EFFECTS OF NANOMETAL POWDERS (Fe, Co, Cu) ON THE GERMINATION, GROWTH, CROP YIELD AND PRODUCT QUALITY OF SOYBEAN (VIETNAMESE HYBRID SPECIES DT-51)

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ABSTRACT

Superdispersive iron, cobalt and copper nanometal powders were synthesized in water-ethanol medium by the reduction method using sodium borohydride as a reducing agent and carboxymethyl cellulose (CMC) as a stabilizer (for Fe and Co nanoparticles). TEM micrograph and XRD analyses of the freshly prepared nanometal powders indicated that they were in zero valent state and with particles size ranged from 20 - 60 nm.

The soybean seeds were treated with an extra low nanometal dose (not more than 300 mg of each metal per a hectare) and then sowed on an experimental landfill plot of 180 m² farming area. This pre-sowing treatment of soybean seeds, which does not exert any adverse effect on the soil environment, reliably changed the biological indices of the plant growth and development. In particular, in laboratory experiment, the germination rate of soybean seeds treated with zerovalent Cu, Co and Fe were 65%, 80% and 80% respectively, whereas 55% germination was observed in the control; in field experiment, for all the nanoscale metals studied, chlorophyll index increased by 7 - 15 % and number of nodules 20 - 49% compared to the control, and crop yield of soybean increased up to 16% in comparison with the control.

Keywords: nanoscale zerovalent, metal powder, germination, soybean, seed.

INTRODUCTION

In recent decades, the nanotechnology products have been intensively applied in agriculture. Nanometals are known as a stimulating agent for plant growth and the activation of metabolic processes in plant and animal organisms. Numerous researchers have studied the biological effects of nanoparticles, as well as their beneficial and harmful effects on plants.

Biogenic nanometals such as Fe, Mn, Zn, Cu, Co, Se etc, among which Fe, Cu, and Co with variable valences are the most bioactive [1], have been widely used in agriculture as they actively participate in different redox processes in plants and are present in the composition of many enzymes and complicated proteins [2-6]. Distinctive feature of metal nanoparticles (MNP)s, so called superdispersive metal powder (SDMP), was the low toxicity compared to their salts and chelates, and the ability to stimulate physiological and biochemical processes in plants by using their extra small doses in pre-sowing treatment of seeds [12,15].

In this report, the effects of nanometal Fe, Co and Cu at extra-low concentration on germination, growth, crop yield and product quality of soybean were studied using nanometal powders produced by chemical aqueous reduction method.

EXPERIMENTAL

Nanocrystalline metals iron, cobalt, and copper of size from 20 - 60 nm were produced following aqueous solution reduction method with sodium borohydride as a reducing agent and carboxymethyl cellulose (CMC) as a stabilizer [7-12]. FeSO₄·7H₂O, CoSO₄·7H₂O, CuSO₄·5H₂O, NaBH₄, CMC were purchased from Merck Chemical Reagent Co.; C₂H₅OH (absolute) – from Beijing Chemical Reagent Co. For laboratory experiment plant agar (Duchefa Co.) and nutrient solution MS (Murashige and Skoog medium) were used.
Preparation of iron, cobalt and copper metal nanoparticles:

For preparation of iron, copper and cobalt nanoparticles NaBH\(_4\) and CMC were used as reducing and capping agents, respectively \([7-12]\).

The obtained nanocrystalline metals in the form of superdispersive metal powders (SDMP) were stored in hermetic flasks filled with argon gas.

Soybean seed treatment:

Soybean seeds of Vietnamese hybrid species DT-51 were procured from Legumes Research and Development Center of Field Crop Research Institute, Vietnam Academy of Agricultural Sciences. Treatment of the seeds with nanoparticles were carried out as follows: A calculated amount of each zerovalent metal (ZVM) nanopowder was dispersed in a closed PE pocket with a certain water volume, sonicated (800 W, 20 kHz) for 30 min and soybean seeds were immersed in this suspension for 45 min. After withdrawing from PE pocket and dried on air the treated seeds were ready (within 24 h) for sowing experiments.

Laboratory experiment:

One set of germination experiments was carried out in laboratory for determining the treatment effect on seed germination and seedling vigor. To investigate the promotory and inhibitory effects of nanoparticles on soybean plant growth, three concentrations of ZVM nanopowders were used: 0.080 g/ha, 0.200 g/ha and 0.320 g/ha. The nanometal-treated seeds were immediately transferred to Petri dishes, each of which contained 80 ml of 7.5% agar solution, for growing to five days. Arrangement of the set I consisted of 3 columns of Petri dishes for 3 nanometals and 3 rows for 3 nanometal concentrations respectively and one row (2 dishes) for the control (8 replicates). For seed germination Petri dishes were sprayed with nutrient solution everyday. 5 days after sowing the soybean plants were uprooted and total length of roots and shoots were measured.

Field experiment:

The field experiments included three Vietnamese iron, cobalt and copper nanometals and one Russian nanocopper, which was produced by low-temperature reduction method \([6]\). The experiments were conducted at Ha Noi Agricultural Promotion Center in randomized complete block design replicated 6 times.

Before sowing soybean seeds were treated with one of the zerovalent metal nanoparticles with a dose of 0.120 g/ha. The total useful plot size 180 m\(^2\) was divided into 30 compartments (4 trials + 1 control) x 6 m\(^2\)/compartment x 6 replicates.

RESULTS AND DISCUSSION

Characterization of the SDMPs:

TEM micrographs of nanocrystalline iron, copper and cobalt prepared by aqueous solution reduction method (Figure 1, upper row) showed that mean particles size ranged from 20 - 60 nm. As aforementioned, for the use in plant cultivation metal nanoparticles should be principally in zerovalent state. Therefore the preparation process of SDMPs requires the use of capping agent to restrict them from oxidation. For this aim carboxymethyl cellulose (CMC) was chosen as a stabilizing agent partially due to its adequate solubility in water. In our work CMC was used for preparation of Fe and Co SDMPs, while for nanocrystalline Cu superdispersive powder was prepared without using capping agent, because nanocrystalline copper metal formed a thin firm oxide layer on its surface. On SEM images presented on the lower row (Figure 1) one can see that nanocrystalline copper possessed smallest particles size. It may be due to the fact that reaction of formation of nanoparticles without capping agent occurred with a higher speed of appearance of crystalline centers, resulted in formation of smaller particles compared to iron and cobalt nanoparticles which were covered with layers of CMC.

Seed germination and seedling vigor (laboratory experiment):

Soybean seeds responded differently towards the treatment at various concentrations of the SDMP and towards various nanometals. Effect of nanocrystalline Fe, Cu and Co on soybean germination and vigor showed that nanometal dose of 0.08 g/ha demonstrated the best result in germination and seedling vigor compared to higher doses. And among the three metals studied cobalt gave the best SVI value, exceeding the control 2.4 times. This seedling vigor value was comparable with that in the work \([13]\), in which peanut seeds were growing in an agar medium in the presence of ZnO nanoparticles.
Figure 1. TEM and SEM micrographs of nanocrystalline Fe (a,a’), Cu (b,b’) and Co (c,c’) prepared by aqueous solution reduction method. Insets show their respective XRD spectra.

Figure 2. Influence of various nanometal powder doses on germination of nanometal-treated soybean seeds.

By increasing the SDMP doses exceeded 0.08 g/ha could inhibit germination rate and seedling vigor. Among the three nanometals tested nano copper exhibited the most strong inhibition effect on germination and seedling vigor (Table 1): at Cu doses 0.20 g/ha and 0.32 g/ha germination rate decreased by 0% and 9.1% compared to the control, respectively, while SVI decreased 5.8% and 41.2% compared to SVI of the optimal dose 0.08 g/ha. Different nanometal doses effected on germination of the treated soybean seeds (Figure 2) showed that inhibition effect of copper nanoparticles was much stronger than that of iron and cobalt nanometals. This result of inhibition effect was in good agreement with other researchers [1,5,13] and implied a possibility to minimize the optimal doses towards the smaller values.

Field experiment:

Results of the field experiment (Table 2, Figures 2) confirmed that, just as in laboratory experiment, cobalt nanopowder exhibited better germination effect than nano-sized iron and copper, wherein all the growth parameters exceeded control ones (with crop yield surpassed the control 16%). In field experiment an additional trial set was conducted on a Russian copper nanopowder, which was produced by low-temperature hydrogen reduction method (Table 2, fourth column). The data showed that all the growth parameters of both “Vietnamese” copper set and “Russian” one were almost the same. This result additionally proved that SDMPs produced by aqueous solution reduction method possessed similar biological activity as those produced by low-temperature hydrogen reduction method and could be used for agricultural application purposes.
Table 2. Basic growth parameters and crop yield of nanometal-treated soybean. Nanometal dose was 0.08g/ha

<table>
<thead>
<tr>
<th>SDMPs</th>
<th>Control</th>
<th>Cu</th>
<th>Cu*</th>
<th>Fe</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll content (mg/100g leaf)</td>
<td>27.0 ± 0.9</td>
<td>29.1 ± 0.6</td>
<td>28.6 ± 0.7</td>
<td>31.7 ± 0.8</td>
<td>29.6 ± 0.8</td>
</tr>
<tr>
<td>Number of nodules/root</td>
<td>13.1 ± 1.6</td>
<td>19.7 ± 4.4</td>
<td>16.2 ± 6.1</td>
<td>12.9 ± 8.7</td>
<td>16.8 ± 7.0</td>
</tr>
<tr>
<td>Number of pods/plant (pod)</td>
<td>76.2 ± 16.4</td>
<td>81.1 ± 18.1</td>
<td>80.3 ± 12.9</td>
<td>63.6 ± 16.1</td>
<td>89.1 ± 18.6</td>
</tr>
<tr>
<td>Pods weight (g)/plant</td>
<td>63.7 ± 14.4</td>
<td>57.0 ± 12.9</td>
<td>60.3 ± 17.4</td>
<td>52.1 ± 23.1</td>
<td>72.1 ± 15.4</td>
</tr>
<tr>
<td>Weight of 1000 grains (g)</td>
<td>162.2 ± 3.1</td>
<td>169.2 ± 2.8</td>
<td>161.9 ± 8.5</td>
<td>162.2 ± 2.9</td>
<td>166.0 ± 5.8</td>
</tr>
<tr>
<td>Crop yield (ton/ha)</td>
<td>2.33 ± 0.06</td>
<td>2.59 ± 0.08</td>
<td>2.46 ±0.06</td>
<td>1.95 ± 0.05</td>
<td>2.71 ±0.08</td>
</tr>
</tbody>
</table>

* Russian copper nanometal powder, produced by physico-chemical method

**CONCLUSION**

Nanocrystalline Fe, Co and Cu with particles size ranged from 20 - 60 nm have been synthesized by aqueous solution reduction method using sodium borohydride as reducing agent and CMC as stabilizer for preventing iron and cobalt ZVMs from oxidation.

It was found that nanometal-treated soybean seeds variously responded towards different concentrations of the SDMP and towards different nanometals: among the studied SPMPs nanocrystalline cobalt exhibited the best biological effects on soybean growth and development, with SVI surpassed 2.4 times and crop yield - about 16% in comparison to the control, while optimal dose for soybean seeds treatment was set equal 0.08 g/ha.

Beyond the dose 0.300 g/ha all the three studied nanometals expressed inhibition effect on germination, especially nanocrystalline copper. SDMPs produced by aqueous solution reduction method with extra low doses can be used for agricultural application.

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**References**