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INFLUENCE OF ZINC OXIDE NANOPARTICLES ON ENZYMES ACTIVITY DURING SEED GERMINATION IN ONION

S. M. Jagtap[1] and S. L. Laware[2]

1. Department of Botany, Hutatma Rajguru Mahavidyalaya, Rajgurunagar. Pune (MS), India
2. Department of Botany, ACS College, Sonai, Newasa, Ahmadnagar , (MS), India

Abstract

Globally, many countries have identified the potential of nanotechnology in the agriculture sector and are trying to explore it significantly through research and development. With this view the present experiment was carried out to investigate the effect of ZnO nanoparticles (NPs) on hydrolytic and antioxidant enzyme activities during seed germination under laboratory condition. In this investigation, graded concentrations (00, 10, 20, 30, and 40 □g mL^{-1}) of ZnO NPs were prepared in distilled water. Surface sterilized onion seeds (25 seed per plate) were germinated with graded concentrations of ZnO NPs in petriplates on Whatman filter paper. Seed germinated without nanoparticles was considered as control. Results showed that ZnO NPs increases the activities of hydrolytic (Amylase and Protease) and antioxidant enzymes (SOD-Superoxidase, CAT-Catalase and POD-Peroxidase) at lower concentration (10-20 μgml^{-1}) while it decelerate the activities in higher concentration (30 and 40 μgml^{-1}). These results also match to the seed germination and early seedlings growth data, where lower concentrations of ZnO NPs showed enhanced seed germination and early seedlings growth and higher concentrations showed adverse effect.

Keywords: ZnO nanoparticles, amylase, protease, SOD, CAT, POD.

Introduction

Increasing uses of NPs in agriculture recommend the assessment of risk of these NPs. As indicated by Nowack and Bucheli (2007) it is required to understand the mobility, reactivity, toxicity and persistency of NPs in plants. Various stresses lead to the overproduction of reactive oxygen species (ROS) in plants. ROS such as superoxide radical (O^-_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) are cytotoxic in nature and damage protein, lipids, pigment and DNA (Halliwell and Gutteridge. 1999). Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APOX) and glutathione reductase (GR) scavenge the ROS in plant (Bowler *et al.,* 1992; Asada, 1992; Foyer, 1993).

ROS generation and oxidative stress induction are the major toxicological mechanisms of ambient NPs. Large amounts of ROS could be generated even when only small amounts of CuO or ZnO NPs are incorporated into cells (Toduka *et al.*, 2012). NPs induced ROS production can lead to various biological responses and such responses are dependent on the quantity of ROS, the type of cellular pathways, and the antioxidants that are engaged in oxidative stress (Xia *et al.*, 2008).

The oxidant defense machinery protects plant against oxidative stress damage. Antioxidant defense system work to control the cascade of uncontrolled oxidation and protect plant cell from oxidative damage by scavenging of ROS. Antioxidant enzymes are more important for plant growth and for plant defense. Hence the present study was planned to study the effect of metal ZnO NPs on Hydrolytic as well as on antioxidant enzyme during seed germination in Onion.

Materials and Methods

Metal NPs of various sizes (18 nm and 21 nm) were obtained from the researchers in the field of nanomaterial synthesis. Seeds of local onion variety were procured from NRC (National Research centre for onion and garlic) Rajgurunagar.

In this investigation, graded concentrations (00, 10, 20, 30, and 40 □g mL^{-1}) of ZnO NPs were prepared in distilled water. Surface sterilized onion seeds (25 seed per plate) were germinated with graded concentrations of ZnO NPs in petriplates on Whatman filter paper. Seed germinated without nanoparticles was considered as control.

Extraction and assay of enzymes: Treated and control seedlings were cut into small pieces. Accurately 1 gm of sample was homogenized and extracted with 3ml of appropriate buffer in pre-chilled mortar and pestle. Extract was centrifuged at 4^0C in cooling centrifuge at 15000 xg for 10 minutes and supernatant was used as source of enzymes. Amylase (α-amylase EC. 3.2.1.1): Amylase activity was calculated by using Jayaraman, (1981) method. Protease: Protease activity was calculated by using Issac and Gokhale (1982) method. Catalase (CAT, EC. 1.11.1.6): Peroxidase: (POD, EC. 1.11.1.7) The POD activity was assayed by Vidyasekharan and Durairaj (1973) method. Superoxide Dismutase **(**SOD, EC1.15.1.1**):** SOD, Superoxide dismutase activity was calculated by using Madamanchi *et al.* (1994).

Result and Discussion

Effect of different concentrations of ZnO NPs on activity of hydrolytic enzymes like Amylase and Protease as well as antioxidant enzymes like SOD, CAT and POD was evaluated during seed germination in *Allium cepa* and results are depicted in table (Table 1and 2) and Fig (1 and 2). Data pertaining to effect of ZnO NPs treatment on amylase activity indicate that amylase activity was increased upto 30 μg ml^{-1} treatment and then decreased significantly over control at 40 μgml^{-1} treatments. Maximum increase (22.95%) in amylase was recorded at 20 μg ml^{-1} of ZNO NPs treatment.

Data with respect to effect of ZnO NPs treatments on protease activity indicate that protease activity was increased upto 30 μg ml^{-1} treatment and then decreased significantly over control at 40 μgml^{-1} treatments (Table-1and Fig 1). The activity of antioxidant enzymes including, SOD, CAT and POD were increased with increasing NPs concentration (Table-2) (Fig 2).

Studies reported both positive and negative effects of NPs on plants, on seed germination, root elongation, cell division, growth and metabolic processes (Lin and Xing 2007; Lin and Xing 2008; Raskar and Laware 2013; Raskar and Laware 2014). Activities of key hydrolytic enzymes like amylase and protease in seedlings of *Allium cepa* that were grown in the presence of Zn ONPs were analyzed in present investigation. Results on enzyme activity revealed that NPs at their lower concentrations promoted enzyme activities, however at higher concentrations inhibited activities of hydrolytic enzymes.

The increased activity of α-amylase during seed germination was probably due to the *de novo* synthesis (Filner and Varner, 1967) of this enzyme. According to Wang *et al.* (1988) amylase activity increases gradually during initial days of germination and convert starch to soluble sugars needed for growth of embryo axis. According to Tully and Beevers (1978) proteins are hydrolysed to free amino acids, which support protein synthesis in endosperm and embryo. Marambe *et al.* (1992) noted a high significant correlation between the α-amylase activities; seed water uptake and subsequent percent seed germination as well as linear correlation of amylase activity with the starch degradation and increase in sugar content of treated sorghum seeds.

It can also be stated that NPs may promote seed antioxidant system (Lu *et al.*, 2002) and reduce the oxidative stress by reducing reactive oxygen species (ROS), malonyldialdehyde content and increase antioxidant enzymes (Lei *et al* ., 2008; Laware and Raskar, 2014.) Antioxidant enzymes are known for ROS scavenging and are more active in the presence of biotic or abiotic stresses; hence, in order to determine the probable mechanism of toxicity of TiO_2 NPs, activities of antioxidant enzymes were assessed in 10 day old onion seedlings. From the results on enzyme activities it is clear that the production of ROS due to NPs is responsible for inducing the antioxidant enzymes.

The activity of key antioxidant enzyme i.e. SOD in *Allium cepa* seeds treated with NPs and seedling grown in the presence of NPs showed increase with increase in concentration of NPs. Superoxide dismutase are responsible to catalyze the dismutation of superoxide (O_2^-) into oxygen and hydrogen peroxide. Hence, SOD enzymes are considered as important antioxidant defense in nearly all cells exposed to oxygen. In a study on wheat seedlings treated with biogenic silver NPs a significant increase in SOD activity was reported by Bhati-Kushwaha *et al.* (2013). Similarly, Wang *et al.* (2011) observed decrease in salt stress due to application of silicon NPs in alfalfa plant and they attributed this decreased salt stress to elevated activities of SOD, POD and CAT. In other study on soybean seed germination, it was found that seeds treated with a mixture of SiO_2 NPs and TiO_2 NPs exhibited more germination and higher activities of nitrate reductase, superoxide dismutase, catalase and peroxidase (Lu *et al.*, 2002). Authors concluded that SiO_2 and TiO_2 NPs would be better for seed germination and early seedling growth in soybean.

Table -1: Effect of ZnO NPs on hydrolytic enzymes during seed germination in Onion

ZnO NPs (μg ml^{-1})	**Amylase (U g^{-1}FW)**	**PIOC/PDOC**	**Protease (U g^{-1}FW)**	**PIOC/PDOC**
Control	42.22	0.00	47.14	0.000
10 μg ml^{-1}	52.42	24.16	52.96	12.346
20 μg ml^{-1}	54.63	29.39	53.84	14.213
30 μg ml^{-1}	48.95	15.94	40.00	-15.146
40 μg ml^{-1}	40.42	-4.26	32.28	-31.523
CD 5%	3.14		3. 82	

Table -2: Effect of ZnO NPs on antioxidant enzymes during seed germination in Onion

ZnO NPs (µg ml-1)	SOD (U g-1FW)	PIOC/ PDOC	Catalase (U g-1FW)	PIOC/ PDOC	Peroxidase (U g-1FW)	PIOC/ PDOC
Control	17.28	0.00	32.24	0.00	22.36	0.00
10 µg ml-1	20.26	17.25	41.76	29.53	28.24	26.30
20 µg ml-1	24.12	39.58	44.24	37.22	31.32	40.07
30 µg ml-1	26.24	51.85	34.42	6.76	20.24	-9.48
40 µg ml-1	27.18	57.29	30.23	-6.23	18.16	-18.78
CD 5%	2.46		2.08		1.48	

CD 5%: Critical difference at 5% probability; PI/PD: percent increase/decrease over control

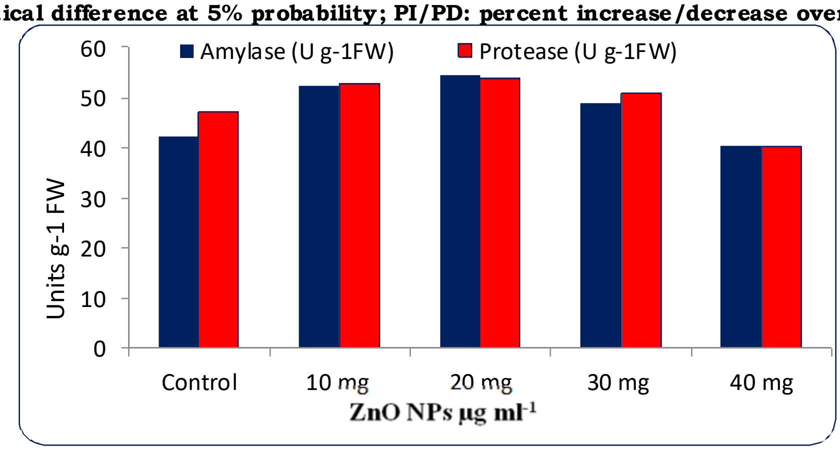

Figure 1: Effect of ZnO NPs on Hydrolytic enzyme

CD 5%: Critical difference at 5% probability; PI/PD: percent increase/decrease over control

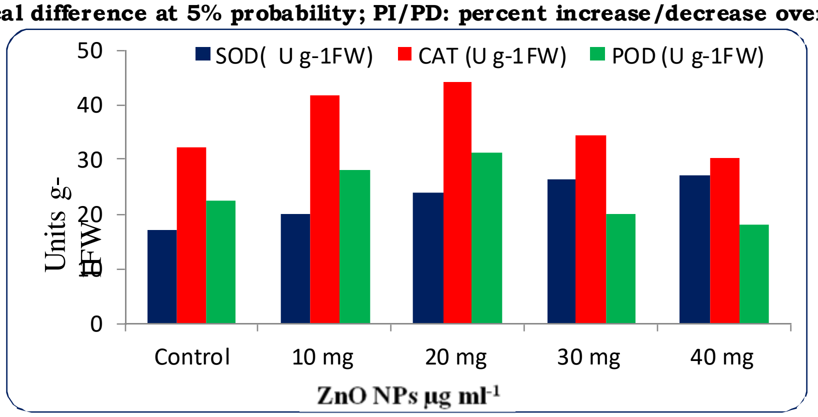

Figure 2: Effect of ZnO NPs on Antioxidant enzyme

Conclusion:

In present investigation, ZnO NPs induced significant changes in hydrolytic and antioxidant enzyme activities. Amylase and protease activities showed enhanced values in lower concentrations, but showed decrease at higher concentration. The activity SOD activity showed concentration dependent increase; however CAT and POD were found to be enhanced in the presences of 10-30 µgml-1 NPs but showed decreased activities in 40 µgml-1 NPs concentrations.

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