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 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$_2$O. 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 oxido-reductase enzyme activities (superoxide dismutase, catalase and peroxidase). Peroxidase catalyzes the oxidation of many organic compounds in plant by hydrogen peroxide,

$$\text{AH}_2 + \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{A}$$

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$_2$O$_2$ to water and molecular oxygen or peroxidatively oxidation of H donors (e.g. methanol, formic acid, phenol etc.) in presence of H$_2$O$_2$.

$$\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$$

RCOOH + AH$_2$ $\rightarrow$ H$_2$O + 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) [13]. 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 oxido-reductase 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 tetra-isopropoxide 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-IIHR) 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°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) [31]. 100 mM potassium phosphate buffer (pH=7), 75 mM H$_2$O$_2$ and 0.2mL of plant tissue extract were used. Enzyme activity was computed by calculating the amount of H$_2$O$_2$ 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$_2$O$_2$, 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 tetraguaiaicol and expressed as units per gram of fresh weight (FW). One unit of POD activity was defined as 1 mmol tetraguaiaicol 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; Jannomhammad et al., 2017; Jaberzadeh et al., 2013; Ebrahim et al., 2016; Kather, 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

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

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

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

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

* 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-TiO₂ 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% (T₉) of anatase showed highest catalase enzyme activity (5.0 μmole H₂O₂ min⁻¹ g⁻¹) which was 78% higher than control. However, 0.5% rutile solution brought about highest catalase enzyme activity (5.6 μmole H₂O₂ 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-TiO₂ particles. 1% anatase solution (T₇) 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](image-url)
**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.

**References**


