drought stress. For example, specific leaf area (SLA; cm^2/gm) ($F_{1,10}=7.88$, $P<0.001$) in Kalar1 treated with silicon dioxide SiO_2 nanoparticles (NPs) had a higher value (435.9 cm^2/gm) than the Kalar2 value (405.25cm^2/gm) treated with the same nanoparticle (Figure 1E). In a similar manner, SiO_2 had a higher impact on SLA (423.4cm^2/gm) under drought stress compared with the other nanoparticle and control ($F_{3,368}=18.332$, $p<0.001$) (Figure 1D).

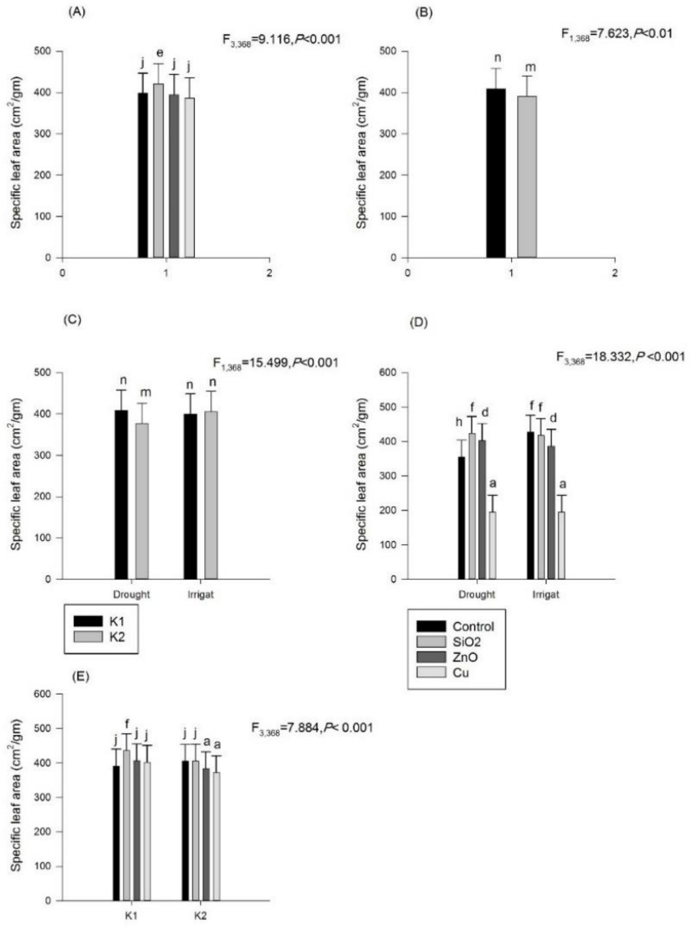

Figure 1: The response of specific leaf area (SLA; cm^2/gm) to: (A) nanoparticles (NPs), (B) varieties (K1 and K2), (C) interaction between drought stress and varieties, (D) interaction between nanoparticles and stress conditions, and (E) interaction between nanoparticles and varieties. Different letters mean significant differences at ($\alpha<0.05$).

SiO_2 nanoparticles treated pots under drought stress had higher chlorophyll content index (13.28) in compare with other treatments (Figure 2A). In addition, chlorophyll content in K1 treated with ZnO and Cu nanoparticles increased (13.44 and 13.03, respectively) compared with control. However, in K2 variety, pots treated with SiO_2 and ZnO nanoparticles showed an increase in chlorophyll content (13.73 and 12.79, respectively) ($F_{3,80}$ =7.884, P=0.01).

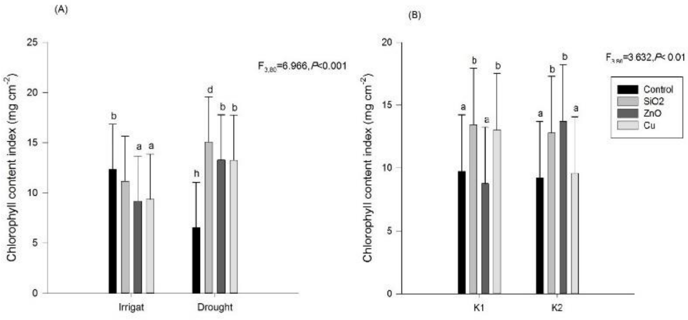

Figure 2: The response of chlorophyll content index to (A) interaction of nanoparticles and drought stress and (B) interaction of nanoparticles and varieties. . Different letters mean significant differences at ($\alpha<0.05$).

The interaction between irrigation treatment (drought vs irrigated) with nanoparticles showed significant differences ($F_{3,80}$=2.791, P=0.04) in the response of soluble carbohydrates to the different nanoparticles. For example, Zinc oxide (ZnO) nanoparticles showed a higher effect on soluble carbohydrate rate (39.57%) under drought stress than the effect of SiO_2 (28.93%) and Cu (32.26%) nanoparticle compared with the control (Figure 3C).

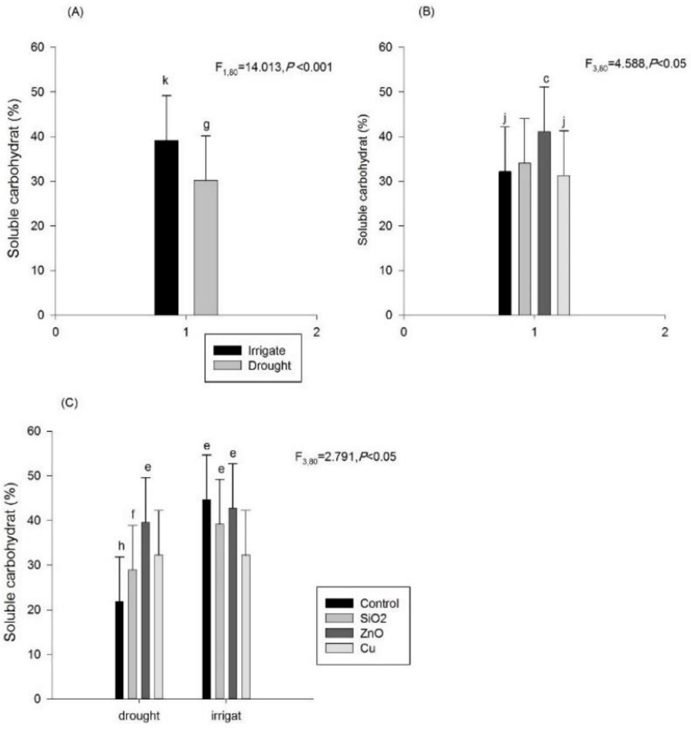

Figure 3: The response of soluble carbohydrate to: (A) drought stress, (B) nanoparticles and (C) interaction between nanoparticle and drought stress. Different letters mean significant differences at ($\alpha<0.05$).

In addition, catalase activity ($F_{3,80}$=3.595, p=0.01) and seedling height ($F_{3,80}$=3.761, p=0.01) of kalar2 treated with SiO_2 nanoparticle were higher (1.11U/g and 18.50cm, respectively) than the similar nanoparticle in kalar1 (0.91U/g, and 12.17cm, respectively) (Figure 4A and 5A). However, the interaction between nanoparticles and drought stress in seedling height showed no significant difference under drought stress (Figure 4C). While catalase activity under drought stress treated with SiO_2 and ZnO (0.88U/g and 0.92U/g, respectively) nanoparticles increased compared with control (0.48U/g) (Figure 5D).

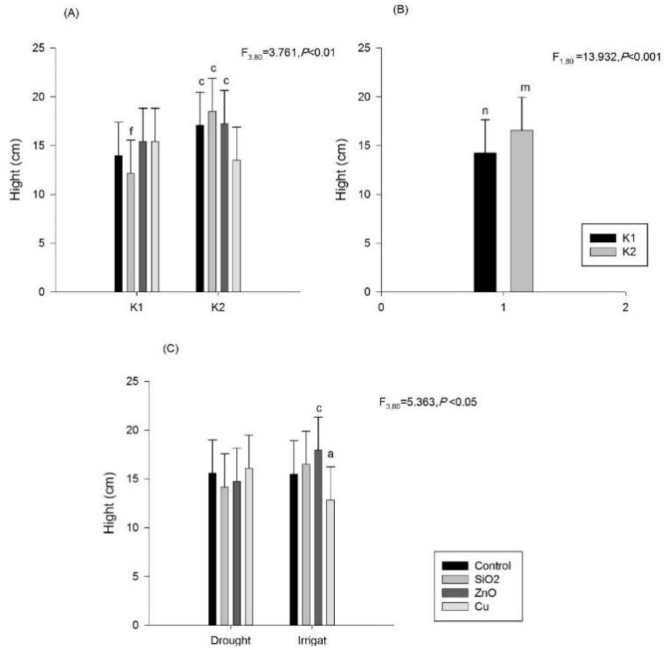

Figure 4: The response of seedling height to (A) interaction between nanoparticles and varieties, (B) varieties and (C) interaction between nanoparticle and drought stress. Different letters mean significant differences at (α<0.05).

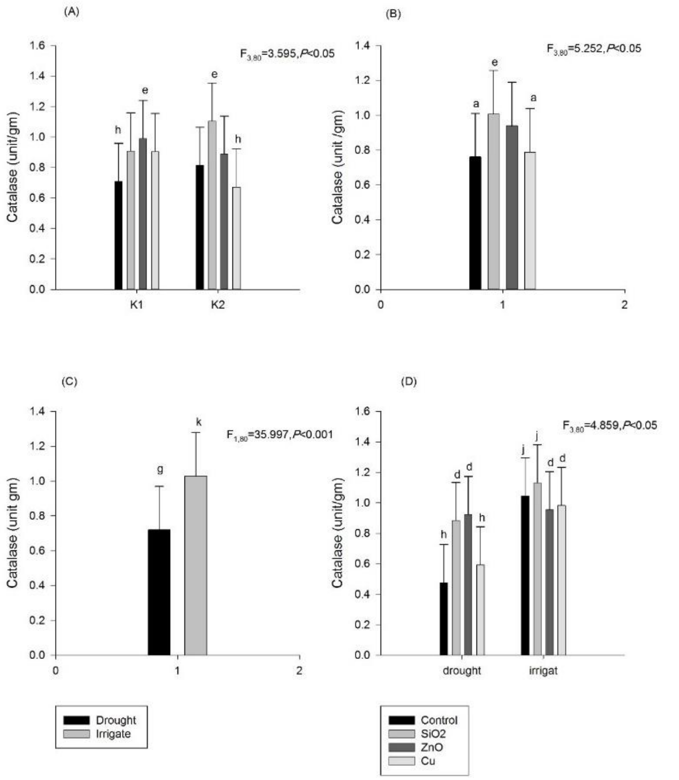

Figure 5: The response of enzyme catalase to (A) interaction between varieties and nanoparticles, (B) nanoparticles, (C) drought stress and (D) interaction between nanoparticles and drought stress. Different letters mean significant differences at (α<0.05).

Compared with irrigated pots, peroxidase activity decreased under drought stress (0.95U/g) (Figure 6A). Contrarily, peroxidase enzyme activity was higher in nanoparticle-treated pots compared to the control ($F_{3,80}$=17.854, *P*<0.0001) (Figure 6B).

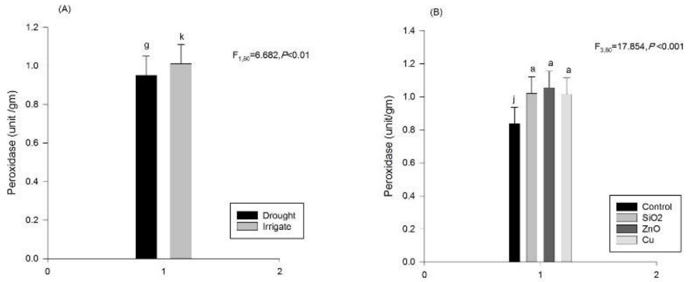

Figure 6: The response of enzyme peroxidase to (A) drought stress and (B) nanoparticles. Different letters mean significant differences at (α<0.05).

Furthermore, the interaction between nanoparticles and drought stress showed that nanoparticles treated pots have higher phosphorus concentration (SiO2 = 7.9%, ZnO = 7.3% and Cu=6.0%) than control (4.5%) (Figure 7D). In addition, the phosphor concentration in Kalar2 was higher than in Kalar1 (7.3% and 6.7%, respectively) (Figure 7C).

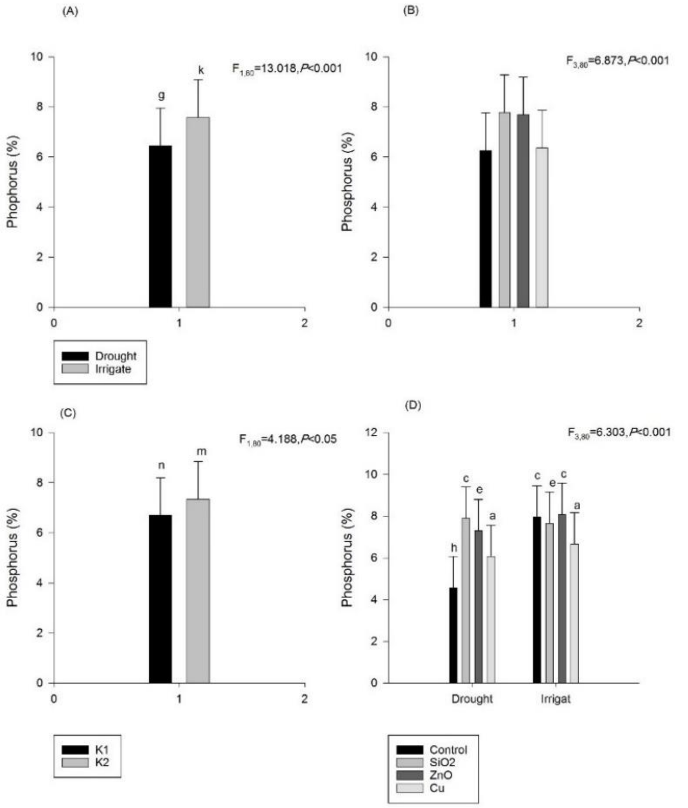

Figure 7: The response of phosphor to: (A) drought stress (B) nanoparticles (C) varieties, and (D) interaction between nanoparticles and water stress.

The concentration of potassium was higher in irrigated pots (0.73%) in comparison with drought (0.61%) (Figure 8A). With the respect to water stress, Zinc oxide and silicon oxide nanoparticles had more impact (0.72 and 0.71% respectively) on potassium concentration than other nanoparticles (figure 8B). In the opposite manner to irrigated pots, nanoparticles showed not significant influence on potassium concentration in pots under drought stress (figure 8C).

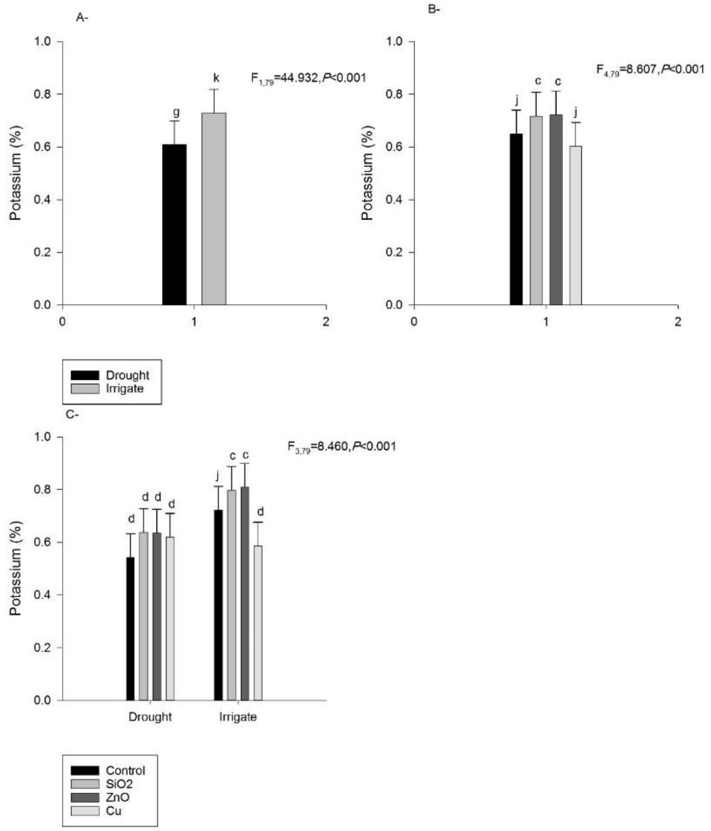

Figure 8: The response of potassium to: A- water stress B- nanoparticle C-interaction between nanoparticle and water stress.

Discussion

Environmental stresses, particularly drought, negatively affect plant growth and productivity worldwide, causing significant yield losses of crops[37]. In comparison to pots treated with nanoparticles, the control pots were decreased in Carbohydrate, Catalase, peroxidase and both potassium (K) and phosphor (P) due to drought stress. The decrease in carbohydrate content occurred mainly due to the decrease in the photosynthesis[45]. Drought stress decreases the yield through changing the amounts and activities of photosynthetic pigments as well as osmolyte content and enzyme activity[46].

The application of SiO_2 nanoparticles can improve the growth via enhancing the root weight. Silicon oxide nanoparticles also facilitate water uptake and its transportation into other plant parts[47]. Leaf area plays a key role in plant development as it indicates the size of stimulatory system, which helps plant growth and development. In this study, it was noticed that the application of SiO_2, Cu and ZnO NPs significantly enhanced specific leaf area (SLA), soluble carbohydrate, chlorophyll, catalase, phosphorous concentrations and plant growth of wheat under drought stress compared with control. Furthermore, SiO_2 had more influence on SLA than other NPs because SiO_2 could alleviate the effect of water stress more than others[48]. In addition, SiO_2 NPs increased chlorophyll content in both varieties and this may due to the role of SiO_2 NPs to improve the photosynthetic pigments by enhancing endogenous levels of cytokinins, which stimulate chlorophyll synthesis and improve chloroplast ultrastructure[46, 49]. Furthermore, increasing carbohydrate contents under drought stress could be attributed to increase osmotic pressure in plants for more absorption of soil water and spraying with solution containing NPs, and increase the levels of osmolytes[50]. Silicon also partially offsets the negative impact of drought on plants by increasing the catalase activity. On the other hand, protein accumulation changes the response to drought stress. Drought stress reduces starch deposition in wheat grain, resulting in an increase in grain protein content[51, 52]. The photosynthetic cells usually have higher copper contents. Therefore, the potential of Cu as an electron carrier in redox reactions of the electron transport chain also helps plants to tolerate drought stress conditions. It is also evident that Cu contributes to plant growth and help in the process of wheat reproduction[53] . In addition, SiO_2 and ZnO nanoparticles improve plant production by increasing the activity of antioxidant enzymes in plant tissues such as CAT, peroxidase and SOD. SiO_2 also plays an important role in balancing the uptake, transport, and distribution of elements in drought-stressed plants through water uptake and development of root[51]. In agreement with our findings, SiO_2 and ZnO nanoparticles have improved the catalase activities, while they had no influence on peroxidase activity. Furthermore, zinc plays an important role in protecting and maintaining the stability of cell membrane structures[54, 55] . The application of nano-formulations of ZnO and Cu also increases P and K uptake and boosts performance of crop plants under stress[56].

Finally, the findings of this study revealed that using nanoparticles can improve crop function and increase drought tolerance in kalar1 and kalar2 variety.

Conclusion

The addition of nanoparticles can help in improving wheat growth in Kalar1 and Kalar2 varieties under drought condition. Therefore, we recommend the use of nanoparticles as a new method to cope drought impacts and enhance wheat growth during seedling stage.

Conflict of interests

None

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