NanoWorld Journal

https://doi.org/10.17756/nwj.2022-s1-002

Research Article

Open Access

Effect of SiO_2 Nanoparticles and MWCNTs Priming on Agroeconomic Important Traits and Biochemical Profile of *Hordeum vulgare* L.: A Comparative Study

Rakhi Poonia[1], Rohit Jain[2], Shamshad Ul Haq[1] and Sumita Kachhwaha[1*]

[1]*Department of Botany, University of Rajasthan, Jaipur, India*

[2]*Department of Biosciences, Manipal University Jaipur, India*

[*]Correspondence to:
Sumita Kachhwaha
Department of Botany, University of Rajasthan, Jaipur, India
E-mail: sumitakachhwaha@gmail.com

Received: August 23, 2022
Accepted: September 29, 2022
Published: October 01, 2022

Citation: Poonia R, Jain R, Ul Haq S, Kachhwaha S. 2022. Effect of SiO_2 Nanoparticles and MWCNTs Priming on Agroeconomic Important Traits and Biochemical Profile of *Hordeum vulgare* L.: A Comparative Study. *NanoWorld J* 8(S1): S6-S10.

Published by United Scientific Group

Abstract

In present study, effect of seed priming with nanomaterials (silicon dioxide nanoparticles; SiO2 NPs and Multi-Walled Carbon Nanotubes; MWCNTs) on agronomic traits of barley (*Hordeum vulgare* L.) was studied. Barley seeds were primed separately with different concentrations (25 - 125 μg/ml) of SiO_2 NPs and OH functionalized MWCNTs. The germination potential increased at higher concentration (50 - 125 μg/ml) of nanomaterials and was maximum in seeds primed with SiO_2 NPs. Plant height showed strong positive correlation (r = 0.75) with SiO_2 NPs concentration and tallest plants (upto 85 cm) developed from seeds treated with higher concentration of SiO_2 NPs (125 μg/ml) and OH-MWCNTs (75 μg/ml). Further, average length of flag leaf (27.8 cm), peduncle (14.4 cm) and spikelet (16.7 cm) were maximum at 25 μg/ml OH-MWCNTs 100 μg/ml SiO_2 NPs and 100 μg/ml OH-MWCNTs respectively. The average grains per spikelet was 4 to 6 times higher in plants developed from SiO_2 NPs primed seeds, while biomass was approximately 1.2 to 1.4 times higher in OH-MWCNTs primed seeds. Maximum yield of 179 grains per spikelet and 2084 mg fresh weight was recorded at 50 - 75 μg/ml SiO_2 NPs and 75 μg/ml OH-MWCNTs respectively. Bio-chemical profiling further confirmed better response of the plants generated from seeds primed with moderate to high concentrations of SiO_2 NPs. The free radical scavenging activity was higher in vegetative stages of all the treated plants and maximum activity was exhibited by plants of SiO_2 NPs treated seeds. Therefore, priming of barley seeds with SiO_2 NPs can be an effective non-invasive approach to enhance growth and yield of such an economically important crop.

Keywords

Nanopriming, Barley, Antioxidant enzymes, DPPH assay, Agronomic yield

Introduction

Hordeum vulgare L is an agro-economically important crop of family Poaceae and one of the 5 major cereals cultivated worldwide. Barley ranks 4th among cereals and 11th among overall crops in terms of its production and is grown across wide geographical range [1]. It is not only used as a source of human food and animal fodder but is also an important substrate in brewing industry mainly for malt production [2]. As per the GMR 533 of 23/06/2022 released by International Grains Council (IGC), the estimated world barley production is 146.1 million tons against the total consumption of 150.8 million tons for 2021/22, out of which ~69% is used for animal feed and ~19% in industries (mainly brewing industries), while only ~4% is used for food.

In India, barley is generally grown under rainfed and/or limited irrigation conditions on resource poor to problematic and marginal soils of northern plains and hills [3]. These cultivation conditions make Rajasthan largest producer of barley in the country, accounting for ~47% of the total annual barley production (USDA Foreign Agriculture Services, Barley Production in India, 2021). The annual barley production has significantly increased over past decade due to increased alcoholic beverage consumption and feed/fodder requirement [4, 5]. Barley has been rendered an important source of feed and fodder over other available sources (such as oat and sugarcane) not only due to its faster growth and ability to grown in water scarce regions, but also due to utilization of both green forage as well as grains for animal feed/fodder [5]. Past decade has witnessed several studies towards development of varieties with improved (agronomic) traits for different uses (including feed/fodder, malt and food) suitable for various agro-climates. Despite of availability of several improved varieties, availability of less cultivable land and the increasing rift between barley demand and supply has generated the need for development of strategies to maximize yield of the crop.

Nanotechnological interventions have extensively been used as an efficient non-invasive approach in agriculture for disease protection, to improve yield and many more [6]. Use of engineered nanomaterials (ENMs) as fertilizer, pesticide, carrier for genetic material/RNA/protein, sensors for detection of contaminants and toxic compounds, plant growth promotor, etc. have been variously reported [6]. Priming of seeds with ENMs has emerged as a newer and user-friendly approach to improve the agronomic traits of the crop.

Out of the numerous types of ENMs available, OH-MWCNTs and SiO_2 NPs are the most explored in terms of their uses/applicability in agriculture. Priming of seeds with these two ENMs have been reported to enhance yield, growth, chlorophyll content, photosynthetic activity and improve stress tolerance of various important crops including oat, wheat [7, 8], rice, great millet [9], and many more.

Therefore, in the present study effect of seed priming with OH-MWCNTs and SiO_2 NPs on agronomic traits and biochemical properties of barley cultivated *ex-vitro* has been studied for its entire life cycle. To our knowledge this is the first report on comparative effect of nano priming of seeds with OH-MWCNTs and SiO_2 NPs on the field performance of barley.

Material and Methods

Collection of plant material

Certified variety of barley seeds (RD 2552) was procured from Rajasthan Agriculture Research Institute, Durgapura, Rajasthan, India. The variety was developed by Agriculture Research Institute, SKRAU, Durgapura, Rajasthan, India and was released by CVRC in 1999. This variety have been rendered highly suitable and adaptable to saline conditions; thus, seeds of this variety were used for the present study.

Priming of seeds with SiO_2 NPs & OH-MWCNTs

Solutions containing different concentrations of nanomaterials (25 ppm, 50 ppm, 75 ppm, 100 ppm, and 125 ppm) were prepared in deionized water. The SiO_2 NPs containing solutions were prepared by mixing appropriate concentration in the desired volume followed by thorough mixing. However, in case of OH-MWCNTs an homogenous suspension was prepared through sonication at 33 KHz, 50 W for about 30 min with 3 sec ON and 1 sec OFF pulse. Seeds (5 g) were soaked in each of these nanomaterial solutions for overnight in an incubator shaker at 100 rpm. Seeds soaked in deionized water (with-out any nanomaterial) were used as control. After overnight soaking, the seeds were thoroughly washed with tap water to remove the excess nanomaterial, dried on a blotting sheet for 24 h at room temperature.

Experimental design

Primed seeds were sown during Rabi season (Oct - Feb) in pots (13 mm diameter) containing soil and organic manure (1:1). The pots were kept in natural condition and were watered daily up to field capacity. Total 10 plants per pot were maintained in triplicates (n = 30) for each treatment. Proper agronomic management practices were followed throughout the experiment to ensure optimum growth in the plants generated from both untreated and nano-primed seeds.

Plant vigour and yield analysis

The agronomic important traits including germination efficiency, plant height, grains per spikelet, fresh and dry weight and length of shoot, root, flag leaf, spikelet and peduncle were recorded after completion of the life cycle of the plants developed from seeds primed with different types and concentrations of nanomaterials. These observations were used to analyze the effect of seed priming with nanomaterial(s) on plant vigour and yield.

Biochemical analysis

The plant samples of each treatment were harvested during vegetative and reproductive stages for biochemical profiling. The chlorophyll estimation was done as per the method given by Arnon [10], free radical scavenging activity and antioxidant enzyme activities namely peroxidase, catalase and superoxide dismutase were performed as per our previously reported methods [11].

Statistical analysis

All the experiments were performed in triplicates. The results were analyzed using SPSS (IBM, US). The means were compared through univariate tests and the significant differences were highlighted using Duncan's post-hoc test. Further pearson's correlation test was also performed to check the influence of nanomaterial and concentration on the variations in different morpho-biochemical parameters. All the tests were performed at 5% level of significance ($P < 0.05$).

Results

Effect on seed germination

Better germination efficiency was recorded in plants generated from seeds treated with SiO_2 NPs than those with OH-MWCNTs. However, the germination pattern was similar among plants generated from both nanomaterials (Table 1). The germination efficiency increased upto 18% and 36% in seeds treated with OH-MWCNTs and SiO_2 NPs, respectively with maximum germination (85 ± 8.7 %) in seeds primed with 100 ppm SiO_2 NP. A significant positive correlation (r = 0.529) at 0.01 level was noted between the germination percentage and SiO_2 NPs concentration used for priming of seeds, while no such significant correlations were detected in case of OH-MWCNTs primed seeds.

Effect on plant vigour

Both shoot and root length increased in plants generated from nano primed seeds than those from untreated (control) seeds (Table 2). The maximum shoot elongation (18.1 ± 1.6 cm) was noted in plants obtained from 75 ppm SiO_2 NPs treated seeds. No significant correlation was detected between the shoot elongation and concentration of nano particles, however shoots obtained from SiO_2 NPs primed seeds were upto 14% taller than those obtained from OH-MWCNT primed seeds. The root length increased upto 3.6 times and 1.5 times in plants obtained from OH-MWCNTs and SiO_2 NPs treated seeds, respectively. Roots of plants treated with SiO_2 NPs were longer (1.9 - 1.25 times) than that with OH-MWCNTs, with longest roots (12.5 ± 1.3 cm) at 50 ppm SiO_2 NPs.

The plant height significantly increased in seeds primed with OH-MWCNTs(1.4 times) and SiO_2 NPs (1.6 times) than their respective controls. No significant difference was recorded in the height of the plants generated from both types of nanomaterials (Table 3, Figure 1). Correlation analysis revealed strong positive and significant correlation (r = 0.75) between the plant height and concentration of SiO_2 NPs used for seed priming indicating an increase in the plant height with increasing concentration of SiO_2 NPs.

Flag leaf length of all the plants was similar irrespective of the treatment given to the seeds prior to germination (Table 4). Similarly, the spikelet and peduncle length of plants generated from the nano primed seeds were similar to that of plants generated from untreated seeds (Table 4). The correlation analysis revealed that the concentration of nanomaterial used for seed priming had no significant effect on the flag leaf, spikelet, and peduncle length of the plant.

Table 1: Effect of OH-MWCNTs and SiO_2 NPs seed priming on germination efficiency.

Conc. (ppm)	Germination efficiency (%) ± SE*	
	OH-MWCNTs	SiO_2 NPs
Control	55 ± 6.5^{bcd}	62.5 ± 6.3^{abc}
25	52.5 ± 4.8^{cd}	62.5 ± 4.8^{abc}
50	50 ± 4.1^{cd}	72.5 ± 4.8^{abc}
75	37.5 ± 4.8^{d}	67.5 ± 4.8^{abc}
100	55 ± 16.6^{bcd}	85 ± 8.7^{a}
125	65 ± 8.7^{abc}	77.5 ± 2.5^{ab}

*Different letters in superscript indicate significantly different values at alpha = 0.05.

Table 2: Effect of OH-MWCNTs and SiO_2 NPs shoot & root length of the seedlings.

Conc. (ppm)	Shoot Length (cm) ± SE*		Root Length (cm) ± SE*	
	OH-MWCNTs	SiO_2 NPs	OH-MWCNTs	SiO_2 NPs
Control	4.8 ± 0.2^{d}	9.6 ± 1.3^{c}	2.8 ± 0.2^{e}	8.4 ± 0.8^{bc}
25	9.4 ± 0.5^{c}	14.5 ± 1.6^{b}	4.7 ± 0.1d^{e}	10.1 ± 0.6^{ab}
50	14.3 ± 0.3^{b}	15.2 ± 1.4^{ab}	7.7 ± 0.2^{bcd}	12.5 ± 1.3^{a}
75	16 ± 0.3^{ab}	18.1 ± 1.6^{a}	10 ± 0.1^{ab}	8.9 ± 1.5^{bc}
100	15.1 ± 0.4^{ab}	14.8 ± 1.2^{b}	5.8 ± 0.2^{cde}	9.6 ± 2.3^{ab}
125	4 ± 0.5^{d}	13 ± 1.2b	5.8 ± 0.1^{cde}	10.3 ± 1.1^{ab}

*Different letters in superscript indicate significantly different values at alpha = 0.05.

Table 3: Effect of OH-MWCNTs and SiO_2 NPs plant height.

Conc. (ppm)	Plant Height (cm) ± SE*	
	OH-MWCNTs	SiO_2 NPs
Control	61.9 ± 3.8^{d}	52.9 ± 3.2^{e}
25	73.8 ± 2.8^{bc}	69.5 ± 3.9^{cd}
50	74 ± 2.6^{bc}	76.3 ± 1.2^{abc}
75	84.8 ± 2.3^{a}	81.2 ± 1.4^{ab}
100	68.4 ± 2.6^{cd}	81.8 ± 2.1^{ab}

*Different letters in superscript indicate significantly different values at alpha = 0.05.

The yield was determined in terms of number of grains per spikelet and weight (fresh and dry) of the plants (Table

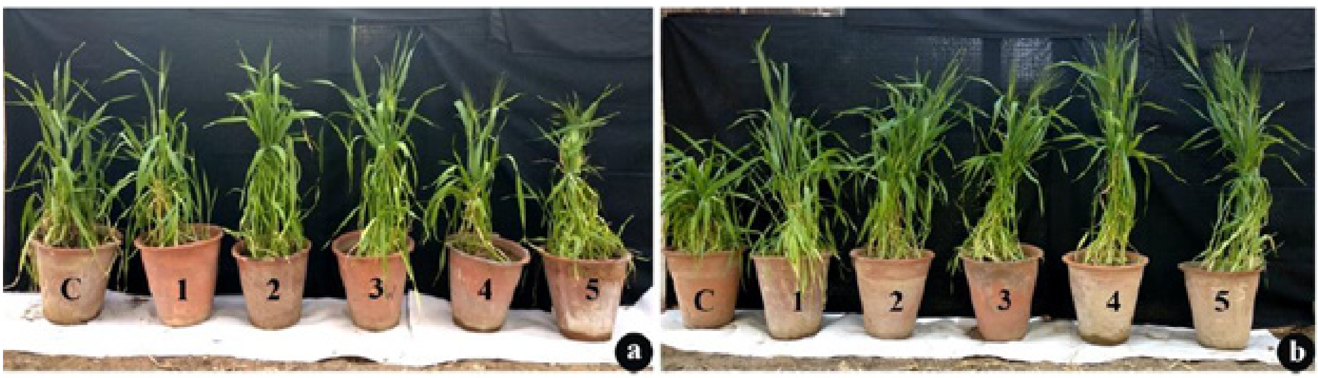

Figure 1: Effect of seed priming with (a) OH-MWCNTs and (b) SiO_2 NPs on morphological traits of barley after 60 days. *C - control, 1 - 25 ppm, 2 - 50 ppm, 3 - 75 ppm, 4 - 100 ppm, 5 - 125 ppm.*

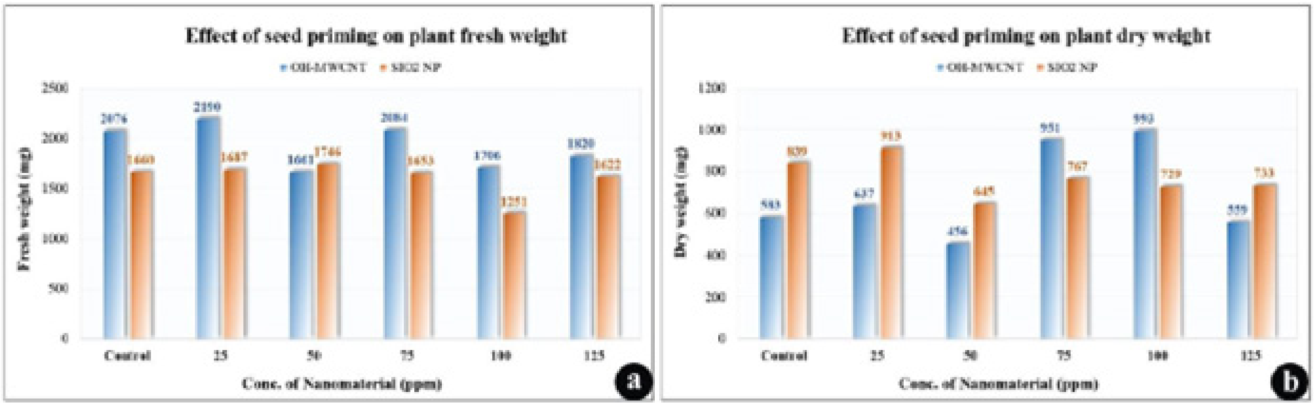

Figure 2: Effect of seed priming with OH-MWCNTs and SiO_2 NPs on (a) fresh weight and (b) dry weight of barley.

Table 4: Effect of OH-MWCNTs and SiO_2 NPs flag leaf, spikelet, and peduncle length.

Conc. (ppm)	**Flag leaf Length (cm) ± SE***		**Spikelet Length (cm) ± SE***		**Peduncle Length (cm) ± SE***	
	OH-MWCNTs	SiO_2 NPs	OH-MWCNTs	SiO_2 NPs	OH-MWCNTs	SiO_2 NPs
Control	21.1^{a}	21.1^{a}	16.4 ± 0.6^{ab}	16.4 ± 0.6	10.1 ± 0.4^{c}	10.1 ± 0.4^{c}
25	27.9 ± 3.9^{a}	27.2 ± 0.3^{a}	15.8 ± 1.1^{abc}	16.4 ± 0.5^{a}	10.6 ± 0.5^{bc}	13.8 ± 1.9 ^{ab}
50	21.9 ± 3.1^{a}	21.7 ± 2.1^{a}	14.6 ± 0.4^{c}	14.7 ± 0.3^{bc}	11 ± 0.7b^{c}	9.8 ± 2.4^{c}
75	22.9 ± 1.4^{a}	23 ± 2.7^{a}	16 ± 0.3^{abc}	15.1 ± 0.4^{abc}	9.4 ± 0.6^{c}	10.3 ± 1.5^{c}
100	26.5 ± 1.9^{a}	23.6 ± 1.8^{a}	14.5 ± 0.6^{c}	16.7 ± 0.2^{a}	14.4 ± 1.1^{a}	8.9 ± 2^{c}
125	25.7 ± 1.2^{a}	21.2 ± 2.1^{a}	16.3 ± 0.4^{ab}	15.5 ± 0.3^{abc}	10.9 ± 0.7^{bc}	12.2 ± 4.2^{abc}

Table 5. Effect of OH-MWCNTs and SiO_2 NPs on grain yield.

Conc. (ppm)	**No. of grains per spikelet ± SE***	
	OH-MWCNTs	**SiO_2 NPs**
Control	35.3 ± 2^{bc}	33.25 ± 3.3^{c}
25	29 ± 2.7^{c}	39.25 ± 5.7^{abc}
50	29.5 ± 2.5^{c}	44.75 ± 2.7^{ab}
75	48.25 ± 4^{a}	44.75 ± 2.8^{ab}
100	28.75 ± 2.1^{c}	35.75 ± 3.5^{bc}
125	35.5 ± 1.9^{bc}	37 ± 5.3^{bc}

*Different letters in superscript indicate significantly different values at alpha = 0.05.

Table 6: Effect of OH-MWCNTs and SiO_2 NPs on grain yield.

Conc. (ppm)	**No. of grains per spikelet ± SE***	
	OH-MWCNTs	**SiO_2 NPs**
Control	35.3 ± 2^{bc}	33.25 ± 3.3^{c}
25	29 ± 2.7^{c}	39.25 ± 5.7^{abc}
50	29.5 ± 2.5^{c}	44.75 ± 2.7^{ab}
75	48.25 ± 4^{a}	44.75 ± 2.8^{ab}
100	28.75 ± 2.1^{c}	35.75 ± 3.5^{bc}
125	35.5 ± 1.9^{bc}	37 ± 5.3^{bc}

*Different letters in superscript indicate significantly different values at alpha = 0.05.

5, Figure 2). The grain yields increased upto ~1.35 times in plants developed from nano primed seeds, such that maximum yield was recorded at 75 ppm for both OH-MWCNTs and SiO_2 NPs treated seeds. The biomass of plants treated with OH-MWCNTs was higher (1 - 1.4 times) than those from SiO_2 NPs, with maximum biomass obtained at 25 ppm OH-MWCNTs (Figure 2).

Effect on chlorophyll content

Chlorophyll content increased upto 1.7 times in plants generated from seeds primed with OH-MWCNTs than the control, whereas no significant increase was observed in plants generated from SiO_2 NPs treated seeds (Figure 3). The chlorophyll content in-creased with increasing concentration of OH-MWCNTs (≤ 75ppm), while it decreased with increasing concentration of SiO_2 NPs (≤ 75 ppm).

Effect on free radical scavenging activity

Antioxidant activity was determined in terms of DPPH radical scavenging capacity of the plants generated from different nano-treated and untreated seeds. The activity was higher (1.45 times) in plants at reproductive stage than in their vegetative stage. The activity was variable and did not show any significant pattern w.r.t changes in concentration or type of nanomaterial used (Figure 4).

Effect on antioxidant enzyme activity

Superoxide dismutase (SOD) activity was higher in reproductive stage than in vegetative stage (Figure 5). Priming of seeds with OH-MWCNTs (25 - 75 ppm) and SiO_2 NPs (50 - 75 ppm) at lower concentrations reduced the SOD activity by

2.3 and 1.7 times, respectively in vegetative stage, whereas no such pattern was noted during reproductive stage of the plants.

Conclusion

The present study aimed to improve agronomic traits of barley through priming of seeds using two different types of nanomaterials. Priming of seeds not only improved the germination potential of the seeds, but it also improved the plant vigour in terms of plant height and length of spikelet, peduncle, and flag leaf. The yield and biomass of the plants developed from seeds primed with nanomaterials was also significantly higher than that of plants developed on non-primed (control) seeds. Among various seed treatments, priming of seeds with SiO_2 NPs at low to moderate levels (25 - 75ppm) was the most effective method for improving agronomic traits of barley. Further, low to moderate levels of nanomaterials also reduced stress, increased chlorophyll content, and enhanced free radical scavenging activity of the plants. These findings thus present first report on the comparative study on the effect of priming of seeds with different types of nanomaterials on complete life cycle of barley in field. The results of this study can be further used to conduct larger field trials for nano-priming seeds of barley and can be used to develop economically viable and efficient strategies for improving crop productivity without using invasive techniques.

Acknowledgment

Authors are thankful to CSIR for providing fellowship to Rakhi Poonia. Rajasthan Agriculture Research Institute (RARI), Durgapura, Jaipur, Rajasthan, India is acknowledged for providing barley seeds for this study. Necessary facilities provided under MHRD sponsored Rashtriya Uchchatar Shiksha Abhiyan (RUSA 2.0) project, component 10: thematic project 3 awarded to Prof. Sumita Kachhwaha, Department of Botany, University of Rajasthan, Jaipur, India is highly acknowledged.

References

1. Distelfeld A, Avni R, Fischer AM. 2014. Senescence, nutrient remobilization, and yield in wheat and barley. *J Exp Bot* 65(14): 3783-3798. https://doi.org/10.1093/jxb/ert477
2. Yadav T, Kothari S, Kachhwaha S. 2011. Evaluation of regeneration potential of mature embryo derived callus in Indian cultivars of barley (*Hordeum vulgare* L.). *J Plant Biochem Biotechnol* 20(2): 166-172. https://doi.org/10.1007/s13562-011-0042-9
3. Sharma N. 2013. Suitability of barley varieties for cultivation in transitional plain of Luni Basin of Rajasthan. *Indian Journal of Plant Genetic Resources* 26(3): 235-237.
4. Kharub AS. 2017. Barley research in India: challenges and opportunities. Haryana, India: Indian Council of Agricultural Research, Indian Institute of Wheat and Barley Research (ICAR-IIWBR).
5. Kharub AS, Singh Verma RP, Kumar D, Kumar V, Selvakumar R, et al.: 2013. Dual purpose barley (*Hordeum vulgare* L.) in India: Performance and potential. *J Wheat Res* 5(1): 55-58.
6. Dhingra P, Sharma S, Singh KH, Kushwaha HS, Kumar J, et al. 2022. Seed priming with carbon nanotubes and silicon dioxide nanoparticles influence agronomic traits of Indian mustard (*Brassica juncea*) in field experiments. *J King Saud Univ Sci* 34(4): 102067. https://doi.org/10.1016/j.jksus.2022.102067
7. Jangir H, Bharadwaj A, Srivastava G, Das M. 2020. Fertilizer-free cultivation of wheat in nutrient-deficient soil by treating the seeds with nanopyrite. *Nanotechnol Environ Eng* 5(9): 1-16. https://doi.org/10.1007/s41204-020-00072-2
8. Mushtaq A, Jamil N, Riaz M, Hornyak GL, Ahmed N, et al. 2017. Synthesis of silica nanoparticles and their effect on priming of wheat (*Triticum aestivum* L.) under salinity stress. *Biological Forum – An International Journal* 9(1): 150-157.
9. Soares C, Branco-Neves S, de Sousa A, Azenha M, Cunha A, et al.: 2018. SiO_2 nanomaterial as a tool to improve *Hordeum vulgare* L. tolerance to nano-NiO stress. *Sci Total Environ* 622517-525: 517-525. https://doi.org/10.1016/j.scitotenv.2017.12.002
10. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* 24(1): 1-15. https://doi.org/10.1104/pp.24.1.1
11. Jatav PK. Verma R, Kothari SL, Jain R, Kachhwaha S. 2021. Relative morpho-physiological responses of millets and oats against lead toxicity. *Environ Exp Bot* 192: 104658. https://doi.org/10.1016/j.envexpbot.2021.104658