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GERMINATION STUDIES AND BIOCHEMICAL PROFILE IN SEEDS OF WHEAT EXPOSED TO MAGNESIUM NANOPARTICLES

A. Pathak [*], Rajwinder Kaur and Neetu Thakur

Goswami Ganesh Dutta Sanatan Dharma College, Sector 32, Chandigarh – 160030, India.

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Correspondence to Author:
Dr. Ashima Pathak

Department of Biotechnology
Goswami Ganesh Dutta Sanatan Dharma College, Sector 32,
Chandigarh – 160030, India.

E-mail: ashima.pathak@ggdsd.ac.in

ABSTRACT: Nanotechnology has gained a lot of importance and application in agriculture in the last decade. The present work was focused on the synthesis and characterization of magnesium oxide nanoparticles and on studying the effect of these nanoparticles on wheat (PBW-677 variety). Magnesium oxide nanoparticles were synthesized by chemical synthesis method and were characterised by using UV- Visible spectrophotometer. The seeds were treated with different concentrations (200 ppm, 500 ppm, and 1000 ppm) of nanoparticles. A Control group was also run along with in which the seeds were treated with tap water. The aim of the study was to see the effect of these nanoparticles on various parameters that include seed germination, estimation of chlorophyll content, and antioxidants. The present study showed that magnesium oxide nanoparticles increase seed germination, growth, phenolics, flavonoids, total antioxidants, chlorophyll and carotene and protein content of PBW-677 Wheat.

INTRODUCTION: Nanoparticles are widely being used in almost all spheres of life. It is gaining great importance in the agricultural system. Nano-particles are now thought of as the potential candidates for modulating the seeds' growth parameters and improving the quality of crop [1] and ultimately, the plants. Magnesium has an important role to play in photosynthesis as it is a component of chlorophyll. It is also involved in plant respiration and energy metabolism, including the generation of reactive oxygen species. However, globally, it is the most limiting nutrient in agriculture.

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Therefore in the present study, the effect of magnesium nano-particles on germination studies and various biochemical parameters was taken into consideration so that the nutrition can be regulated in seeds as well as in plants.

MATERIALS AND METHODS:

Synthesis of Magnesium oxide Nanoparticles: 5 g of magnesium sulphate ($MgSO_4$) was added to 100 ml of distilled water in a beaker, and 5 g of sodium hydroxide (NaOH) was added to 100 ml of distilled water in another beaker. $MgSO_4$ and NaOH solutions were mixed in 1:1 to make the total volume up to 100 ml. The solution was kept on a magnetic stirrer and was stirred for 2 h. Then the solution was placed in oven and heated at 100 ºC for 2 h. The precursor of magnesium oxide nanoparticles *i.e.*, magnesium hydroxide was produced. The precursor was kept in silica crucible and was placed in muffle furnace, then heated at 300 ºC for 3 h.

After 3 h dried Mg Oxide nanoparticles were obtained. The synthesis of magnesium oxide nanoparticles was confirmed [3] by using UV-Visible spectrophotometer.

Seed Germination: After surface sterilisation, the seeds were sown in the pots containing soil. Various sets of plants were maintained which include control seeds, seeds treated with magnesium sulphate salt and seeds treated with different concentrations of magnesium nanoparticle (*i.e.,* 200, 500 and 1000 ppm).

The nanoparticles were first suspended directly in distilled water and dispersed by ultrasonic vibration and then used to treat seeds.

20 seeds were germinated in each concentration of different groups. All these treatments continued for a period of 3 weeks.

The number of seeds germinated each day was recorded after every two days up to 10^{th} day of experiment. On 21^{st} day, seedlings were separated into shoots and roots.

Plantlets were re- moved from the soil and roots were carefully washed to remove soil particles. Washed roots were analysed for root length. Leaf length, leaf lamina, shoot length and root length were also measured using ordinary meter scale.

Biochemical Estimations: After noting down various germination parameters, the aqueous extracts were prepared and various biochemical estimations were performed.

Chlorophyll estimation was done by the method of Kamble *et al.* [4]. Phenolic and flavonoid content was determined by the method of Chandraetal [5]. Ascorbic acid determination was done following the method of Hassan *et al.* [6] Total antioxidant capacity was assayed by the method of Rubio *et al.* [7] Lowry method [8] was used to determine the protein content in the aqueous extract of seeds.

Results: MgO nanoparticles were successfully synthesized using magnesium sulphate and were confirmed by using a UV-VISIBLE spectrophotometer at a wavelength in the range of 200-500 nm. The UV-VISIBLE spectrum of these nanoparticles showed a maximum peak at 305.6 nm, as shown in **Fig. 1**, which indicates that MgO nanoparticles are synthesized successfully.

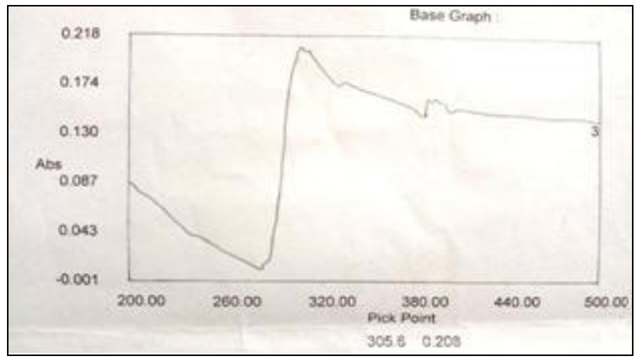

FIG. 1: PEAK POINT AT 305.6 NM

Seed Germination: Leaf lamina, leaf length, and shoot length were found to increase when the seeds were treated with magnesium sulphate salt and with 200 ppm nanoparticles. But treatment with 500 ppm nanoparticles led to a decrease in these parameters. Seeds treated with 1000 ppm of nanoparticles showed the least germination **Table 1**. However, the root length was maximum when treated with magnesium sulphate, and was found to decrease on increasing the concentration of nanoparticles up to 500 ppm and then increases when treated with 1000 ppm solution.

TABLE 1: SEED GERMINATION PARAMETERS IN PBW-677 VARIETY OF WHEAT

Treatment	Lamina	Leaf Length	Shoot length	Root length
Control	0.34 ± 0.02	12.13 ± 0.57	5.35 ± 0.13	9.42 ± 1.59
$MgSO_4$ salt	0.35 ± 0.02	12.4 ± 0.32	6.93 ± 0.14	11.34 ± 0.77
200 ppm NP	0.38 ± 0.01	17.1 ± 0.82	5.76 ± 0.35	8.47 ± 1.52
500 ppm NP	0.37 ± 0.02	14.31 ± 0.85	6.16 ± 0.15	6.98 ± 0.58
1000 ppm NP	0.36 ± 0.06	13.08 ± 1.45	5.5 ± 0.26	7.38 ± 0.50

Values are expressed as Mean ± SD.

Chlorophyll and Carotene Content: When the seeds were treated with different concentrations of nanoparticles (200, 500, 1000 ppm), chlorophyll a, chlorophyll b, total chlorophyll, and carotene content was found to be high at 200 ppm, decreased at 500 ppm, and reached a maximum in the plants treated with 1000 ppm **Table 2**.

TABLE 2: CHLOROPHYLL AND CAROTENE CONTENT IN PBW-677 VARIETY OF WHEAT

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotene
Control	6.53±0.82	11.69±0.65	16.88±1.63	0.695±0.06
MgSO4 salt	6.5±0.104	11.82±0.39	14.82±1.002	0.701±0.01
200 ppm NP	6.27±0.34	13.42±1.12	19.69±1.47	0.798±0.07
500 ppm NP	5.05±0.21	9.28±0.38	14.32±0.6	0.409±0.016
1000 ppm NP	7.44±0.71	14.91±1.36	22.67±1.56	0.868±0.05

Values are expressed as Mean ± SD.

Phenolic Content: Upon treatment with different concentrations of nanoparticles, the plantlets treated with 1000 ppm show maximum phenolic content. However, $MgSO_4$ salt caused a decrease in the phenolic content **Table 3**.

TABLE 3: PHENOLIC CONTENT IN PBW-677 VARIETY OF WHEAT

Treatment	Phenolics
Control	0.881±0.01
MgSO4 salt	0.697±0.01
200 ppm NP	0.957±0.008
500 ppm NP	0.913±0.01
1000 ppm NP	1.011±0.01

Values are expressed as Mean ± SD

Flavonoid Content: When the plantlets were treated with $MgSO_4$, the flavonoid content was found to increase.

However, upon treatment with 200 ppm solution of nanoparticles, the flavonoid content started decreasing, and upon treatment with 500 ppm solution, the flavonoid content again increased gradually and reached maximum when the plants were treated with 1000 ppm solution **Table 4**.

TABLE 4: FLAVONOID CONTENT IN PBW-677 VARIETY OF WHEAT

Treatment	Flavanoid
Control	0.51±0.012
$MgSO_4$ salt	0.57±0.013
200 ppm NP	0.37±0.014
500 ppm NP	0.28±0.007
1000 ppm NP	0.39±0.019

Values are expressed as Mean ± SD.

Ascorbic Acid Content: The ascorbic acid content was found to increase when the seeds were treated with magnesium sulphate.

Upon treatment with solution of nanoparticles, the ascorbic acid was found to in- crease at 200 ppm concentration but the concentration of ascorbic acid was found to decrease with increasing concentration of nano-particles **Table 5**.

TABLE 5: ASCORBIC ACID CONTENT IN PBW-677 VARIETY OF WHEAT

Treatment	Ascorbic acid
Control	150.33±3.14
MgSO4 salt	186.95±1.22
200 ppm NP	152.46±7.02
500 ppm NP	151.69±11.24
1000 ppm NP	146.47±2.01

Values are expressed as Mean ± SD.

Total Antioxidant Capacity: When the plantlets were treated with $MgSO_4$, total antioxidant was found to increase.

Upon treatment with a solution of nanoparticles, the antioxidant capacity was found to decrease gradually at low concentration of nano-particles, and maximum antioxidants were present in plants treated with 1000 ppm solution of nano-particles **Table 6**.

TABLE 6: TOTAL ANTIOXIDANT CAPACITY IN PBW-677 VARIETY OF WHEAT

Treatment	Total Antioxidant
Control	1.905±0.04
MgSO4 salt	2.016±0.09
200 ppm NP	1.86±0.06
500 ppm NP	1.84±0.02
1000 ppm NP	2.006±0.34

Values are expressed as Mean ± SD

Protein Content: Protein content was found to decrease when the seeds were treated with $MgSO_4$. Protein content was found to decrease in nano-particles treated group upto 500 ppm, and then it was found to increase at 1000 ppm nanoparticle treatment **Table 7**.

TABLE 7: TOTAL PROTEIN CONTENT IN PBW-677 VARIETY OF WHEAT

Treatment	Protein
Control	82.61±3.34
MgSO4 salt	60.6±3.19
200 ppm NP	79.80±1.35
500 ppm NP	70.76±2.01
1000 ppm NP	82.26±2.05

Values are expressed as Mean ± SD

DISCUSSION: The study was performed to examine the effects of MgO nanoparticles on PBW-677 Wheat. The positive effect of MgO nanoparticle on seed germination at 200 ppm can be due to the reason that the size of nanoparticle is small, which enable them to penetrate the seed coat and change the biochemical characteristics to avoid the metal stress [9].

Another reason behind the increase in seed germination can be higher water uptake by the seeds, which is due to the ability of nanoparticle to penetrate the seed coat [10]. The chlorophyll and carotene content of plants increased with the treatment of MgO nanoparticles due to the enzymes that get activated with the help of Mg.

There is a significant increase in protein content when the plants are treated with MgO nano-particles. Upon treatment with MgO nano-particles, plants do not show an increase in ascorbic acid content as the nanoparticle has no significant effect on plants if soil is rich in $MgSO_4$. Plants grown in the presence of heavy metals produce phenolics and flavonoids so that they can protect themselves against toxicity caused by metals [11, 12].

Plants treated with higher concentrations (1000 ppm) of nanoparticles lead to the production of higher flavonoids and phenolic content. The reason behind this can be stress caused due to heavy metal accumulation inside the plants [12]. Plants grown in the presence of high concentration (1000 ppm) of MgO nanoparticle show high total antioxidant which is due to resistance of plants to heavy metals. This increase in antioxidant may lead to an increase in triggering the production of secondary metabolites in response to nanoparticle stress [12].

CONCLUSION: The study revealed that MgO nanoparticles have a positive effect on plant growth and biochemical parameters of plants.

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CONFLICTS OF INTEREST: Nil

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