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A Novel Nanomaterial to Enhance Growth and Yield of Wheat

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ABSTRACT

Careful and judicious use of nanotechnology can ensure food security through boosting agriculture production. Nanoparticles have potential to improve growth and yield of wheat crop. This paper reports the effect of silver nanoparticles on germination, seedling growth and yield of wheat cultivar NARC-2009. Silver nanoparticles (10-20 nm size) were synthesized by chemical reduction of silver nitrate with tri-sodium citrate. Wheat seeds were soaked in 0, 25, 50, 75, 100, 125 and 150 ppm solutions of silver nanoparticles for germination test. To determine the effect on seedling growth silver nanoparticles were applied with MS medium and through soil in small plastic pots in two different experiments in lab. To evaluate yield response silver nanoparticles were applied to soil at the time of transfer of seedlings to clay pots. Application of silver nanoparticles either did not affect or decreased germination and germination index. However, number of seminal roots increased significantly with 25 and 50 ppm silver nanoparticles in comparison to control. Leaf area, root biomass, fresh weight and dry weight increased significantly at 25 ppm as compared to control during first week of exposure but decreased during the following two weeks. Higher concentration of silver nanoparticles in solution and longer exposure proved toxic to the seedlings. All levels of silver nanoparticles (0, 25, 50, 100 and 150 ppm) applied to soil in pots significantly enhanced fresh weight, dry weight and chlorophyll content over control. Nonetheless, effect of 25 ppm was more pronounced as compared to other levels of SNPs. Maximum number of grains per spike was recorded with 25 ppm followed by 50 ppm whereas maximum 100-grain weight was obtained for 25 and 125 ppm soil applied silver nanoparticles. Highest grain yield per pot was obtained with 25 and 50 ppm. Silver nanoparticles have definite potential to increase crop growth and yield. Nonetheless, comprehensive experimentation is required to establish the most suitable concentration, size and mode of application of silver nanoparticles for higher growth and maximum yield of wheat.

1. Introduction

Nano technology is the science of small things less than 100 nm in size. It is the exploration of properties of materials at nano scale. Nanotechnology is expected to play a vital role in various disciplines. It is becoming the most innovative scientific field. Exploring beneficial uses of nanoparticles in plant sciences is becoming an increasingly important area of interest [1]. Nanotechnology is being visualized as a rapidly evolving field that has potential to revolutionize agriculture and food systems and improve the conditions of the poor. Nanotechnology will improve agricultural yields for mushrooming population in Asian countries. It has potential to provide food security by enhancing crop production through precision farming, efficient utilization of water, protection against insects and diseases, providing new tools for molecular and cellular biology, new materials for pathogen detection and protection of the environment. Advances in nanotechnology are being integrated into biology that has led to emergence of a new exciting discipline called nanobiotechnology. Nanotechnology can address the most critical sustainable development problems of agriculture in an environment friendly manner. It may provide efficient means for application of agrochemicals thereby reducing amount of chemicals introduced into the environment [2]. There might be several possible applications of nanotechnology in agriculture starting from crop production, fertilizer and irrigation management, crop protection and crop improvement for quality and agronomic traits. Nanotechnology-based reorientation of agriculture can boost production of quality food.

Nanoparticles are atomic aggregates with size range of 1-100 nm. Nanoparticles can have both growth promoting or harmful effects on crops. Uses of nanoparticles in crop sciences are consistently increasing. Silver nanoparticles have remarkable uses in crop production. Plants grown in nutrient medium provided with nanosilver can uptake and accumulate nanoparticles [3]. Application of iron nanoparticles improved agronomic traits of soybean [4]. Soaking of cotton seeds in silver nanoparticles produced favourable effects and reduced the amount of fertilizers applied through roots by half [5]. Application of nanoparticles has been found to improve germination, enhance growth and physiological activities [6], increase water and fertilizers use efficiency [7]. Silver nanoparticles have catalytic effects [8] and increase chlorophyll [9].

Silver nanoparticles also have strong antimicrobial effects [6]. They can control and avoid plant diseases. These particles in concentrations of 0.5 to 1000 ppm cause faster growth of plants and control pathogens [10]. Silver nanoparticles are beneficial for seed growth and germination [7], and act as growth simulators [11]. Silver nanoparticles can enable plant to inhibit senescence caused by reactive oxygen species (ROS) generated due to oxidative stress. Senescence induced by oxidative stress and ROS generation triggered by 2,4-D in mungbean was repressed by application of 100 microliter of silver nanoparticles [12] .

Effects of nanoparticles on higher plants depend on their reactivity and size [13]. Nanoparticles were found to affect seed germination in lettuce and cucumber [6, 14], Germination inhibition effects were observed for ryegrass, barley and flax when placed in 10 mg/L silver nanoparticles [15]. Nano silver can also interact with carbohydrates assimilation, protein and starch production [16]. Toxic effects of silver nanoparticles were reported for *Lolium multiflorum* [17]. Higher concentrations of nanosilver hinder root growth and dry weight but increase root branching in rice. While lower concentrations hasten root growth, branching, dry weight and plant photosynthetic pigments [18]. Nanosilver inhibits action of ethylene in

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plants by binding silver on the sites which are bound normally by ethylene [19].

In fact, nanotechnology is a rapidly developing discipline substantially influencing every field of science and biology. Exploring comprehensive application profile of nanoparticles may revolutionize research in crop science and ensure food security by increasing crop productivity. Sifting the potential benefits of silver nanoparticles present research was conducted to document the role silver nanoparticles can play in germination, growth and yield of wheat.

2. Experimental Methods

2.1 Synthesis of Silver Nanoparticles

Silver nanoparticles (SNPs) were synthesized by reduction of silver nitrate ($AgNO_3$) with tri-sodium citrate ($Na_3C_6H_5O_7.2H_2O$) according to the methods described [20] with little modification. Silver nitrate (510 mg) was dissolved in 500ml distilled water and heated for 15 minutes at 75-80 °C with continuous stirring at 7000 rpm on magnetic stirrer. Then 500 mL solution containing 300 mg of trisodium citrate was added slowly. The solution was kept at 75-80 ^{0}C with continuous stirring for about one hour. When the solution turned golden yellow (indication of silver nanoparticles) the reaction was stopped. Ascorbic acid @ 1.0 mg/L was added to stabilize the nanoparticles. Aggregation of SNPs was not observed. Furthermore, reaction conditions and concentration of the reactants were adjusted in such way to ensure that no silver ions were left in the solution. The solution so prepared was used in experiments.

2.2 Size of Silver Nanoparticles

Nanoparticle crystallite size was determined by using X-Ray Diffraction (XRD) method at School of Chemical and Material Engineering, National University of Science and Technology (SCME-NUST), Islamabad. SNPs were solidified using 5.0% agarose and the solidified samples were heated at 120 °C which converted samples into dry form which were then tested. XRD analysis indicated that size of SNPs was in the range of 10 to 20 nm with maximum number of particles with 11 nm size (Fig. 1).

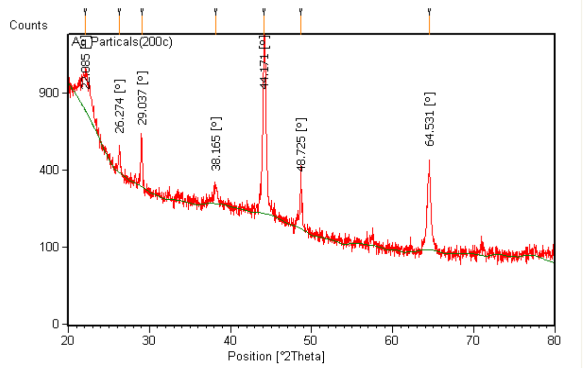

Fig. 1 XRD Peak for SNPs

2.3 Germination Test

Seeds of wheat cv NARC-2009 were sterilized by washing with ethanol for about five minutes followed by thorough washing with distilled water. The seeds were placed on three layers of filter papers fitted in petri-dishes soaked with 25, 50, 75, 100, 125 and 150 ppm solutions. Distilled water was used as check to determine the effect of nanoparticles on germination of wheat seeds. Completely randomized design with three replications was employed for germination test with ten seeds in each plate. Germination percentage was measured after 7 days by following formula:

Germination Percentage = (Number of seed germinated/ Total Number of Seeds) x 100

Germination index was calculated by following formula:

$$\text{Germination Index (GI)} = \Sigma\, Gi/Di$$

Where Gi is the difference in number of seeds germinated between the count i and the preceding count, and Di is the day of each count

2.4 Seedling Growth

To document the effect of SNPs on seedling growth, germinated seedlings were transferred to small plastic pots filled with MS medium blended with 0, 25, 50, 75, 100, 125 and 150 ppm of SNPs in subsequent experiments. Completely Randomized Design (CRD) was used for experimental layout. Solutions were changed after every 15 days. Seedlings were allowed to grow for four weeks. Data was recorded for leaf area (using leaf area meter), fresh weight (FW), dry weight (DW) and root dry weight at 2nd, 3rd and 4th week of growth in solution culture.

Second experiment was conducted to determine the effect of soil applied SNPs. Germinated seedlings were transferred to small plastic pots filled with homogenous soil. Pots were soaked with 25, 50, 100 and 150 ppm SNP solution equivalent to field capacity water. Check pots were soaked with simple water. Seedlings were allowed to grow up to four weeks and FW, DW and chlorophyll content (by acetone extraction method) of seedlings were determined.

2.5 Grain Yield

Third experiment was carried out to monitor the effect of SNPs on wheat yield. Completely homogenized soil was filled in pots and field capacity of soil was determined by gravimetric method. Wheat seedlings germinated in lab were transferred to the earthen pots. At the time of the seedlings transfer SNP solution containing (25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm and 150 ppm) equivalent to field capacity was applied in pots. Simple water was applied to control pots. Equal amount of water in pots was applied when need. Thinning was done in 3 to 4 weeks and only five plants per pot were kept. Completely Randomized Design (CRD) with four replications was used. When plants were mature and ready for harvesting data on number of grains per spike, 100 grain weight and grain yield per pot was recorded.

Statistical analysis of data was done using the computer based statistical package MSTATC and treatment means were compared using LSD Test at 5 % level of probability.

3. Results and Discussion

3.1 Silver Nanoparticles and Germination/Seminal Roots

Effect of SNPs on germination, germination index and seminal roots are presented in Table 1. SNPs either had no effect on germination with 25 to 75 ppm or caused significant reduction in germination with 100-150 ppm in comparison to control. Almost similar trend was observed in case of germination index. However, SNPs had pronounced effect on number of seminal roots. Significantly higher number of seminal roots as compared to control was produced with application of 25 and 50 ppm followed by 75 ppm of SNPs (Fig. 2). Further increase in concentration of SNPs (100, 125 and 150 ppm) significantly decreased number of seminal roots.

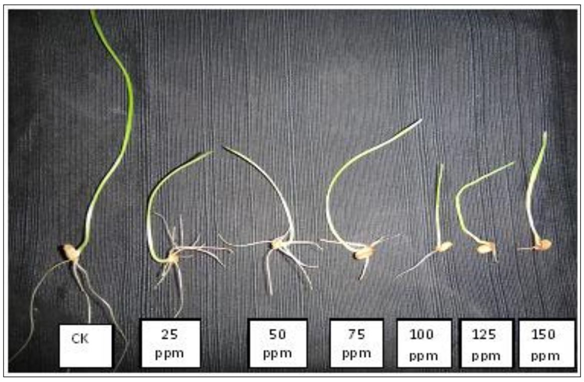

Fig. 2 Effects silver nanoparticles on number of seminal roots

Table 1 Effect of SNPs on germinations and number of seminal roots

SNPs conc.	Germination %	Germination Index	Number of Seminal Roots
0 ppm	87.5 a*	0.9188 a*	3.6682 c*
25 ppm	97.5 a	0.8900 ab	6.3698 a
50 ppm	90.0 a	0.7728 cd	6.3748 a
75 ppm	92.5 a	0.7783 cd	5.8000 b
100 ppm	57.5 b	0.76 d	2.3708 d
125 ppm	37.5 c	0.839 bc	1.2000 e
150 ppm	25.0 c	0.8375 bc	1.1000 e

**Means not sharing a letter in common differ significantly at 5% probability level*

SNPs were found to have no effect affect on germination on wheat seed at lower concentrations. Similar effects of SNPs on germination have been reported in other crops. SNPs did not affect seed germination of *Baccopa monnieri* and zucchini plant [16, 21]. Higher concentrations (more than 75 ppm) were found to inhibit germination in this study. Germination inhibition effects of SNPs have reported for cucumber, lettuce, barley and ryegrass [6, 14, 15]. However, silicon oxide and titanium oxide nanoparticles increased germination rate and index of soybean and spinach [22, 7]. Germination rates of tomato seeds treated with CNTs [23] were dramatically higher. No favorable effects of SNPs on germination/germination index of wheat seed were found in our experiment.

SNPs at low concentration, in this study, favorably affected number of seminal roots. No study, so far, reported the effect of SNPs on number of seminal roots. However, increased root branching of rice was seen at 30 and 60 ppm concentration of SNPs [18]. SNPs can help reduce phytotoxic effect of Pb (Plumbum). Minimum damage to roots was observed in plants treated when 50 ppm SNPs under Pb toxicity [24]. Other nanoparticles like nanoAl_2O_3 have growth promoting effects on roots of rape and radish. In our experiment maximum increase in number of seminal roots was recorded at 25 ppm SNPs. Increase in the number of seminal roots is a trait desirable for crops sown in low soil moisture condition [25]. Consequently, positive effect of SNPs on number of seminal roots may be exploited for early crop establishment in areas with limited moisture availability in soil at sowing time.

3.2 Silver Nanoparticles and Seedling Growth in MS Medium

Efficacy of SNPs applied through MS medium on growth parameters of wheat is presented in Fig. 3 (A, B, C & D). Significantly higher leaf area, fresh weight, dry weight and root biomass was observed with 25 ppm SNPs as compared to control after one week of growth in nutrient medium. Further increase in SNPs (50-150 ppm) caused progressive reduction in these growth parameters. Concentration and duration of exposure to SNPs had significant impact on seedling growth. Increase in concentration and longer exposure caused decrease in leaf area, fresh weight and dry weight. Root biomass produced with 25 ppm SNPs was significantly higher than the plants from control or treated with other levels of SNPs during 1st week but became similar to root mass from control and the plants treated with 50 ppm SNPs. This indicated progressive reduction in root growth with increased duration of exposure to SNPs. Favorable effects on FW, DW and root biomass of wheat seedlings were observed after one week of exposure to 25 ppm SNPs applied in MS medium. However, fresh and dry weight decreased during next two weeks. The results of this experiment indicate that SNPs at low level (25 ppm) have no phytotoxic effects. Toxicity occurs only with longer exposure or higher concentration of SNPs.

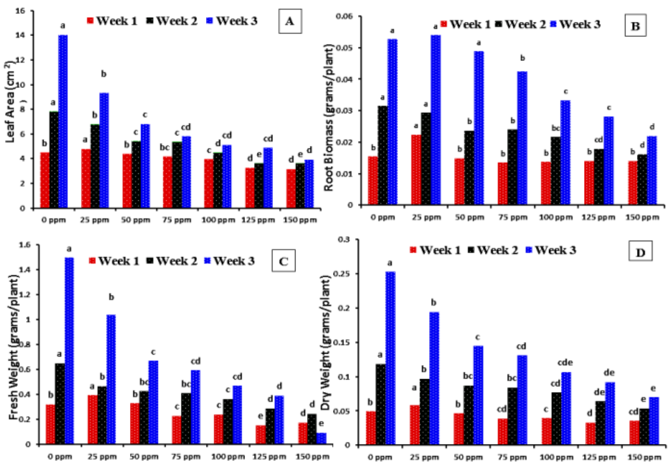

Fig. 3 Effect of SNPs in MS medium on leaf area (A), root biomass (B), fresh weight (C) and dry weight (D) of wheat seedlings

SNPs had no severe toxic effects at low concentration [16] and may enhance seedling growth. Najafi et al found significant increase in shoot FW, plant height and root FW in response to 50 ppm SNPs [24]. Unfavorable effect of SNPs on growth in MS medium might be due to enhanced bioavailability and more accumulation in plants leading to adverse effects on growth. Higher levels of metal nanoparticles cause increased bioavailability and massive accumulation in roots/shoots [26] leading to toxic effects that might be due to DNA damage induced by metal nanoparticles [27]. SNPs reduced plant dry weight of rice[18]. Decrease in root growth of Phaseolous radiatus and Sorghum bicolor and root length and biomass of *Lolium multiflorum* when exposed to SNPs has also been reported [3, 17]. In general the studies reporting inhibitory/unfavorable effects of SNPs [28-30] either used higher concentration or conducted the experiment in solution culture. Nanoparticles have inhibitory effects in aqueous suspensions [15]. In our experiment seedling FW, DW, leaf area and root biomass declined with higher concentration or three weeks of exposure to 25 ppm SNPs in MS medium. SNPs in MS medium have inhibitory effects on growth of wheat seedling and are more pronounced with longer exposure.

3.3 Soil Applied Silver Nanoparticles and Seedling Growth

FW, DW and chlorophyll content of wheat seedlings in response to soil applied SNPs in pots is presented in Fig. 4 (A & B). All levels of SNPs viz. 25, 50 and 100 ppm significantly increased FW and DW over control. Nonetheless, 25 ppm SNPs remarkably increased FW and DW over control plants. Further increase in concentrations to 50 and 100 ppm progressively decreased FW and DW. Almost similar trend was observed for chlorophyll content in response to soil applied SNPs. Significantly higher total chlorophyll and chlorophyll a & b were accumulated in response to 25, 50 and 100 ppm as compared to control. Nonetheless, effect of 25 ppm SNPs was more pronounced and produced maximum chlorophyll a, b and total chlorophyll contents. Increase in SNPs concentration caused progressive reduction in chlorophyll contents of the seedlings.

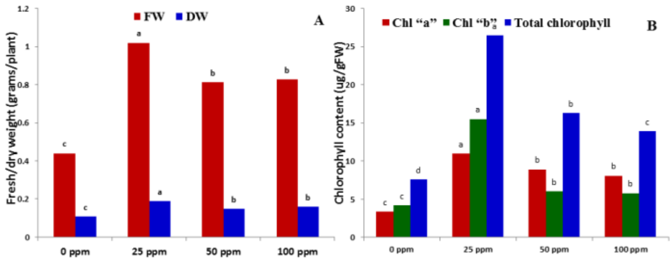

Fig. 4 Effect of soil applied silver nanoparticles on FW/DW (A) and chlorophyll content

Variable responses of different plants to SNPs have been reported by different investigators. SNPs induce significant changes at physiological and molecular level thereby affect plant growth. Soil applied SNPs positively affect growth of wheat. Application of 50 ppm SNPs increased plant height, fresh and dry weight, length of root and shoot of wheat seedlings [24] and fresh weight, root and shoot length, and vigor index and chlorophyll contents of seedlings of *Brassica juncea* [9]. SNPs (0.5 to 1000 ppm) can cause faster growth and 25-50 ppm has the potential to significantly increase plant height, fresh and dry weight over control in wheat [10]. SNPs at 50 ppm was observed to increase remarkably total chlorophyll, chl-a, chl-b, root FW in mug bean plants but did not affect shoot FW [24]. Application of 50 ppm nanosilver in combination with nitrogen and nitroxin increased weight and yield of potato tubers. We found significant increase in FW, DW and chlorophyll content of wheat with 25 ppm soil applied SNPs. Favorable effects of soil applied SNPs on growth may be due to less bioavailability and accumulation in plants thereby stimulating growth. Same concentration in MS medium had inhibitory effect after two weeks.

Several studies reported negative and unfavorable effects of SNPs. Significant reduction in root elongation, chlorophyll content, shoot and root fresh weight was noted on exposure of rice seedlings to SNPs. Chlorophyll, shoot fresh/ dry weight and root dry weight of *V. radiata* and *B. campestris* was significantly inhibited at 1000 ppm SNPs [29]. Absorbed AgNPs disrupt the thylakoid membrane structure and decreased chlorophyll content, inhibited root length and fresh weight [30]. Nonetheless, adverse effects are related with higher concentration and more bioavailability. All the studies reporting negative effects conducted experiments in solution or used higher concentration. Investigators who used SNPs in soil at lower concentration [24, 9] found positive effects of SNPs. SNPs had no severe toxic effects and enhanced peroxidase and catalase activity [16] whereas SNPs applied to duckweed (*Spirodela polyrhiza*) in nutrient solution significantly decreased plant biomass and chlorophyll content [28].

Effect of SNPs seems concentration dependent. Nanoparticles promote growth at low dose but retard growth at high dose [31]. Other nanoparticles also have similar effect. Iron based nanoparticles at low concentration promoted growth of maize but retarded at high concentration [32]. We also observed similar results effects of SNPs in our study. Higher concentrations had inhibitory effects while lower concentrations enhanced growth of wheat. Salama et al also reported similar results [33]. They observed that increasing concentration of SNPs from 20 to 60 ppm led to an increase in shoot and root lengths, leaf surface area, chlorophyll, carbohydrate and protein contents of common bean and corn. Additional increase in level of SNPs resulted in reduction of these parameters. Soil application of SNPs at low concentration can enhance growth of plants while SNPs applied through nutrient solution increase the bioavailability and accumulation in plants thereby inhibiting growth.

3.4 Soil Applied Silver Nanoparticles and Yield

Effect of SNPs on yield parameters is (Fig. 5) revealed significant differences among treatments.

Significantly more number of grains per spike was produced with 25 ppm SNPs, followed by 50 ppm in comparison to control. While a decreases in number of grains per spike was observed from 75 to 150 ppm. The 100-grain weight was significantly more for 25 and 125 ppm compared to other treatments and control. Grain weight obtained by other treatments was at par with control. It was observed that leaves of plants treated with 125 ppm remained green for longer time than any other treatment. Grain yield per pot was significantly higher with 25 ppm SNPs followed by 50 and 75 ppm than control and other concentrations of SNPs. Maximum yield reduction were recorded with SNPs at 150 ppm.

Increase in yield by application of nanoparticles has been postulated. Silver is an excellent growth simulator [11]. Effect of SNPs on wheat yield has not been reported so far. Highest yield and chemical composition was obtained with application of SNPs to mung [24]. Application of iron oxide nanoparticles increased grain yield of soybean [4]. We first time report highly favorable effects of SNPs at 25 and 50 ppm on number of grains/spike, 100-grain weight and grain yield per pot in wheat.

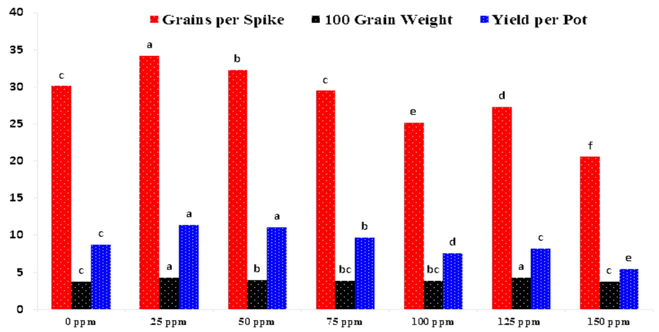

Fig. 5 Yield response of wheat to soil applied silver nanoparticles

4. Conclusion

SNPs had no effect on germination but increased number of seminal roots. Silver nanoparticles can stimulate wheat growth and yield. High concentration and long exposure of wheat seedlings to SNPs in nutrient medium result in negative effects. Soil applied 25 ppm SNPs had highly favorable growth promoting effects on wheat growth and yield. Judicious use of soil applied SNPs can improve yield of wheat crop. However further experiments are needed to explore precise concentration, suitable mode and best time of application to realize the growth and yield enhancing potential of SNPs for wheat and other crops in eco-friendly manner.

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