Zinc oxide nanoparticles alleviate drought-induced alterations in sorghum performance, nutrient acquisition, and grain fortification

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Abstract

Drought is a major environmental event affecting crop productivity and nutritional quality, and potentially, human nutrition. This study evaluated drought effects on performance and nutrient acquisition and distribution in sorghum; and whether ZnO nanoparticles (ZnO-NPs) might alleviate such effects. Soil was amended with ZnO-NPs at 1, 3, and 5 mg Zn/kg, and drought was imposed 4 weeks after seed germination by maintaining the soil at 40% of field moisture capacity. Flag leaf and grain head emergence were delayed 6-17 days by drought, but the delays were reduced to 4-5 days by ZnO-NPs. Drought significantly ($p < 0.05$) reduced (76%) grain yield; however, ZnO-NP amendment under drought improved grain (22-183%) yield. Drought inhibited grain nitrogen (N) translocation (57%) and total (root, shoot and grain) N acquisition (22%). However, ZnO-NPs (5 mg/kg) improved (84%) grain N translocation relative to the drought control and restored total N levels to the non-drought condition. Shoot uptake of phosphorus (P) was promoted (39%) by drought, while grain P translocation was inhibited (63%); however, ZnO-NPs lowered total P acquisition under drought by 11-23%. Drought impeded shoot uptake (45%), grain translocation (71%) and total acquisition (41%) of potassium (K). ZnO-NP amendment (5 mg/kg) to drought-affected plants improved total K acquisition (16-30%) and grain K (123%), relative to the drought control. Drought lowered (32%) average grain Zn concentration; however, ZnO-NP amendments improved (94%) grain Zn under drought. This study represents the first evidence of mitigation of drought stress in full-term plants solely by exposure to ZnO-NPs in soil. The ability of ZnO-NPs to accelerate plant development, promote yield, fortify edible grains with critically essential nutrients such as Zn, and improve N acquisition under drought stress has strong implications for increasing cropping systems resilience, sustaining human/animal food/feed and

nutrition security, and reducing nutrient losses and environmental pollution associated with N-fertilizers.

Keywords: Drought stress; Food and nutrition security; Nutrient use efficiency; Resilient agriculture; Zinc oxide nanoparticles.

1. Introduction

Increasing spates of drought events are negatively affecting agricultural production globally. In the United States, 80% of all farm losses in 2012 were due to drought, heat, and wind, according to the USDA (http://ers.usda.gov/topics/in-the-news/us-drought-2012-farm-and-food-impacts.aspx#.VDMwNvldVrU) and the US Natural Resources Defense Council (NRDC; https://www.nrdc.org/media/2013/130827). Notably, this involved more than 60% of the country being in a severe drought condition covering over 1000 counties, with billions in losses reported.

Sorghum grain is a staple food for populations in rural and resource-poor regions, ranking as the fifth most important cereal in the world. The United States, led by Kansas and Texas, is the largest sorghum producer in the world; India and Nigeria are among the other top producers. Harvested sorghum is used primarily for feed and ethanol production in the United States, while it is an important food security crop in India and other producing countries (FAO, http://www.fao.org/3/W1808E/w1808e02.htm; accessed 2019; NSP, (https://sorghumgrowers.com/sorghum-101/). Although sorghum is a relatively drought- and heat -tolerant species, it can be affected by drought (Downing, 2015); there is a threshold for plant tolerance to drought under prolonged and severe events. Therefore, with recurrent adverse agro-environmental perturbations such as drought becoming more common as the climate continues to change, there is a clear need to improve the sustainability of crop production, so as to ensure global security of food, feed, and agro-industrial production systems.

Importantly, use of conventional micronutrients have been shown to mitigate drought stress in agricultural crop plants (reviewed in Karim and Rahman, 2015; Dimkpa and Bindraban, 2016; Lamaoui et al., 2018). Micronutrients mitigate drought effects in plants by increasing water use efficiency; maintaining cell membrane stability which otherwise causes tissue flaccidity due to drought-induced wilting; and detoxifying toxic free radicals that accumulate in plant cells during water scarcity (Cakmac, 2000; Karim and Rahman, 2015). These actions are related to the multiple roles of micronutrients in stimulating a number of enzymes and plant physiological processes related to abiotic stresses, water balance, or nutrient uptake (Karim and Rahman, 2015; Dimkpa and Bindraban, 2016; Lamaoui et al., 2018). Specifically, zinc (Zn) activates metabolic processes regulating water dynamics in plants. Under limited water condition, plants produce increased quantities of abscisic acid (ABA) to optimize stomatal closure to conserve water (Karim and Rahman, 2015; Lamaoui et al., 2018). Zn has been shown to increase ABA production in plants (Zengin, 2006), thereby enhancing stomatal regulation by ABA under water stress conditions.

Given the physiological roles of Zn in agricultural crops, significant agronomic outcomes have been realized with Zn fertilization under drought stress. For example, wheat grain yield which decreased on average by 25% due to drought was recovered by 16% upon fertilization with Zn (as Zn ions) (Bagci et al., 2007). This implies a mitigation of yield loss due to water shortage from 25% to 13%. Similarly, application of Zn (ions) under drought stress increased wheat grain yield by 15%, relative to drought-impacted plants not treated with Zn (Karim et al., 2012). Drought-induced reduction in soybean grain yield was more pronounced in the absence (54%) than in the presence (37%) of Zn ions (Dimkpa et al., 2017a). Concomitant with direct effects on crop productivity, water limitation also negatively affects nutrient acquisition by plants as a result of impaired soil processes, including reduced nutrient mobility within soil, increased soil pH, and decreased soil organic matter levels (He and Dijkstra, 2014; Moreno-Jiménez et al., 2019). Drought-induced reductions in shoot uptake of N (43%) and K (38%) in soybean were recovered 28% and 19%, respectively, upon fertilization with mixtures of Zn and other micronutrients (Dimkpa et al., 2017a). Not surprisingly, the yield increase reported with Zn application in wheat by Karim et al., (2012) occurred with increased grain accumulation of Zn (29%) relative to plants not amended with Zn. Indeed, fertilization has been recognized as a strategy for achieving fortification of edible plant tissues with Zn, a nutrient that is critically lacking in human diets around the world due to low Zn stocks in agricultural soils (Joy et al., 2015; Oliver and Gregory, 2015) that is worsened in dry soils (Moreno-Jiménez et al., 2019). Accordingly, grain Zn contents can be significantly impacted by drought, which could depend on severity and crop species. However, as discussed above, this can be improved by Zn fertilization under drought conditions. Therefore, Zn induction of tolerance to drought stress in plants can result in multiple benefits including yield improvement, enhancement in nutritional value of crop produce, and better nutrient management.

Nanoparticles (≤100 nm in at least one dimension) of micronutrients are increasingly being assessed as fertilizers for quantitative and qualitative crop improvement (Tolaymat et al., 2017; Dimkpa and Bindraban, 2018; White and Gardea-Torresdey, 2018). Nanoparticles are more reactive than their bulk scale counterparts due mainly to the possession of enhanced surface area and the potential to engineer specific properties through coatings and/or functionalization to enhance nutrient delivery. Evidence from both short and full-term crop growth studies indicate that nanoparticles of micronutrients can facilitate the protection of crops against abiotic stresses such as drought; however, outcomes can be similar as those evoked by ionic micronutrients (Dimkpa et al., 2017a). In a short-term, wheat development and metabolism under drought stress in a sand matrix was positively altered (viz, increased lignification, modified root morphology, increased nitric oxide production, gene expression, and rigid shoot with high water content) due to synergistic effects between ZnO or CuO nanoparticles and a drought tolerance-imparting soil microbe, *Pseudomonas chlororaphis* O6 (Jacobson et al., 2018; Yang et al., 2018). Similarly, in a full-term study, foliar application of a composite nano-formulation of ZnO, CuO and B_2O_3 significantly enhanced soybean biomass growth, grain yield and uptake of macro and micro nutrients under drought stress (Dimkpa et al., 2017a).

Whereas the sole role of Zn salts (ions) in mitigating drought stress in plants has been demonstrated, for example, in the studies of Bagci et al., (2007) and Karim et al., (2012) noted above, relatively little is known when presented in nanoscale form, particularly throughout the crop's growth cycle. Moreover, compared to other cereals such as wheat, rice and maize, sorghum is relatively understudied with respect to Zn fertilization with nanoparticles. Although ZnO nanoparticles have been demonstrated to be phytotoxic; studies indicating such toxicity have been conducted with high doses (up to 500 mg/kg) of the nanoparticles, and in some cases in non-soil media where the modulating effects of soil chemistry are precluded (e.g., Dimkpa et al. 2012a, 2013; Watson et al. 2014). In studies conducted in soil at reasonable doses of Zn from ZnO nanoparticles, no toxic effects were observed (Raliya et al. 2016; Dimkpa et al. 2017b, 2018, 2019; Zhang et al. 2018). Even, at high doses (up to 500 mg/kg), phytotoxic effects were not observed with ZnO nanoparticles, depending on soil property (Priester et al. 2012; Wang et al. 2013; Watson et al. 2014). Thus, it was previously demonstrated in a system devoid of drought stress that grain yield, shoot Zn accumulation and grain translocation, and accumulation of N and K are positively affected by fertilization with ZnO nanoparticles via soil and foliar treatments (Dimkpa et al., 2017b). This suggest that fertilization with ZnO nanoparticles could be a useful nutrient management tool for the production of sorghum and other crops, as well as for grain quality improvement for human health, that could be particularly suited for adverse environmental production conditions. Therefore, the objectives of the present study were to (i) evaluate the effect of drought on the vegetative and reproductive performance and nutrient acquisition in sorghum; and (ii) assess the role of Zn as ZnO nanoparticles in modulating sorghum performance, nutrient acquisition and tissue partitioning, and grain fortification with Zn under drought stress.

2 Materials and methods

2. 1. Chemicals and soil

The ZnO nanoparticle (18 nm) product used in this study was purchased from US Research Nanomaterials, Inc., Houston, Texas. As the present study was conducted in soil, the ZnO nanopowder was used ‘‘as is’’, without any characterization. This is due to the complexity of soil as a natural growth medium, the likelihood that nanoparticle properties change dramatically upon addition to soil, as well as the current inability to characterize nano-scale structures in soil. For instance, the presence of natural nano-size colloids in soil will obfuscate the interpretation of nanoparticle characterization, relative to in vitro characterization in a pure aqueous system. The soil used for the study was collected from Plains, Texas. It is a sandy loam with a very low organic matter content of 0.92%; a pH of 6.87, and a bioavailable (DTPA-extractable) Zn level of 0.1 mg/kg, indicating a Zn status well below the critical soil level for most crops, 0.5-1.0 mg/kg. The levels of bioavailable inorganic nitrogen (N), phosphorus (P), and potassium (K) in the soil were 4.0 (low), 2.05 (low), and 246 (0.63 cmol/kg) (high) mg/kg, respectively. However, the total N (inorganic + organic [and thus, immediately nonbioavailable]) level was high, 400 mg/kg soil (Dimkpa et al., 2019). Triplicate pots per treatment were filled with 8 kg of the dry soil and basally amended with P (from mono calcium phosphate) and K (from sulfate of potash) at 100 and 275 mg/kg soil, respectively. Thereafter, N and ZnO nanoparticles were amended into the soil based on treatments outlined below. A single urea briquette (1.68 g) containing ≈ 0.78 g N (briquette weight x 46.4% N/100) was added per pot as the N source. As such, each 8-kg potted soil received ≈ 98 mg N/kg soil. Three concentrations of the ZnO nanoparticles, corresponding to 1, 3, and 5 mg Zn/kg soil, were added to designated pots as treatments. Prior to deep placing the urea briquettes, the pots were tumbled on a mechanical mixer to facilitate uniform distribution of the nanoparticles into the soil. A control treatment lacked the ZnO nanoparticle amendment.

2.2. Plant growth conditions

A greenhouse pot experiment with sorghum (*Sorghum bicolor* var. 251) was conducted in Muscle Shoals, Alabama (34.7448° N, 87.6675° W). Greenhouse conditions were as follows: average temperature, 30 °C; relative humidity, 44-96%; and daylight duration, 13-15 h. Three sorghum seeds were planted per pot, which was thinned down to one post seedling emergence. Approximately one month after sowing, the plantlets were exposed to drought condition. To this end, a control treatment without Zn amendment and three treatments with Zn at 1, 3, and 5 mg/kg were subjected to drought stress based on sustained maintenance of soil moisture at 40% of the field moisture capacity (FMC). FMC was determined by saturating 8-kg potted test soil without plants with water, and letting the water drain off completely over time. Thereafter, the pot was weighed, and 80% of the amount of water retained in the potted was taken as the non-drought condition for this study. Half of the amount of water considered as non-drought condition was taken as the drought condition (see also Dimkpa et al., 2017a). Thus, 40% FMC represented the drought (D) condition used and was maintained throughout the duration of the study by periodic weighing of the pots and manually adjusting the amount of water added to the pots. A separate treatment of ZnO nanoparticle-amended plants at 3 mg/kg was provided with adequate water at 80% FMC to serve as the non-drought (ND) condition control. This non-drought condition was maintained by an automated watering regime via drip irrigation. Subsequently, vegetative growth (tiller number, panicle number, and plant dry biomass) and reproductive (grain) yield were monitored. Upon maturity, plants were harvested and separated into root, shoot, and grain. The separated plant tissues were oven-dried (60^{O} C) until constant weight, ground into powder using a Model 4 Thomas Wiley Laboratory Mill (Pennsylvania, U.S.A.) for shoot and root, or a Zn 100 Retsch grinder (Retsch GmbH, Haan, Germany) for the grain.

2.3. Plant analyses

A subset of ground tissue was acid-digested (3 mL sulfuric acid + 1 mL H_2O_2), heated for one h at 350 °C and cooled to ambient temperature, before being equilibrated with d-H_2O and subjected to the following analytical procedures to determine elemental content: Skalar segmented flow analysis for N and P, and inductively coupled plasma-optical emission spectroscopy (ICP-OES; model Spectro Arcos, SPECTRO Analytical Instruments GmbH, Kleve, Germany) for K, Ca, Mg, Zn, and Fe. In addition, total S analysis with another subset of the ground plant tissues was performed using a nitric and perchloric acid (5 mL; 1:1, v: v) digestion that oxidizes the plant S to sulfate (SO_4) (Blanchar et al., 1965). Digestion was done for 1 hr. at 150 ºC, followed by capping with a funnel, and increasing the temp to 200 ºC until only 1 mL of solution is left. The concentration of S in the digested solution was then determined by means of ICP-OES. For quality assurance (QA) and quality control (QC), standard operational procedures were followed in these analyses, including calibration and standardization, quality laboratory water, replicate analyses, and sample spiking. Standards were used as QC samples to verify method appropriateness for sample handling. In all cases, the matrix solution (without the sample under investigation) is used as blank, to provide information on contamination levels of the test elements from non-sample sources. All blanks were below the detection limit and there was no loss of the test elements to laboratory glassware.

2.4. Data Analysis

A two-way analysis of variance (ANOVA; OriginPro 2018) was used to determine significant differences in crop response to treatment and block effects for each parameter, including plant vegetative and reproductive production, and nutrient content in plant tissues. A Fisher LSD means comparison was performed to further explore the differences with significant ($p < 0.05$) ANOVA.

3 Results and Discussion

3.1. ZnO nanoparticles regulate sorghum phenological development under drought stress

Provision of adequate water at 80% FMC and amendment of the soil with ZnO nanoparticles (3 mg Zn/kg) promoted early plant development. Under this non-drought condition, the emergence of the flag leaf (i.e., the top-most leaf on the stem of a cereal crop critical for grain filling) was observed 43 days after planting (DAP). However, under drought stress, this duration was significantly extended to 59 DAP without Zn amendment, but Zn amendment shortened the duration to 47 DAP, which was not significantly different from the non-drought condition (Fig. 1 A). Similarly, in the non-drought control, the grain head was already fully formed 46 DAP. In contrast, grain head formation was significantly delayed, and had not emerged 63 DAP in the drought-affected plants. Conversely, when amended with Zn under drought, grain heads emerged 51 DAP, which was significantly different from other treatments (Fig. 1 A). Fig. 1 B shows a representative image of plants under drought and non-drought growth condition, with and without

Zn amendment, whereby the differential emergence of the grain head is visually evident. Thus, whereas drought caused a delay in sorghum phenological development, the addition of Zn to a Zn-deficient soil mitigated this inhibition of phenological development. A prior study in soil whose Zn status was undetermined indicated only modest effect of Zn salt (2-4 kg/ha; ≈ 1-2 mg/kg) in shortening time to flowering in sorghum (Bhoya et al., 2014) under adequate water regimen. Similarly, Sher et al., (2018) reported that Zn salt (1-3 kg/ha) did not affect the number of days to anthesis in wheat grown in soil that contained 0.17 mg/kg Zn. However, the authors did report significant effect of N and Zn interaction on time to anthesis. In the present study, N was not under investigation and thus, was supplied to all treatments. As such, the effects of its interaction with Zn on sorghum phenological development could not be evaluated. Mechanistically, it is conceivable that drought stimulated the production of hormones in the root, such as indole acetic acid (IAA), that modulate root growth for improved adaptation to drought stress (discussed in Defez et al., 2017). However, although IAA is generally known to prolong plant vegetative growth, ZnO nanoparticles exposure could remodel this effect by lowering plant IAA levels, as reported for bacterial IAA (Dimkpa et al., 2012b), while potentially stimulating ABA production. Hence, reproductive development of sorghum was facilitated in the presence of ZnO nanoparticles. Taken together, our findings highlight the potential of ZnO nanoparticles to facilitate plant phenological development under the stress of drought. Our findings show that although Zn did not completely reverse the developmental inhibition associated with drought, the negative impacts of water stress were significantly alleviated by its amendment. Future investigations will be focused on understanding the developmental mechanisms driving ZnO nanoparticles effects under drought stress, in comparison with other Zn types.

3.2. ZnO nanoparticles improve sorghum vegetative and reproductive growth under drought stress

Drought significantly ($p<0.05$) reduced above-ground vegetative and reproductive parameters. Tiller number, panicle number, shoot biomass and grain yield were all reduced due to drought stress. Notably, shoot biomass and grain yield were, respectively, lowered by as much as 39% and 76% under water stress. In contrast to these above-ground endpoints, there was no significant effect of drought on root biomass, presumably due to mitigating affects from hormonal (IAA) regulation (Table 1). Importantly, amendment of Zn under drought resulted in a dose-independent alleviation of drought effects on tiller (65-85%) and panicle (59-76%) production, as well as a dose-independent mitigation of drought-induced inhibition of shoot biomass (17-24%). Furthermore, grain yield showed a dose-dependent response to Zn amendment under drought; compared to the drought control, yield was significantly higher with Zn amendment at 1, 3, and 5 mg/kg by 22, 64, and 183%, respectively. Indeed, ZnO nanoparticles at 5 mg Zn/kg reversed yield loss due to drought from 76% to 33%. In contrast to shoot tissues, root biomass production under drought was increased by ZnO nanoparticles, especially at the higher doses where biomasses were significantly higher than both the non-drought condition and the control (Table 1). Again, we note that hormonal regulation may have been driving this effect of increased root biomass due to Zn application, to mitigate drought stress. Bhoya et al. (2014) previously reported that sorghum shoot biomass was significantly increased by fertilization with Zn salt (ions). In contrast, in our previous study involving the same soil as the present one but under non-drought conditions, Zn ions, but not ZnO nanoparticles, increased sorghum shoot biomass (Dimkpa et al., 2017b). As noted earlier, drought is one of the most important climatic events affecting crop productivity and production. Collectively, our findings here are significant given the utility of using ZnO nanoparticles as a strategy for mitigating drought effects in sorghum production, for both human food and animal feed.

3.3. ZnO nanoparticles improve nitrogen acquisition by sorghum under drought stress

The accumulation of N by sorghum roots from soil was unaffected by drought but importantly, was enhanced by ZnO nanoparticles under drought stress (Fig. 2). Root N accumulation under drought was significantly greater with Zn amendment, compared to both the non-drought (37-62%) and control (24-46%) treatments, except for the 3 mg/kg Zn which was not significantly different from the control. In contrast to root, N content in the shoot was unaffected by both drought and ZnO nanoparticle treatments. However, translocation of N from shoot to grain was significantly inhibited (56%) by drought, compared to the non-drought control. Notably, ZnO nanoparticle amendment at the highest concentration (5 mg/kg Zn) under drought significantly increased (84%) grain N content compared to the control, allowing for a 36% mitigation of drought-induced inhibition of grain N translocation by drought. Ultimately, the increased grain N content at the 5 mg/kg ZnO amendment level resulted in a significantly higher total (root + shoot + grain) N content than other drought treatments; levels were statistically comparable to the non-drought control (Fig. 2). These results support our previous observations for sorghum under non-drought conditions, where shoot accumulation of N was largely unaffected by ZnO nanoparticles amendment, whereas grain content and total N accumulation were significantly enhanced (Dimkpa et al., 2017b). Nitrogen is known to be preferentially allocated into the grain of cereal crops, including wheat (Dimkpa et al., 2018) and maize (Chen et al., 2015). Similarly, more N was reported to also be allocated to sorghum grain than to the shoot (Dimkpa et al., 2017b), although data for root N partitioning was not measured in that previous study, precluding an assessment of overall nitrogen dynamics in this species. In the present study, the overall distribution of N in sorghum roots, shoots and grain was inconsistent with observations in wheat, where significantly more N was partitioned in the grain, with lower levels of N being present in the roots and shoots

(Dimkpa et al., 2018). Here, in the non-drought control plants, N levels were 81% and 69% greater in sorghum shoot than root, and grain than shoot, respectively. In contrast, under drought, 94% more N was partitioned in the shoot than root and 37% less N was present in the grain than shoot, respectively. This shoot-grain allocation pattern under drought contrasts with our previous study when measured under adequate water supply (Dimkpa et al., 2017b). The current data indicate that lack of adequate water in the sorghum xylem stream transporting soil-acquired nutrients strongly affects shoot to grain N translocation, whereas the effect on N mobilization from root to shoot is less impacted. However, grain N translocation is also affected by reduced grain sink size (panicle number and grain yield). Taken together, the extent to which N partitions in sorghum grain appears to be strongly dependent on the water regime, as has been noted for other crops (Devries et al., 1989). Importantly, the presence of ZnO nanoparticles, and Zn ions for that matter, did not alter the pattern of shoot-grain N allocation in sorghum under adequate water availability, or in soybean under drought (Dimkpa et al., 2017a, b). In contrast, the pattern of shoot-grain N allocation was altered in wheat by ZnO nanoparticles (Dimkpa et al., 2018). Here, while ZnO nanoparticles at 5 mg/kg significantly affected sorghum N shoot-to-grain remobilization under drought, with 37% more N contained in the grain than the shoot, the lower doses (1-3 mg/kg) did not exert this effect. Therefore, the degree to which ZnO nanoparticles influence N translocation to sorghum grain under drought will likely be dependent on dose, as well as perhaps other factors such as timing or Zn availability in the soil. Concerning overall N acquisition by the plants, it is well known that N use in agriculture is characterized by low efficiency (Hill et al., 2019). Notably, increased total N acquisition facilitated in this study by adequate soil moisture and ZnO nanoparticles (3 mg/kg), and by ZnO NPs alone under drought (5 mg/kg), has significant implications for N management and for the ability to minimize N losses in agricultural systems. Accordingly, N recovery efficiency

(RE) as a function of ZnO nanoparticle amendment was determined. The data show that the combination of adequate water and ZnO (3 mg/kg) resulted in a RE (= $N_{t\,(+Zn)} - N_{t\,(-Zn)})/N_p$ x 100; where N_t = total N, and N_p = total N amount per pot) of 19%, while lower REs of 4.5% and 4.7%, respectively, were obtained with 1 and 3 mg/kg Zn application under drought. In contrast, at 5 mg/kg, Zn under drought completely restored the RE back to 19%. Thus, Zn at relatively high application rates can compensate for the reduced N recovery efficiency under drought conditions. A recent study estimated that 4300 human deaths, as well as associated economic losses of $39 billion, occur annually in the U.S. due to air pollution associated with ammonia released from N in intensively fertilized corn fields (Hill et al., 2019). The authors noted that reducing N application rates or devising strategies to increase N uptake efficiency by plants are important interventions likely to reduce these negative impacts. Previously, it was shown under adequate water supply that application of ZnO nanoparticles (6 mg Zn/kg) increased overall N accumulation in sorghum by 8% and 16% under N application rates of 100 and 200 mg/kg, respectively (Dimkpa et al., 2017b). Here, under drought, ZnO nanoparticles increased overall N accumulation in this plant between 7% (insignificant) and 28% (significant) when N is applied at 98 mg/kg soil. Investigations evaluating the potential of ZnO nanoparticles to enhance N use efficiency and yield in a variety of crops under drought at lower N application rates (<100 mg/kg) are warranted, particularly in Zn-deficient soils. Nevertheless, the current findings suggest that enhancing N acquisition via ZnO nanoparticle amendment is a straightforward intervention with strong potential to promote N use efficiency in crops. Such efforts could significantly mitigate some risk to humans and the environment associated with large-scale N-fertilizer use in agriculture.

3.4. ZnO nanoparticles impede sorghum acquisition of phosphorus under drought stress

Root accumulation of P was unaffected by drought but ZnO nanoparticles under drought significantly increased P acquisition in the root, regardless of dose; increases ranged between 52 and 80%. In contrast, translocation of P from roots to shoots was significantly enhanced (39%) under drought, relative to the non-drought control. Conversely, shoot P content was significantly reduced (37-52%) upon ZnO nanoparticle amendment under drought condition; the reduction was dose dependent (Fig. 3). The translocation of P to the grain under drought imposition differed from shoot content; drought reduced grain P content. Importantly, this outcome was mitigated by Zn exposure at the highest level, where grain P content was only 27% less than in the non-drought treatment, whereas other drought treatments had grain P contents that were 58-70% less. The overall pattern of P root-shoot-grain distribution resulted in the highest level of total plant P occurring in the non-drought and drought control treatments (Fig. 3). This suggests that drought alone had little or no effect on the overall acquisition of P by sorghum. However, the presence of Zn under drought significantly lowered total P content in the plants. Overall, inhibition of P acquisition in plants with Zn amendment, which has been reported for most Zn types (salts, nanoparticle and bulk particles), is a phenomenon that has been widely observed both under adequate and limited water regimes (e.g., Dimkpa et al., 2017a,b, 2018, 2019). Therefore, the present drought-focused study further confirms the strong role of ZnO nanoparticles in this regard, given that the control plants lacking adequate water contained higher shoot P than the non-drought plants amended with Zn from ZnO nanoparticles. Whereas adequate water availability in soil should have facilitated P mobilization in the non-drought treatment (He and Dijkstra 2014.), the presence of Zn in this treatment appeared to inhibit this process. It seems plausible that under drought condition, the potential for the formation of Zn-phosphate resulted in immobilization and potentially in accumulation on or in the root, effectively reducing the availability of this nutrient for shoot uptake. On the other hand, moisture enhances the complexation of P in soil aggregates (Li et al., 2016) which, ostensibly, allows for more interaction with Zn in soil, creating conditions for the formation of Zn-phosphate. Accordingly, P uptake into shoot was affected in the presence of Zn, regardless of the water status of the plants. In a previous study, the ratio of shoot-grain P in sorghum in the absence of drought and Zn depended on the concentration of P: at a ''low'' P level (50 mg/kg), the ratio was 1, and at a ''high'' P concentration (100 mg/kg), the ratio was 2.5 (Dimkpa et al., 2017a). Here, at a P rate of 100 mg/kg, the ratio of shoot-grain P under drought stress is 1.6, which clearly demonstrates the need for adequate water to remobilize P to reproductive tissues (grain, seed). Interestingly, although shoot-to-grain P translocation was unaffected under drought stress upon exposure to low Zn concentrations, the grain P content was increased at the highest Zn level by an average of 102%, relative to lower Zn treatments. This indicates a dose effect of Zn on P translocation and agrees with a previous report showing high grain P content at 6 mg/kg ZnO nanoparticle exposure, despite the inhibition of shoot P content (Dimkpa et al., 2017a). Notably, unlike ZnO nanoparticles, Zn salt at same exposure level from soil did not enhance P grain translocation in sorghum in a previous study (Dimkpa et al., 2017a). Whether ZnO nanoparticles at certain doses uniquely induces pathways related to P mobilization to sorghum grains remains unknown. Nevertheless, the overall pattern of P distribution in the different sorghum organs that was observed, specifically with more P partitioned in the shoot than in the root or grain, was different from the pattern in rice and wheat, where the vast majority of plant-internalized P was distributed to the grain (Ye et al., 2014; Dimkpa et al., 2018). Perhaps not surprisingly, this suggests species differences in above-ground tissue P partitioning among grain crops. The inhibition of P acquisition in fertilized systems by Zn has two contrasting implications for the environment and human health. On the one hand, it is a potential impediment to soil P management efforts, as significant amount of P is left in soil rather than taken up by plans. Notably, however, Zn application in unfertilized soils has been reported to facilitate the mobilization of legacy P by enhancing the populations and activities of P-solubilizing soil microbes (Raliya et al., 2016). On the other hand, it might be beneficial in terms of reducing the formation of Zn-phytate and increasing available Zn in grains (Dimkpa et al., 2019). Zn-phytate is an insoluble Zn complex that lowers the bioavailability of Zn for humans.

3.5. ZnO nanoparticles promote acquisition of potassium by sorghum under drought stress

As with N and P, drought did not affect K uptake into the root; however, fertilization with ZnO nanoparticles under drought significantly increased root K uptake in a dose-independent manner; increases were between 29% and 56%. In contrast to root tissues, drought strongly inhibited (45%) shoot K content and amendment with ZnO nanoparticles did not affect this reduction. As a result, grain translocation of K was significantly higher in the non-drought treatment, relative to the drought control and the drought treatments with Zn amendment (Fig. 4). Notably, amendment with ZnO nanoparticles under drought significantly increased grain K content, but only at the highest dose. Calculating the total plant K content in all three plant organs shows that drought significantly reduced K acquisition, but that this negative impact was mitigated by ZnO nanoparticle amendment, particularly at the 1 and 5 mg/Kg treatment level (Fig. 4). The majority of K in the sorghum plant was partitioned to the shoot tissue; this agrees with prior findings in sorghum under non-drought stress (Dimkpa et al., 2017b), as well as with previous observations in soybean (under drought) and wheat (under adequate water availability) (Dimkpa et al., 2017a, 2018). The greater presence of K in the plant shoot relative to other tissues is not surprising, given its direct role in regulating photosynthesis and CO_2 exchange in the leaf. However, it is notable that neither drought nor ZnO nanoparticle amendment altered the distribution of K in sorghum. ZnO nanoparticles have been consistently reported to enhance overall K accumulation in different species, including wheat, bean, and sorghum (Medina-Velo et al., 2017; Dimkpa et al., 2017b). Moreover, in studies involving the omission of Zn (Zn ions, ZnO nanoparticles, and ZnO bulk), efficient accumulation of K was negated in rice and soybean plants (Dash et al., 2015; Dimkpa et al., 2019). These findings indicate that the stimulation of K accumulation in plants by Zn occurs irrespective of Zn type. Clearly, the dissolution of ZnO nanoparticles results in the release of divalent Zn ions. Once in the root, divalent metal ions could alter potential across the plasma membrane, thereby facilitating the uptake of monovalent cations such as K (Haynes, 1980).

3.6. Drought reduces sorghum acquisition of zinc at low Zn exposure

After plant harvest, the soil pH was reduced to 6.24 under the non-drought condition; to 6.22 in the drought condition without Zn amendment; and to between 6.11 and 6.29 in the drought conditions with Zn amendment. Thus, it appears that ZnO nanoparticle amendment did not by itself influence soil pH to regulate Zn accumulation by the plant. Rather, plant root exudates, likely organic acids, lowered soil pH, which potentially facilitated Zn uptake. As expected, root Zn content was significantly higher (46%) in the non-drought than in the drought treatment lacking Zn amendment. This is due to both the presence of added Zn, as well as adequate moisture which facilitated Zn transport into the plant. Similarly, among the drought-affected plants, root Zn content was significantly increased by Zn amendment. The highest root Zn level was recorded at the highest application dose of 5 mg/kg, which was significantly higher than the non-drought treatment (Fig. 5). Translocation of Zn from root to shoot presented a different scenario, with the effect of water being highly pronounced. Shoot Zn was significantly reduced (88%) in the drought control treatment, followed similarly by the Zn treatments under drought, 74-78%. Collectively, the level of shoot Zn in the non-drought treatment with Zn application was 307% higher than the drought treatments with Zn application. Accordingly, grain Zn content was greatest in the presence of adequate water, but notably, the reduction of grain Zn under drought stress was significantly mitigated by Zn fertilization at all treatment levels, especially at the highest dose. Zinc accumulation in the different plant organs resulted in a total Zn content in the plant that was significantly greater at the highest Zn fertilization under drought, followed by the non-drought treatment, low Zn treatments, and the control (Fig. 5).

In our previous study in this soil involving sorghum grown under non-drought condition, the control treatment without Zn amendment contained up to 0.6 mg Zn/plant (above-ground tissues) (Dimkpa et al. 2017b). Here, we show that the above-ground Zn amount in drought-stressed sorghum without Zn amendment is 0.29 mg/plant. Notably, this 50% reduction as a result of drought could be mitigated by the application of Zn under drought condition. Nevertheless, above-ground tissue Zn bioaccumulation efficiency, based on the ratio of [shoot + grain] to root Zn, was compromised under drought conditions with Zn amendment. The lowest efficiency of 30% was found with 5 mg/kg Zn, while efficiencies of 69% and 74% were found with Zn at 1 and 3 mg/kg, respectively. In contrast, above-ground tissue Zn bioaccumulation efficiency increased to 88% in the drought control treatment lacking Zn addition, and perhaps not surprisingly, to 349% under non-drought condition with Zn addition. These findings indicate that (i) adequate soil moisture is an important factor controlling Zn uptake from nanoparticles into above-ground tissues; (ii) drought-stressed plants deficient in Zn can apparently access mechanisms to improve Zn transfer from root; and that (iii) excessive root Zn may inhibit the efficiency of Zn uptake, ostensibly to prevent Zn toxicity in the shoot tissues. In contrast to root-to-above-ground Zn transfer, grain Zn translocation (i.e., ratio of grain to shoot Zn) was inefficient under non-drought conditions; under drought, the highest efficiency was recorded at the highest Zn level. Grain Zn translocation efficiencies were 32% for the non-drought treatment, 49% for drought control, 46% for Zn at 1 mg/kg, 39% for Zn at 3 mg/kg, and 84% for Zn at 5 mg/kg. The later value implies that under drought, high shoot Zn may be required to maximize grain Zn content. Furthermore, by specifically comparing the translocation efficiency between the non-drought with 3 mg/kg Zn and the drought condition with same amount of Zn, it is evident that water was more important in driving Zn uptake from root to shoot than it was for translocation to the grain. The higher grain Zn translocation efficiency in the drought control than the non-drought treatment with Zn, or even the drought treatments with lower Zn exposure, can be related to the enhanced Zn remobilization capacity of Zn ions by plants under Zn deficiency (Dimkpa et al., 2018, 2019). Our results indicate that presenting Zn to plants as ZnO nanoparticles is unlikely to significantly alter grain Zn remobilization dynamics due to the high dissolution rate of ZnO nanoparticles in the rhizosphere prior to plant acquisition by (Wang et al., 2013).

3.7. Drought and Zn fertilization differentially influence nutrient concentrations in sorghum grain

Based on the observed effects of drought and ZnO nanoparticles on shoot and grain Zn acquisition, the grain concentrations of N, P, K, as well as secondary and micronutrients critical for human health, namely Zn, iron (Fe), calcium (Ca), magnesium (Mg), and sulfur (S), were examined. Among the added nutrients, grain N and P concentrations were unaffected by drought; whereas grain K concentration was significantly reduced by drought (Table 2). The effect for Zn was expected, given that no Zn was applied in that treatment. Among the other nutrients, drought significantly reduced grain Fe and Mg concentrations; increased Ca concentration; and had no effect on S concentration (Table 2). Under drought stress, ZnO nanoparticle amendment did not significantly affect grain N and P concentrations; notably however, grain N concentration was higher in the ZnO nanoparticle treatments of 1 and 3 mg/kg, relative to the non-drought treatment.

In contrast, grain K concentration was significantly increased relative to the drought control, but this only occurred with the treatment of 3 mg/kg of Zn. increased grain. Under drought stress, Zn amendment at 1 mg/kg increased grain Fe concentration, compared to the control. The value was similar to Zn treatments at 3 mg/kg, regardless of soil moisture status. Notably, Zn amendment at 1 mg/kg mitigated drought effect on grain Fe. Grain Ca concentration was significantly reduced under drought by Zn treatments of 3 and 5 mg/kg; grain Mg concentration was increased by low Zn amendment, compared to other treatments; and grain S concentration was increased by Zn at 3 mg/kg, compared to other treatments (Table 2). These results indicate that drought has strong and mostly deleterious effect on sorghum grain nutritional content, but that Zn nanoparticle amendment can moderate many of these negative impacts. Apparently, biomass dilution due to drought did not play a substantial role in the concentrations of these nutrients, comparing the non-drought control and the drought control. Except for N and Ca, the drought control plant had lower grain yield and correspondingly lower (even if not significantly so in all cases) grain concentrations of P, K, Zn, Fe, Mg and S.

N, P, K, Zn, Fe, Mg, S, and Ca are the elements investigated in the current study for their grain contents as a function of drought and Zn exposure. Importantly, the concentrations of these nutrients in edible crop tissues have been reported to be reduced by elevated CO_2 as a function of climate change (Loladze, 2014; Myers et al., 2014; Smith and Myers, 2018). Drought is another climate-related event that can negatively impact grain concentrations of these nutrients (Ettiene et al., 2018). As demonstrated with soybean, Zn and B concentrations were reduced; N, P, K, Mg, Ca and S concentrations were unaffected; and Cu and Fe concentrations were increased (Dimkpa et al., 2017a). In the current study, sorghum grain N, P, and S were also unaffected by drought, whereas K, Zn, Fe, and Mg concentrations were strongly reduced by drought. In contrast, grain Ca was increased. Notably, the presence of Zn under drought stress had different degrees of positive (N, K, Zn, Fe, Mg, and S) or negative (Ca) outcomes for grain nutrient quality. The effect of Zn on the concentration of specific nutrients may be related to synergistic or antagonistic interactions orginating in the soil or at the root cell interface, and which depends strongly on nutrient ratios (Dimkpa et al., 2017b; Rietra et al., 2017). The current findings with sorghum, combined with the previous study in soybean (Dimkpa et al., 2017a), indicate that the effect of drought on grain nutritional quality can be quite deleterious; however, there likely is considerable variability based on crop species and it appears that Zn amendment can significantly moderate some of these impacts.

Zinc is one of the most commonly deficient nutrients in human diets globally; its deficiency in humans has been linked to Zn deficiency in soils, and correspondingly, in plants (Joy et al., 2015; Oliver and Gregory, 2015; Dimkpa and Bindraban 2016); a good example is the soil used in this study. Several studies conducted under non-drought conditions report improved grain Zn concentration in different crops upon exposure to ZnO nanoparticles (e.g., Subbaiah et al., 2016; Dimkpa et al., 2017b, 2018, 2019). A review of a number of human nutrition websites indicates that sorghum grain contains 3.2 mg Zn per serving of 192 g. It is unclear whether the sorghum plants were fertilized or not with Zn. However, that amount of Zn supplies only 22% of the daily recommended dietary allowance (RDA), leaving a deficit of 78%. In the current study, the average grain Zn concentration in the non-drought plants fertilized with Zn was 45 mg/kg grain; this is approximately 8.6 mg Zn per 192 g serving, and provides about 60% of the daily RDA. Importantly, drought imposition (with no Zn amendment) reduced Zn supply per sorghum serving to about 3.6 mg Zn (19 mg/1000 mg x 192 g), and thus, lowered the daily RDA to 36%. Importantly, under drought, amendment of ZnO nanoparticles (1-3-5 mg Zn/kg soil) increased the grain Zn content to between 6.9 and 7.3 mg Zn per 192 g serving, and hence, supply between 48 and 50.2% of the daily RDA. Taken together, this implies that grains from food crops grown under drought stress and fertilized with Zn could supply up to 50% of the daily human Zn RDA, representing a significant fortification of this important nutrient that could be critical for populations that depend on sorghum grain as a staple food. As noted above, many of these populations are coincidentally in areas most affected by Zn deficiency in soils and humans.

4. Conclusion

This study represents the first evidence of mitigation of drought stress in full-term plants solely by exposure to ZnO nanoparticles in soil. Overall, the findings of this study add to the growing body of knowledge on the effectiveness of Zn, specifically ZnO nanoparticles, in stimulating crop development, and increasing grain yield in cereal crops, while fortifying edible plant parts with Zn for human and animal nutrition. Importantly, under drought conditions where the availability and use of N, Zn and other essential nutrients by plants are impeded, supplemental Zn promoted the fortification of a number of important nutrients. The soil used in the current study has a very low organic matter content (Dimkpa et al., 2019). Soil organic matter can modulate micronutrient availability in both arid and non-arid soils (Medina-Velo et al., 2017; Moreno-Jiménez et al., 2019); this likely contributed in part to our findings. The role of organic matter in modulating ZnO nanoparticle use by plants in dry soils, and its implication for N use and crop yield are currently under investigation. Strategies that minimize N loss via increasing uptake by plants and without compromising yield are sorely needed. Herein, we demonstrate for the first time that the use of ZnO nanoparticles as a soil fertilizer amendment at judicious doses can increase the resilience of cropping systems to climate change events, increase the use efficiency of N, while promoting both the yield and Zn nutrition of crops under otherwise adverse production conditions. It is clear that the management of N and other nutrients must become much more efficient; efforts such as those described in this paper will be critical to agricultural sustainability and food and nutrition security.

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Table and Figure Legends

Table 1: Effects of drought and ZnO nanoparticles (1, 3, and 5 mg Zn/kg) on the vegetative and reproductive performance of sorghum. Values are means (g dry weight; dw) and standard deviations. Different letters after values indicate significant differences among treatments (rows), separately for each variable ($p < 0.05$; n = 3). D = drought; ND = non-drought.

Table 2: Effects of drought and ZnO nanoparticles (1, 3, and 5 mg Zn/kg) on sorghum grain concentrations of zinc, iron, calcium, magnesium and sulfur. Values are means and standard deviations. Different letters after values indicate significant differences among treatments, separately for each variable ($p < 0.05$; n = 3). D = drought; ND = non-drought.

Fig. 1: (A) Days to development of flag leaf and grain head (GH) in sorghum under drought stress and ZnO nanoparticle fertilization (3 mg Zn/kg). Bars on graphs are means and standard deviations. Different letters on bars indicate significant differences among treatments, separately for flag leaf and grain head ($p < 0.05$; n = 3). (B): Representative sorghum plants at 51 days after planting showing the influence of drought and ZnO nanoparticle fertilization (3 mg Zn/kg) on the development of grain head.

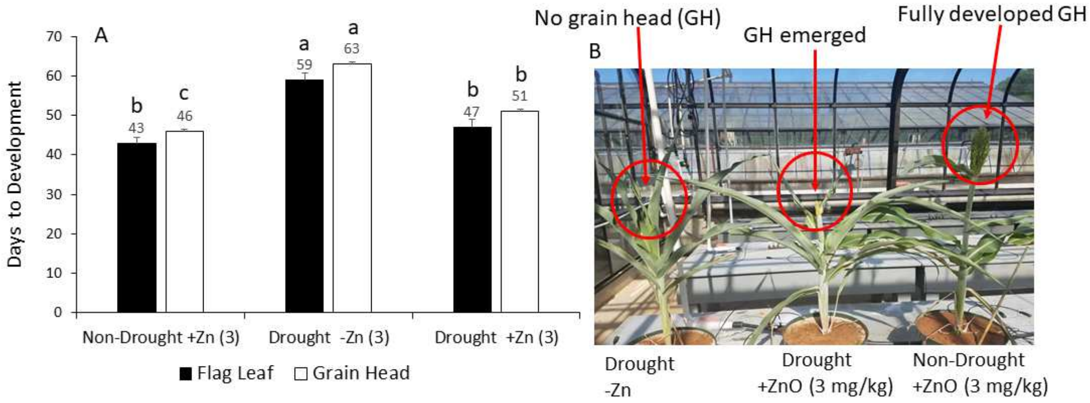
Fig. 2: Effects of drought and ZnO nanoparticles (1, 3, and 5 mg Zn/kg) on nitrogen acquisition and partitioning in sorghum plant organs (root, shoot and grain). Bars on graphs are means and

standard deviations. Different letters on bars indicate significant differences among treatments, separately for each organ and total acquisition ($p < 0.05$; n = 3). D = drought; ND = non-drought.

Fig. 3: Effects of drought and ZnO nanoparticles (1, 3, and 5 mg Zn/kg) on phosphorus acquisition and partitioning in sorghum plant organs (root, shoot and grain). Bars on graphs are means and standard deviations. Different letters on bars indicate significant differences among treatments, separately for each organ and total acquisition ($p < 0.05$; n = 3). D = drought; ND = non-drought.

Fig. 4: Effects of drought and ZnO nanoparticles (1, 3, and 5 mg Zn/kg) on potassium acquisition and partitioning in sorghum plant organs (root, shoot and grain). Bars on graphs are means and standard deviations. Different letters on bars indicate significant differences among treatments, separately for each organ and total acquisition ($p < 0.05$; n = 3). D = drought; ND = non-drought.

Fig. 5: Effects of drought and ZnO nanoparticles (1, 3, and 5 mg Zn/kg) on zinc acquisition and partitioning in sorghum plant organs (root, shoot and grain). Bars on graphs are means and standard deviations. Different letters on bars indicate significant differences among treatments, separately for each organ and total acquisition ($p < 0.05$; n = 3). D = drought; ND = non-drought.


A
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a
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a
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47
b
51
Non-Drought +Zn (3)
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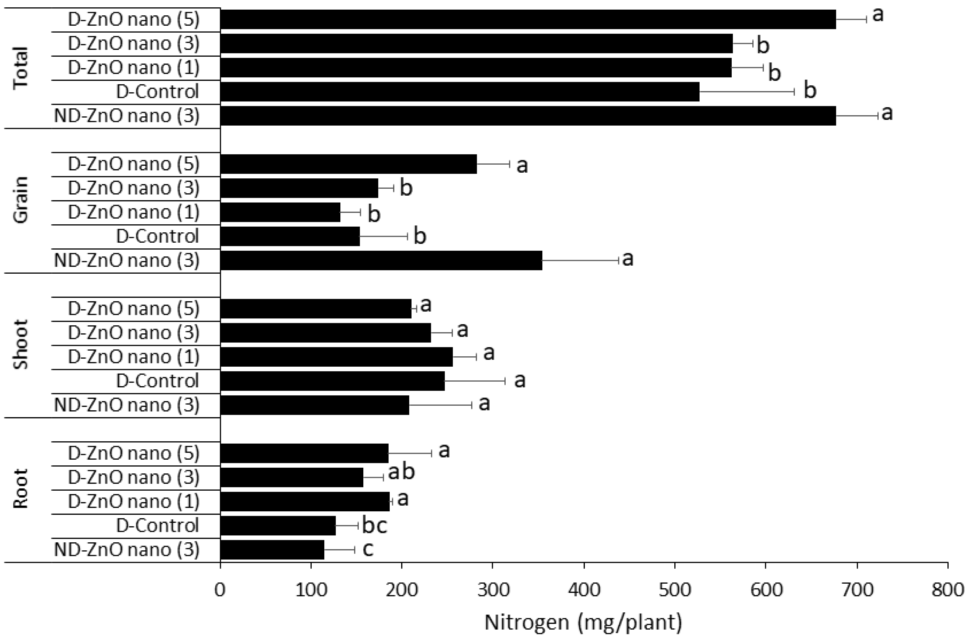
Drought +Zn (3)
■ Flag Leaf
☐ Grain Head
B
No grain head (GH)
GH emerged
Fully developed GH
Drought
-Zn
Drought
+ZnO (3 mg/kg)
Non-Drought
+ZnO (3 mg/kg)

Fig. 1


Total
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D-ZnO nano (3)
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ND-ZnO nano (3)
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Grain
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D-ZnO nano (3)
D-ZnO nano (1)
D-Control
ND-ZnO nano (3)
a
b
b
b
a
Shoot
D-ZnO nano (5)
D-ZnO nano (3)
D-ZnO nano (1)
D-Control
ND-ZnO nano (3)
a
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a
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a
Root
D-ZnO nano (5)
D-ZnO nano (3)
D-ZnO nano (1)
D-Control
ND-ZnO nano (3)
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100
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300
400
500
600
700
800
Nitrogen (mg/plant)

Fig. 2

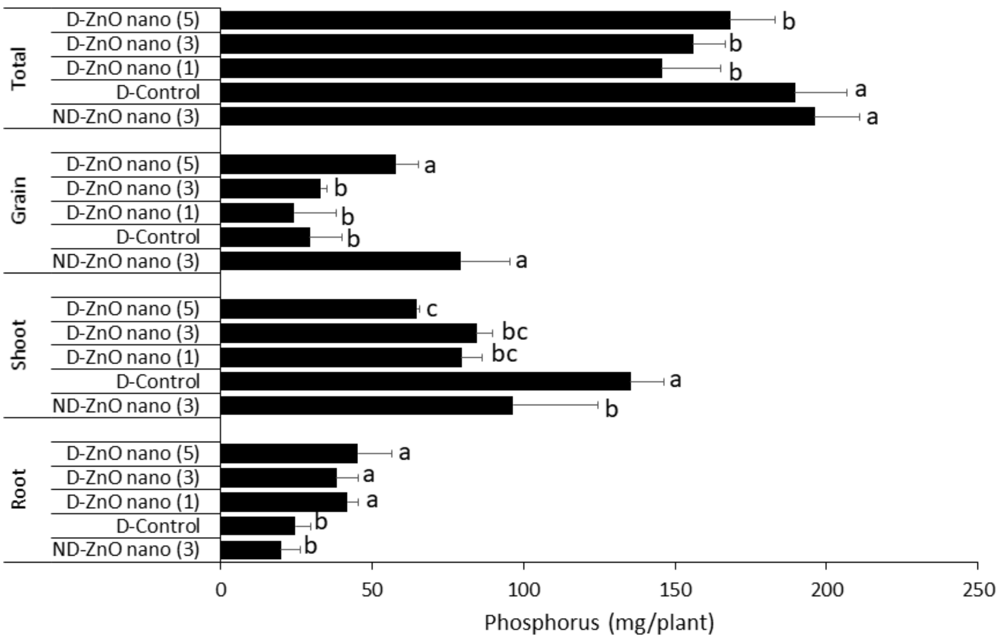


Figure 3

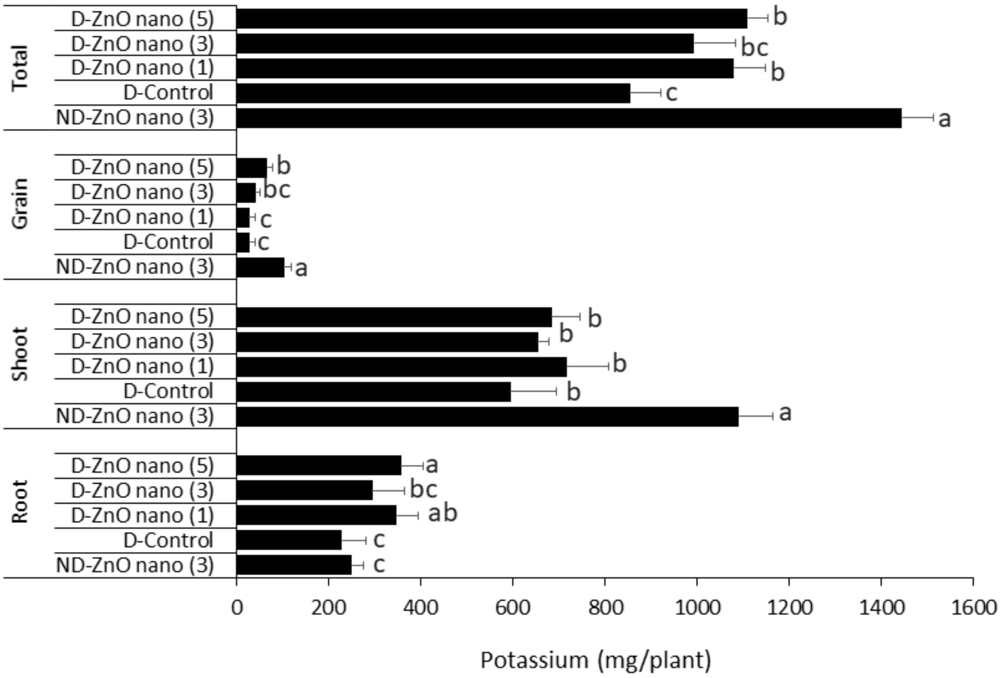
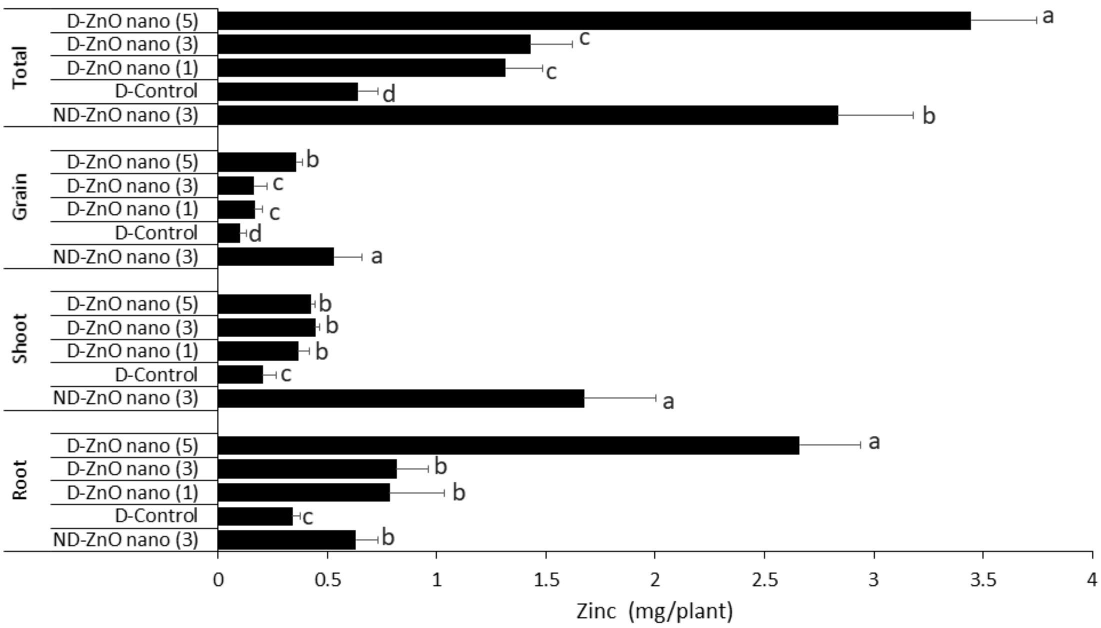


Figure 4


Total
Grain
Shoot
Root
D-ZnO nano (5)
D-ZnO nano (3)
D-ZnO nano (1)
D-Control
ND-ZnO nano (3)
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c
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b
c
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a
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c
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a
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c
b
0
0.5
1
1.5
2
2.5
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3.5
4
Zinc (mg/plant)

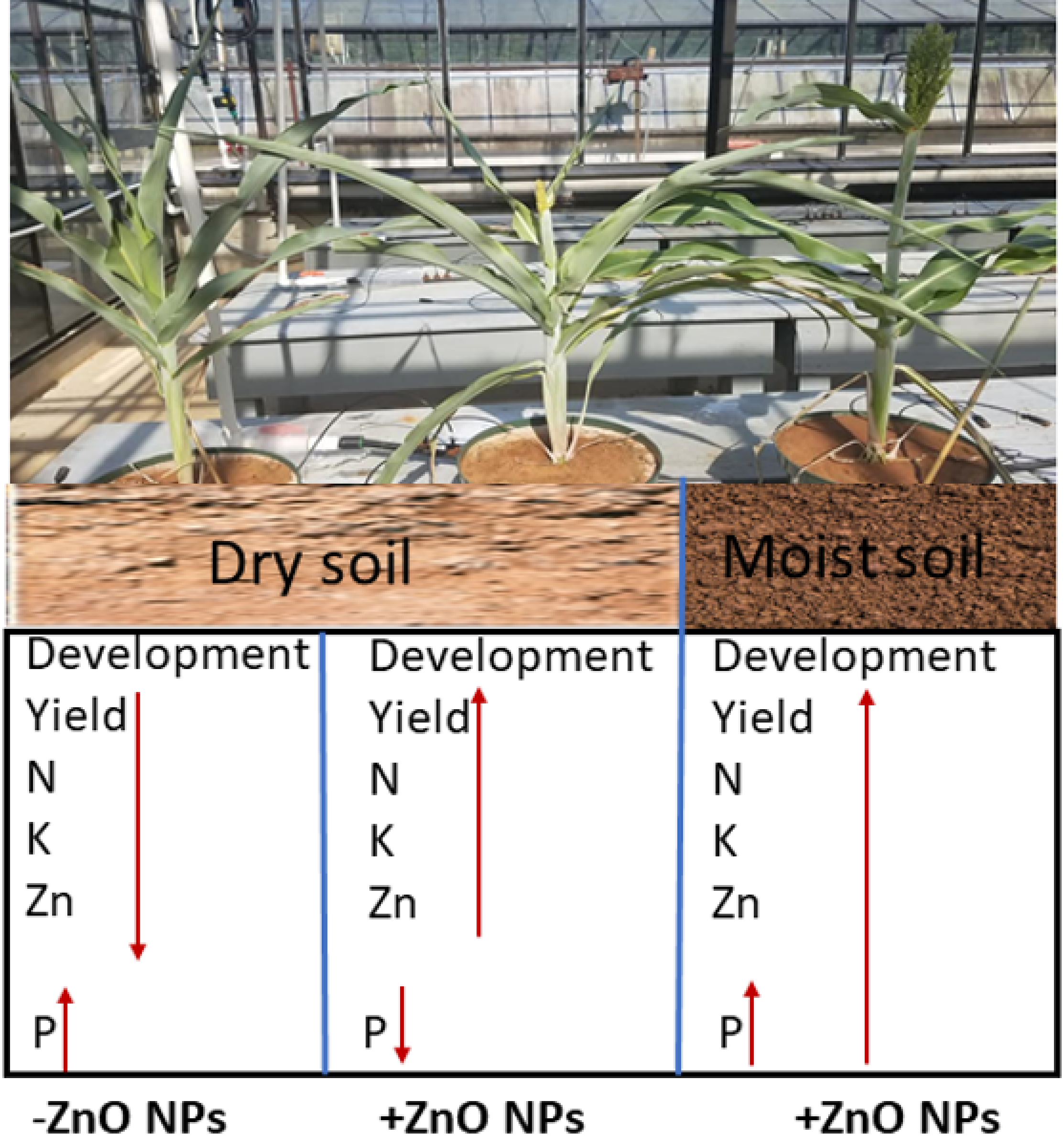
Figure 5

Table 1

Variable	Treatment				
	ND-ZnO nano (3)	D-Control	D-ZnO nano (1)	D-ZnO nano (3)	D-ZnO nano (5)
Tiller number	3.3 ± 0.6a	2.0 ± 1.0b	3.3 ± 0.6a	3.3 ± 0.6a	3.7 ± 0.6a
Panicle number	2.7 ± 0.6a	1.7 ± 0.6b	3.0 ± 0.0a	2.7 ± 0.6a	3.0 ± 0.0a
Shoot biomass (g dw)	38.6 ± 1.4a	23.6 ± 1.6c	27.7 ± 2.3b	29.2 ± 1.9b	27.6 ± 0.6b
Grain yield (g dw)	15.3 ± 6.1a	3.6 ± 2.7c	4.4 ± 2.8bc	5.9 ± 0.5b	10.2 ± 2.3a
Root biomass (g dw)	16.1 ± 2.4bc	14.9 ± 0.6c	26.5 ± 6.3abc	29.6 ± 3.4a	36.5 ± 13.9a

Table 2

Grain nutrient (mg/kg)	Treatment				
	ND-ZnO nano (3)	D-Control	D-ZnO nano (1)	D-ZnO nano (3)	D-ZnO nano (5)
N	27474 ± 1540b	29391 ± 1316ab	29953 ± 398a	30097 ± 988a	28261 ± 842ab
P	5685 ± 290a	5589. ± 108a	5756 ± 457a	5851 ± 280a	5715 ± 515a
K	7225 ± 189a	5694 ± 302b	6859 ± 1276ab	7220 ± 889a	6703 ± 332ab
Zn	44.7 ± 2.6a	19.0 ± 1.5c	37.0 ± 3.2b	38.0 ± 4.6b	35.6 ± 3.0b
Fe	48.5 ± 2.0a	43.3 ± 2.4c	47.5 ± 2.0ab	44.8 ± 2.1bc	42.7 ± 0.7c
Ca	171.9 ± 7.9bc	215.8 ± 13.9a	195.1 ± 11.7ab	174.0 ± 8.7bc	167.6 ± 20.2c
Mg	1697.8 ± 0.6ab	1586.8 ± 23.6c	1758.3 ± 84.0a	1671.8 ± 68.1ac	1628.9 ± 22.0bc
S	1648.1 ± 34.7ab	1608.1 ± 175.5b	1717.7 ± 102.3ab	1791.3 ± 44.5a	1638.9 ± 18.8b


Dry soil
Moist soil
Development
Yield
N
K
Zn
P
Development
Yield
N
K
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Development
Yield
N
K
Zn
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-ZnO NPs
+ZnO NPs
+ZnO NPs