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Changes in carbon partitioning in pearl millet (*Pennisetum glaucum*) and clusterbean (*Cyamopsis tetragonoloba*) in response to ZnO nanoparticle application

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There is growing interest to study the effect of nano-particles to break productivity barriers of agricultural and horticultural crops. But variations in the response of plants to nano-particles have been reported in the literature. The effects of nanomaterials on the plant growth and development were dependent upon the type of nanoparticles, concentration and plant species besides specific conditions of experiments (Gao *et al*. 2006, Hong *et al.* 2005, Ma *et al.* 2010). Nano-ZnO is being extensively used because of its ultraviolet scattering ability in cosmetics industries besides being used in semiconductors. So chances of its release in environment during industrial processing or dumping are high wherein nano-particles predominantly may get incorporated into sewage sludge and are subsequently applied to agricultural field. Both negative (Lin and Xing 2008) and positive (Adhikari *et al.* 2010) effects of ZnO nano-particles on roots have been reported. However, studies so far have focused on plant growth but there is no study to our best knowledge that describes the effect on carbon partitioning with emphasis on root exudates. We have therefore studied the effect of nano-ZnO solution as foliar spray on root exudates of important crop plants.

Plants were raised in 3 cm diameter glass columns filled with acid washed sand to 40 cm length having G-0 disc at the lower end and fitted with tap at the bottom. Outer surface of the column was covered with black paper followed by aluminum foil to prevent algal growth and to provide dark zone for normal root growth. Columns were vertically arranged on an iron stand as depicted in Fig 1.

Columns were initially leached with one-fourth strength of Hoagland solution (Hoagland and Arnon 1950). Two visibly uniform size pearl millet seeds [*Pennisetum glaucum* (L.) R. Br. emend Stuntz var CZP 9803] surface sterilized with 0.1% mercuric chloride and then soaked in double distilled water for 12 hours were placed at 2 cm depth from surface in the column and covered with moist sand. After seedling emergence, for first seven days, 50 mL Hoagland solution of one-fourth strength was added every day. The leachates were collected in a 100 mL capacity beaker placed at bottom of the column embedded in ice. The tap of the column was kept open. After seven days 50 mL Hoagland solution of complete strength was added and leachates collected every day**.** Leachates were kept in the deep freeze till analysis. Two weeks after emergence when the plants were at 5-6 leaf stage, 10 mL aqueous solution of 100 mg spherical nano-ZnO/L (prepared and characterized by IIT Mumbai of size range 16-30 nm) was sprayed under pressure in an illuminated enclosure to completely drench. The spray was repeated for

Fig 1 Experimental set up showing pearl millet and clusterbean grown in glass columns

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three consecutive days. Plants were then grown under natural conditions in a screen house for another three weeks**.** During this period Hoagland solution of complete strength was added every day and the exudates were collected in the manner as mentioned above. An identical set of plants sprayed with equal quantity of water plus surfactant served as control. After five weeks of growth plants were harvested washed under flowing deionized water, shoot and root separated, oven dried at 72°C for 48 hr and dry biomass recorded.

The level of lipid preoccupation was measured in control and nano ZnO sprayed leaves after seven days using the thiobarbituric acid assay. The TBARS content was calculated according to Heath and Packer (1968).

Harvested plant materials were thoroughly washed in distilled water and separated into stem and roots, and finally oven dried at 72°C. Dried plant materials were powdered and wet digested in a 9:1:4 mixture of nitric acid: sulphuric acid: perchloric acid at 160°C. Zn concentrations were determined using AAS. Carbon in plant and exudates was measured by wet oxidation method (Singh *et al.* 2005).

Similar experiment was also carried out with clusterbean [*Cyamopsis tetragonoloba* (L) Taub] var RGC 936. Six replications were maintained for each treatment. Statistical significance was accepted when the probability of the results assuming the null hypothesis (p) was less than 0.05.

All the plants grew normally and leaves did not show any deficiency or toxicity symptoms. Zn in shoot and root was higher in plants sprayed with nano-Zn compared to control (Table 1). Though ZnO is sparingly soluble in water (Windholz *et al.* 1983) but reducing particle size and pH can increase its solubility (Franklin *et al.* 2007). The pH of the nano-Zn solution in our experiment was 6.2. Due to low size and pH concentration of soluble Zn in the 100 mg nano-ZnO/ L used in our experiments was 1.7 mg/L. Thus, a part of nano-Zn could have moved through as solution possibly through stomata. Nano-ZnO may have also moved inside the cell possibly either by increasing permeability of the cell wall (Brayner *et al.* 2006) or by regulated the activity of aquaporins (Tyerman *et al*. 2002). This possibility may apply for both stabilized bio-available nano-particles in solution and also their small aggregates in aqueous solution.

Shoot biomass of both pearl millet and clusterbean increased after the nano-particle application. Root biomass, however, showed opposite trend (Table 1). Nano-ZnO was also reported to increase shoot growth of maize plants (Adhikari *et al.* 2010). Higher shoot growth could normally be attributed to higher photosynthesis as also observed after TiO_2 application on spinach (Gao *et al.* 2006, Hong *et al.* 2005). High photosynthesis would require steady supply of water and nutrients that is primarily through roots. Thus, a better root system would be logically expected to support the higher photosynthesis. But in our experiment, dry weight of pearl millet and clusterbean roots decreased by 17 % and 38%, respectively after application of nanoparticles.

Total C in shoot and root was 28.8% and 19.9% higher in pearl millet and clusterbean plants treated with nano-ZnO. Reasons for higher C fixation in presence of nano-ZnO are not clear. But, two indirect effects of ZnO on plants may influence photosynthesis (i) high biocompatibility and fast electron transfer kinetics of nano-ZnO (Kumar and Shen-Ming 2008) which may increase rate of some biochemical reactions and (ii) protection of plant membrane from the damage by the UV radiations due to lipid peroxidation. In our experiment too, malonialdehyde content, an indicator of lipid peroxidation, was 4.3 and 10.7% less in nanoZnO treated pearl millet and clusterbean leaves, respectively than the corresponding controls.

Application of nano ZnO significantly decreased the C exuded from roots in both pearl millet and clusterbean (Fig 2). C exuded through roots was 2.19% of total C accumulated in plants for pearl millet and 0.81% for clusterbean**.** Such a low C exudation from roots compared to 5-20% reported in literature may be due to varying growing conditions. Moreover as exuded C is primarily reported to be mechanism employed by plants to modify soil conditions for better nutrient uptake they may serve limited purpose in our experiment as nutrients were supplied in readily available form (Hoagland solution). Application of nano-ZnO further

Table 1 Effect of nano-ZnO on zinc concentration, biomass and total carbon in root and shoot of pearl millet and clusterbean

Crop	Control		Nano-ZnO	
	Shoot	Root	Shoot	Root
Zinc concentration (μg/g biomass)				
Pearl millet	27.69±0.51	41.51±0.83	30.00±0.56	43.75±0.61
Clusterbean	107.44±0.87	159.31±1.37	111.27±2.37	161.39±2.39
Dry weight (g/column)				
Pearl millet	3.19±0.19	1.35±0.13	4.78±0.25	1.12 ±0.06
Clusterbean	1.27±0.07	0.71±0.02	2.01±0.10	0.41±0.03
Total carbon (g)/column				
Pearl millet	1.0559±0.05	0.4200±0.03	1.5550±0.05	0.3461±0.01
Clusterbean	0.4752±0.01	0.2279±0.02	0.7012±0.01	0.1421±0.01

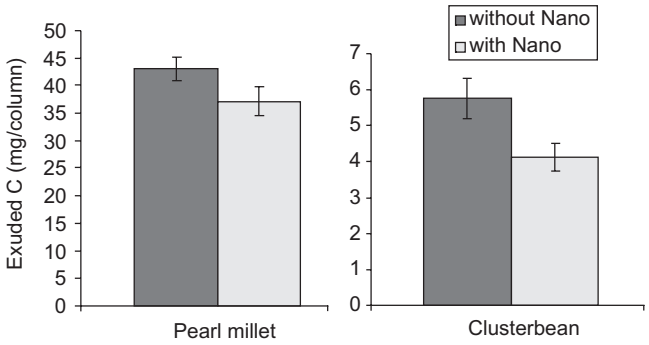

Fig 2 Changes in the carbon exuded from roots of pearl millet and clusterbean after application of ZnO nano particles

decreased the exudates to 1.9 and 0.49% of total C accumulated in pearl millet and clusterbean, respectively. One reason for this could be reduction of the root mass after application of nano-ZnO. Lin and Xing (2007) also reported severe damage to epidermal and cortical cells and even impairment of the endodermal and vascular cells of the ryegrass by nano-Zn and subsequent inhibition of root growth. A similar possibility could explain reduction in root mass in our experiment. Disruption in vascular cells could also explain lower root exudation after treating plants with nano-ZnO. Therefore, it is possible that portion of photosynthate–C, thus saved, was possibly diverted for the observed increase in shoot growth.

SUMMARY

Pearl millet and clusterbean foliar sprayed with 100 µg/mL Zn as nano-zinc oxide increased shoot while root biomass decreased. The amount of total C exuded through roots also decreased. Reduction in root biomass and exudates together accounted for only about 16 and 38% of the additional C accumulated in shoot biomass. Therefore there is all likelihood that part of the enhanced C accumulated in shoot may be due to enhanced photosynthesis. Such a positive influence of nano-ZnO could have been due to protection of the cellular membrane from the UV-radiation as evident from less malondialdehyde content in the treated plants in our experiment

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