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Impact assessment of engineered nanotitanium dioxide seed priming on oxidoreductase enzyme activities in seedlings of Kidney bean (*Phaseolus vulgaris* L.)

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Abstract
A laboratory experiment was performed to study the effects of nano-TiO_2 polymorphs priming on seedling growth and antioxidant enzyme activities of Kidney bean (*Phaseolus vulgaris* L.). Nano-TiO_2 polymorphs *i.e.* anatase and rutile, were synthesized by the sol-gel method using titanium tetraisopropoxide as Ti-precursor and 2-propanol as solvent. Seeds of Kidney bean were treated with nine different concentrations (in water) of each nano-TiO_2 polymorphs (0, 0.10, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 and 2.50%) by soaking in different concentrations suspension (prepared by ultrasonication method) for 24 hours. The growth of seedlings and activity antioxidant enzymes *i.e.* catalase and peroxidase were assessed at three weeks following paper towel method of germination. The results showed that increase in concentration of each nano-TiO_2 polymorphs caused a significant increase in root and shoot length (79-97%) as well as number of lateral roots of the seedlings of kidney bean. The findings illustrated the beneficial effect of nano-TiO_2 polymorphs priming on seedling growth and antioxidant enzyme activities *viz*. catalase (78-80%) and peroxidase (75-162%) of Kidney bean.

Keywords: TiO_2 nanoparticles, kidney bean, seed priming, seedling, catalase, peroxidase

Introduction
Nanotechnology has revolutionized the world with tremendous advancements in many fields of science like engineering, biotechnology, medical, analytical chemistry and agriculture. Nanotechnology has been found to solve many of the agriculture related problems with tremendous improvement, as compared to conventional agriculture systems. Nanoparticle engineering is one of the latest technological innovations that demonstrate unique targeted characteristics with elevated strength. Nanoparticles irrespective of natural or manmade, are materials with at least two dimensions between 1 and 100 nm (ASTM, 2012) [2]. Among various type of engineered nanoparticles (artificially produced) metal oxides are very popular introduced in agricultural technology (Monica and Cremonini, 2009; Haghighi and de Silva, 2014; Ivani *et al.*, 2018) [24, 11, 13]. It makes use of the manipulation of materials for their novel, physical as well as chemical properties at nano-scale.

Titania (TiO_2) or Titanium dioxide nanoparticles are some of the most widely manufactured nanoparticles (Niska *et al.*, 2015) [26] and are proved to enhance seed germination (Feizi *et al.*, 2012) [6], photosynthesis (Gao *et al.*, 2006) [7] and crop yield (Owolade and Ogunleti, 2008) [27]. Titania (TiO_2) exists in three main crystallographic structures, namely, rutile, anatase and brookite. Titania-based nanocatalysts are being increasingly used in photocatalysis. Titanium (Ti) is considered a beneficial element for plant growth (Kuzel *et al.*, 2003) [19]. Titanium applied via roots or leaves at low concentrations has been documented to improve crop performance through stimulating the activity of certain enzymes, enhancing chlorophyll content and photosynthesis, promoting nutrient uptake, strengthening stress tolerance, and improving crop yield and quality. Khan (2016) [16] reported mitigation of salt stress by nano-TiO_2-particles in tomato by improving yield, leaf chlorophyll content, and antioxidant enzyme activities. Antioxidant enzymes are fundamental, they catalyze or participate directly in generation of reactive oxygen species (ROS) (Gill and Tuteja, 2010; Gill *et al*., 2013) [9, 8]. TiO_2 photocatalyze the oxidation-reduction reactions and thereby release high energy electrons (Yand *et al.*, 2008) [32]. In presence of light and water, photocatalyst create strong oxidizing agents (superoxide/hydroxide ions) and electronic holes to breakdown the organic molecules to CO_2 and H_2O. Lu *et al.* (2002) [21] found that TiO_2 nanoparticles can promote root activity of soybean and leaf nitrate reductase activity, enhance water and nitrogen use efficiency and can increase oxidoreductase enzyme activities (superoxide dismutase, catalase and peroxidase). Peroxidase catalyzes the oxidation of many organic compounds in plant by hydrogen peroxide,

$$AH_2 + H_2O_2 \rightarrow 2H_2O + A$$

AH_2 (e.g. amines, phenols, hydrogen quinone etc.) is a hydrogen donor and A is its oxidized form.

Catalase catalyzes the breakdown of H_2O_2 to water and molecular oxygen or peroxidatively oxidation of H donors (e.g. methanol, formic acid, phenol etc.) in presence of H_2O_2.

$$H_2O_2 \rightarrow 2H_2O + O_2$$

$$RCOOH + AH_2 \rightarrow H_2O + ROH + A$$

Seed priming is a technique that partially hydrates seeds in natural or synthetic compounds under specific environment to a point where germination-related metabolic processes begin, but radicle emergence does not occur (Ibrahim, 2016) [12]. Seed priming has been found to be useful for enhancing seed germination rate, seedling establishment and crop yields as well as increasing tolerance to environmental stresses (Chen and Arora, 2013; Ibrahim, 2016) [3, 12]. Kidney bean (*Phaseolus vulgaris* L.), is an herbaceous annual plant which is distributed worldwide. Beans are an important food among the *fabaceae* family for people of all income categories as a source of dietary protein, vitamins, fibre, complex carbohydrates (Kutos *et al*., 2003) [18] and bioactive compounds with antioxidant capacities (Granito *et al*., 2008) [10]. Objective of this study is to find the effects of engineered nano-TiO_2 particles seed priming on growth and oxidoreductase enzyme activities of the seedlings of Kidney bean.

Materials and Methods

Synthesis and characterization of polymorphs of nano-titanium dioxide

Polymorphs of nano-titanium dioxide were synthesized in sol-gel method by using Titanium tetraisopropoxide and 2-propanol as precursor materials (Sharma *et al.*, 2014) [29]. The synthesized polymorphs were characterized by X-ray diffraction (XRD) and Transmission Electron Microscope (TEM). The size of the polymorphs *i.e.* anatase and rutile were found to be 14 and 52 nm respectively.

Seed Priming by nano-TiO_2 and seedling growth of Kidney bean

Paper towel method was used for germination test. 100 seeds of Kidney bean (*var.* Kashi Rajhansh, ICAR-IIVR) of similar size were selected randomly, and surface-sterilized by 10% sodium hypochlorite solution. Then, seeds were washed in deionized water, and soaked in TiO_2 nanoparticle suspension (treatment) at eight different concentrations (0.10, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 & 2.50%) of nano- TiO_2 two polymorphs, viz. rutile & anatase each for 24 hours or in distilled water (control samples). Treated seeds were transferred to germination paper folded with butter paper to germinate in controlled environmental conditions (25°C temperature & 65% relative humidity). Three replicates of each concentration were prepared. Observations for seedling growth were taken 21 days after germination. Length of both shoot and root of the seedlings were taken by 1m scale. Then, seedling samples were transferred into hot air oven after air drying and oven dried at 65^{0}C. The dry weight of seedlings was taken in electronic pan balance.

Assay of redox enzymes in seedlings of Kidney bean

Activity of two redox enzymes *viz.* catalase and peroxidase activities in 3 weeks seedlings of Kidney bean were studied in this particular experiment. Catalase (EC. 1.11.1.6) activity was measured by the procedure described by Aebi (1984) [1]. 100 mM potassium phosphate buffer (pH=7), 75 mM H_2O_2 and 0.2mL of plant tissue extract were used. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed.

Peroxidase activity (EC. 1.11.1.7) was measured using modification of the procedure of McAdam *et al.* (1992) [22]. Guaiacol was used as the substrate. Peroxidase (POD) activity was measured in a reaction mixture containing enzyme extract, 12 mM H_2O_2, and 7.2 mM guaiacol in 50 mM phosphate buffer (pH 5.8). The kinetics of the reaction were measured at 470 nm. Activity was calculated using extinction coefficient (26.6 mM^{-1} cm^{-1} at 470 nm) for tetraguaiacol and expressed as units per gram of fresh weight (FW). One unit of POD activity was defined as 1 mmol tetraguaiacol produced per minute.

Statistical analysis

Experiment was conducted by following completely randomized design. Experimental data were analyzed by using SPSS 24 for their test of significance at 5% level of significance.

Results and Discussion

Effect of engineered nanoparticles on growth of Kidney bean seedlings

Seed-priming with engineered nano-TiO_2-particles (ENP-TiO_2) significantly improved the growth of Kidney bean seedlings. Length, dry weight, ratio of shoot and root length and lateral root density of Kidney bean seedlings were significantly improved with the application of engineered nano-anatase and rutile particles. Length of seedlings ranged 24.16-48.16 cm by seed priming with ENP-TiO_2. T8 and T6 treatments of anatase showed significantly highest seedling length which were 97% and 79%, respectively higher than control (T1). Similarly, T9 treatment of rutile showed 90% higher seedling length than control treatment. Similar trends were obtained for weight (dry) of seedlings as T8 treatment of anatase and T9 treatment of rutile performed best. Root growth of the kidney bean seedlings were also enhanced upon seed-priming with engineered nano-TiO_2 particles. Ratio of shoot and root length is a robust parameter to demonstrate vigour of seedlings. T8 treatment of anatase and T9 treatment of rutile showed highest shoot length/root length ratio. Lateral root density of Kidney bean was also positively effected upon seed-priming with engineered nano-TiO_2 particles. T8, T9 treatments of anatase and T6 treatment of rutile showed 125%, 122% and 102%, respectively increment in lateral root density than control. Similar results were also observed in other experiments (Palmqvist *et al.*, 2015; Janmohammadi *et al.*, 2017; Jaberzadeh *et al.*, 2013; Ebrahimi *et al.*, 2016; Khater, 2015) [28, 15, 14, 5, 17]. Growth promoting effect of nano-TiO_2 particles are attributed to type of particles, specific surface area, concentration, plant species etc. These tiny particles penetrate seed coat and create new passages for water entry. Thus, seed germination rate enhances and consequently the vigour of seedlings. Deposition and penetration of nano TiO_2 supports and allows water uptake inside the seeds. Larue *et al.* (2012) [20] documented that small sized nano-TiO_2 particles with high surface reactivity may enlarge the existing root pores or create new root pores, which facilitate greater hydromineral flow in root. Subsequently, this elevated water and nutrient uptake results in improved root as well as plant growth.

Table 1: Effect of anatase nanoparticles on growth of seedlings of Kidney bean

Treatment	Length of seedlings (cm)	Dry weight of seedlings (mg)	Shoot length (cm)/root length (cm)	Lateral root density*
T1 (Control)	24.46	94.7	0.60	2.60
T2 (0.10%)	33.30	148.3	0.70	3.26
T3 (0.25%)	36.50	158.0	0.64	4.90
T4 (0.50%)	32.12	143.0	0.68	3.97
T5 (0.75%)	37.75	165.3	0.77	4.27
T6 (1.00%)	43.83	189.3	0.73	5.21
T7 (1.50%)	37.50	164.4	0.76	5.78
T8 (2.00%)	48.16	197.3	0.84	5.85
T9 (2.50%)	40.75	173.9	0.70	4.08
CD (p=0.05)	6.20	29.3	0.09	0.64

* Number of lateral roots/unit length of tap root (cm)

Table 2: Effect of rutile nanoparticles on growth of seedlings of Kidney bean

Treatment	Length of seedlings (cm)	Dry weight of seedlings (mg)	Shoot length (cm)/root length (cm)	Lateral root density*
T1 (Control)	24.16	100.7	0.65	2.63
T2 (0.10%)	32.00	121.7	1.01	4.84
T3 (0.25%)	38.41	145.3	0.79	4.89
T4 (0.50%)	33.43	128.6	1.13	5.21
T5 (0.75%)	29.75	125.0	1.01	4.98
T6 (1.00%)	33.23	126.3	1.06	5.32
T7 (1.50%)	37.45	143.9	1.07	4.47
T8 (2.00%)	38.25	150.0	1.15	4.78
T9 (2.50%)	46.00	153.7	1.21	4.92
CD (p=0.05)	6.10	20.3	0.12	0.83

* Number of lateral roots/unit length of tap root (cm)

Effect of engineered nanoparticles on oxidoreductase enzyme activities of Kidney bean seedlings

The activities of redox enzymes in Kidney bean seedlings were significantly influenced by seed priming with nano-TiO_2 particles. Activity of catalase was significantly (p<0.05) enhanced by the seed priming with both anatase and rutile (Figure 7 and Figure 8). The solution @ 2.50% (T_9) of anatase showed highest catalase enzyme activity (5.0 µmole H_2O_2 min^{-1} g^{-1}) which was 78% higher than control. However, 0.5% rutile solution brought about highest catalase enzyme activity (5.6 µmole H_2O_2 min^{-1} g^{-1}) in Kidney bean seedlings. Another important redox enzyme i.e. peroxidase activity was also significantly (p<0.05) enhanced upon seed with by nano-TiO_2 particles. 1% anatase solution (T_7) and 2.00% rutile solution treatment accounted 75% and 163% increase in peroxidase activity, respectively in kidney bean seedlings. Exposure of plants to unfavorable environmental conditions can increase the production of ROS (reactive oxygen species) to protect themselves against these toxic oxygen intermediates, plant cells and its organelles like chloroplast, mitochondria and peroxisomes employ antioxidant defense systems (Tuteja, 2007) [30]. Nano anatase and rutile particles has a tendency to activate antioxidant enzyme activity *viz.* peroxidase, catalase and superoxide dismutase among different life forms like marine microalgae (Dalai *et al*., 2014; Xia *et al*., 2015) [4, 31], higher plants (Mattiello *et al*., 2015) [23] and in human (Niska *et al*., 2015) [26].

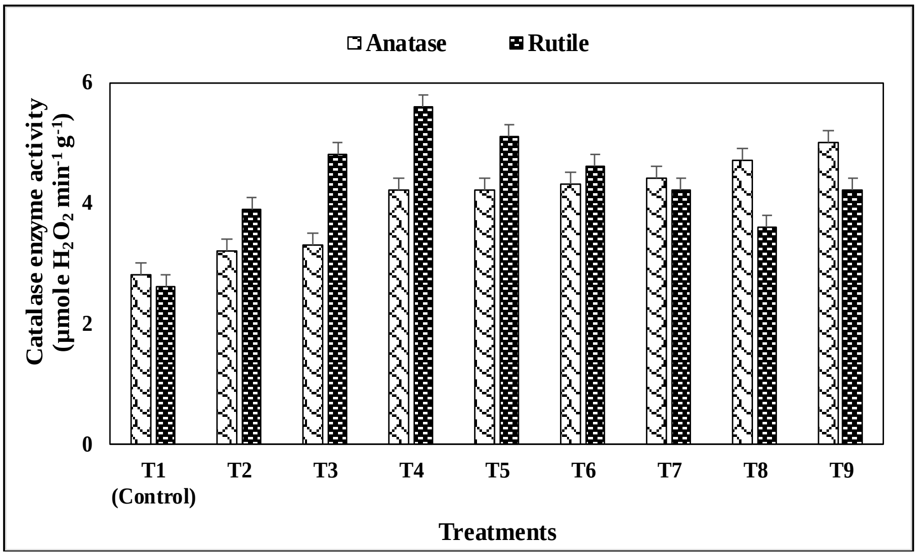

Fig 1: Effect of anatase and rutile nanoparticles on catalase enzyme activity of seedlings of Kidney bean

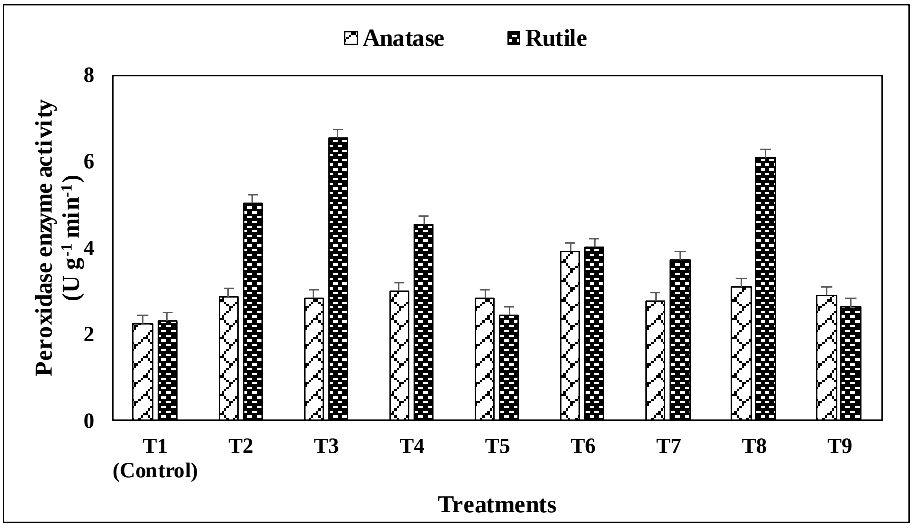

Fig 2: Effect of anatase and rutile nanoparticles on peroxidase enzyme activity of seedlings of Kidney bean

Conclusion

Seed priming is very useful for better crop performance. Seed priming with nano-TiO_2 particles viz. anatase (14 nm) and rutile (52 nm) remarkably improved the shoot length, root length seedlings, ratio of shoot and root, lateral root density and redox enzyme activities viz. catalase and peroxidase in seedlings of Kidney bean. Thus, seed priming with nano-TiO_2 particles polymorphs, particularly @ 2.00% of anatase solution could be a useful technology for Kidney bean crop.

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