Int. J. Nano Dimens., 12 (2): 128-134, Spring 2021

ORIGINAL ARTICLE

Physiological and molecular responses of wheat following the foliar application of Iron Oxide nanoparticles

Elham Rostamizadeh[1], Alireza Iranbakhsh[1,], Ahmad Majd[2], Sedigheh Arbabian[2], Iraj Mehregan[1]*

[1]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Biology, Tehran North Branch, Islamic Azad University, Tehran, Iran*

Received 18 August 2020; revised 30 December 2020; accepted 12 January 2021; available online 22 January 2021

Abstract

This study investigates the possible effects of iron oxide nanoparticles (FeNPs) on plant growth, expression of *bZIP*, *DREB*, and *WRKY1*, and biofortification efficacy in wheat (*Triticum aestivum*). The seedlings were treated with bulk iron oxide (bulk-Fe) or FeNPs at 100, 200, 300, and 400 mgL^{-1}. FeNPs significantly improved the fresh and dry weights of shoot and root compared to the control. Likewise, different concentrations of bulk-Fe caused an increase in biomass accumulation in shoot and root. Moreover, Fe content was increased in response to the foliar application of FeNPs and bulk-Fe. The expression of *bZIP*, *DREB*, and *WRKY1* in the FeNP-treated plants was markedly up-regulated compared to the control. The increase in Fe contents and biomass, as well as upregulation in *bZIP*, *DREB*, and *WRKY1* genes, indicate that FeNPs could be a promising strategy to encounter iron deficiency in the human diet and to improve plant protection against biotic and abiotic stress conditions.

Keywords: *bZIP; DREB; Iron Oxide Nanoparticles; Plant Nutrition; Transcription Factor; Triticum Aestivum; WRKY1.*

How to cite this article

RostamizadehE., Iranbakhsh A.R., Majd A., Arbabian S., Mehregan I. Physiological and molecular responses of wheat following the foliar application of Iron Oxide nanoparticles. Int. J. Nano Dimens., 2021; 12(2): 128-134.

INTRODUCTION

Nanotechnology has gained increasing importance for the utilization in various practical applications including agriculture (as a nano fertilizer and nano pesticide), pharmacology, electronics, and medicine [1-9]. Iron is one of the essential nutrients for adequate plant growth, performing different cellular processes, including photosynthesis. It is also present in the Fe(II)/2-oxoglutarate-dependent oxygenases and cytochrome P450s as a cofactor [10]. Iron deficiency symptoms in plants occur due to low solubility of Fe^{3+} ion in the soil, resulting in iron deficiency in human and animal diets [11]. Fe deficiency in calcareous soils is a vital factor that can reduce plant growth and crop yield leading to lower quality of crops. The application Fe fertilizer is the most beneficial method to ameliorate Fe deficiency in plants [12].

Triticum aestivum is one of the widely consumed crops all over the world owing to its nutritional values for animals and humans [11]. Foliar spraying is one of the most effective methods that provide a functional approach to improve crop yield as well as to increase plant stress tolerance. Growing evidence demonstrated that iron oxide nanoparticles (FeNPs) were more useful in improving plant growth than the bulk-Fe [11, 13]. It has been reported that FeNPs have an effective impact on plant growth but dependent on the dose, plant species, and exposure time manners [7]. Furthermore, Iannone *et al.* [14] showed that the application of Fe_3O_4NPs (up to 20 mgL^{-1}) in the hydroponic did not influence lipid peroxidation and growth in *Triticum aestivum* whereas it enhanced the biomass application of 2000 mgL^{-1} Fe_3O_4 NPs [15]. Moreover, Pariona *et al.* [16] indicated that the application of Fe_2O_3 and $5Fe_2O_3{\cdot}9H_2O$ nanoparticles improved maize growth. Likewise, another study indicated that Fe_2O_3 nanoparticles were found to affect the seedling growth of *Citrullus lanatus* [17]. However, little

* Corresponding Author Email: *iranbakhsh@iau.ac.ir*

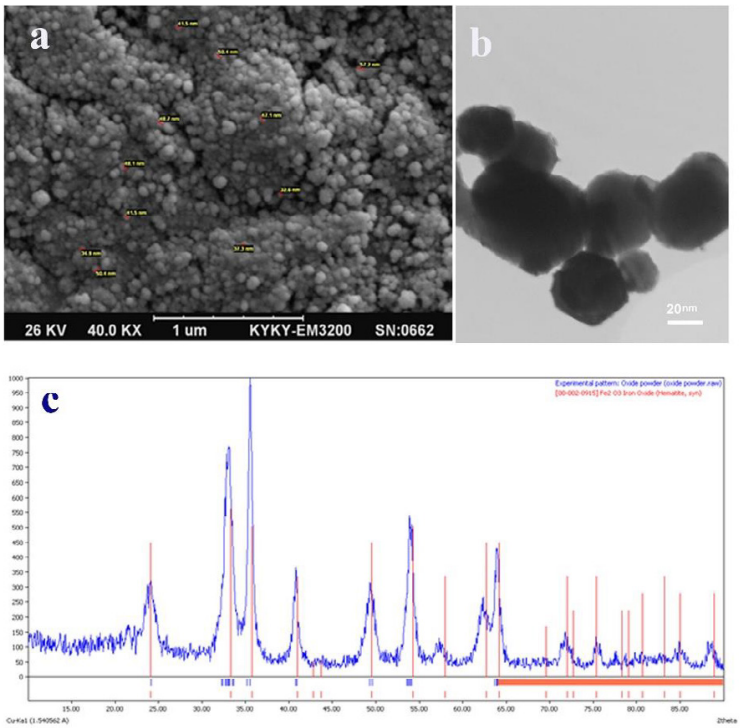

Fig. 1. (a) SEM, (b) TEM, and (c) XRD powder pattern of FeNPs.

is known about its effect on transcription factors and phytotoxicity of iron nanoparticles in different crops. FeNPs also improved growth in peanuts [18], chlorophyll content in soybean [19], iron level in black-eyed peas [20] whereas led to detrimental effects at higher concentrations [21-24]. FeNPs have been, therefore, suggested as an efficient fertilizer to alleviate iron-deficient soil [25].

Plant bZIP transcription factors (bZIPs) play a pivotal role in the abscisic acid signaling pathway and confer abiotic stress tolerance, including freezing [26, 27], drought, and salt stress [28]. The role of DREB in abiotic stress responses such as low temperature, salinity, and drought tolerance in plants has been well documented [29]. The WRKY transcription factors are implicated in regulating abiotic and biotic stress responses and different physiological and developmental processes [30-33]. This study aimed to investigate the effect of different concentrations of FeNPs on growth, Fe content, and gene expressions in wheat plants.

MATERIAL AND METHOD

Characterization of FeNPs

Scanning electron microscopy (SEM; TESCAN, VEGA 3, USA), Transmission electron microscopy (TEM; FEI Tecnai F20 Super-Twin), and X-ray powder diffraction (XRD; 3003 PTS, Seifert, Germany) were used to characterize the microstructure and particle size of FeNPs (Fig. 1).

Treatments and experimental conditions

The seeds of wheat (*Triticum aestivum* L.) were provided by Pakan Bazr. Seeds were sterilized by the sodium hypochlorite solution. Then, for the removal of excess sodium hypochlorite contents, the seeds were rinsed with distilled water. The seeds were placed in every pot with a completely randomized design with three replicates per treatment. It should be noted that the soil was composed of peat and perlite and seedlings were irrigated with Hoagland solution every other day. After 10 days, bulk-Fe and FeNPs at different concentrations (100, 200, 300, and 400 mgL^{-1}) were applied every other day. The wheat plants were harvested 21 days after the first treatments for the evaluation of growth, physiological, and molecular traits. After this, parts of plants oven-dried at 70 °C for 48 hours for obtaining dry weight and estimating of Fe content.

Fe content

Concentrations of Fe were determined using atomic absorption spectroscopy (Varian SPECTRAA-200, USA) approach.

Expression of WRKY1, DREB, and bZIP1

Expression of WRKY1, DREB, and bZIP1 as

Table 1. The sequences of forward and reverse primers for 18s rRNA, WRKY1, DREB, and bZIP1 genes.

Primer name	Sequence (5-3)	Tm	Length	Amplicon size (bp)
18s-F	GGAGTATGGTCGCAAGGCTGAAAC	61	20	133
18s-R	CTCAATCTGTCAATCCTCACTATGTCTGG	61	20	
***WRKY1*-F**	GCCCGTCATCCTCCATCAAT	60	20	129
***WRKY1*-R**	CCTGCCCATCATCTGTTGGT	60	20	
***DREB*-F**	GGGGCCGACTTTTCTTTCTC	61	20	80
***DREB*-R**	TCGCAATCTTGTCGCCTGTT	61	20	
***bZIP1*-F**	AGAGCTGGGAATCTTGGTGC	61	20	139
***bZIP1*_R**	AATGCAACCACCCAAGCCTA	61	20	

target genes were assessed in leaves. Trizol-based RNA extraction was performed on the liquid nitrogen-grounded leaves and then followed by the synthesis of complementary DNA (cDNAs) using a thermocycler (PeQlab company). The sequences of forward and reverse primers for 18s rRNA, *WRKY1*, *DREB*, and *bZIP1* genes are presented in Table 1. In this study, 18s rRNA was a housekeeping gene. The expressions of target genes were quantified using the real-time quantitative RT-PCR method (Applied Biosystems, StepOne™) and a commercial kit under the cycle of 94 °C for 120s, 94 °C for 30 s, 58 °C for 30s, and 72 °C for 20s. The relative expression levels of the target genes were explained in a fold change unit [26, 31].

Statistical analysis

All data were statistically analyzed using GraphPad software. The mean values were compared based on the Tukey test.

RESULTS AND DISCUSSION

SEM, and XRD of FeNPs are depicted in Fig. 1. Physicochemical traits of FeNPs were characterized by XRD (Fig. 1(a)) and SEM (Fig. 1(b)). The particle sizes of FeNPs are ranged from 20 nm to 40 nm. The XRD pattern analysis was employed to show the crystal structure of FeNPs. The characteristic peaks (2θ) of FeNPs were observed at 18.3, 30.2, 35.5, 43.1, 53.5, 57.04, and 62.6. There was a significant increase in shoot fresh weight ($p < 0.05$) in the Fe-treated plants compared to control and the highest shoot fresh weight was observed at the concentration of 400 mgl^{-1} FeNPs (Fig. 2(a)). When compared with the controls, shoot fresh mass increased by 20%, 27%, 39%, and 46% with application of 100, 200, 300, and 400 mgl^{-1} BFe, respectively, and increased by 37%, 46%, 53%, and 63% under the application of 100, 200, 300, and 400 mgl^{-1} Fe-NPs, respectively.

Root fresh weight increased by 8%, 14%, 21% and 28% in BFe100, BFe 200, BFe 300, BFe 400 respectively. With the treatment of FeNPs (100, 200, 300, and 400 mgl^{-1}), the root fresh weight improved by 12% and 26%, 36%, and 43%, respectively, as compared to the control (Fig. 2(b)).

Interactions between a different concentration of FeNPs and bulk Fe and plant dry weight were also under investigation. With the increment in concentration of FeNPs and BFe, dry weights of leaves and roots were enhanced gradually, and the highest shoot and root dry weight was found at the FeNP400 and BFe400 treatments with an increment of 58% and 52% in shoot dry weight as well as 55% and 51% in root dry weight, respectively (Fig. 3(a,b)). Fe concentrations in the shoot and roots of wheat sprayed with FeNPs or bulk Fe were significantly higher at all FeNPs treatments (100, 200, 300, and 400 ppm) than the control (Fig. 4(a,b)). In leaves, exposure to 100, 200, 300, and 400 mgl^{-1} FeNPs resulted in 25%, 30%, 37.1%, and 37.3% increase in the Fe content, respectively (Fig. 4(a)). The addition of 100, 200, 300, and 400 mgl^{-1} FeNPs enhanced the root element content by 15%, 25%, 34, 35%, indicating the high potency of FeNPs to improve nutritional status (Fig. 4(b)). These results confirmed that the FeNPs displayed higher potency to improve plant growth characteristics and Fe biofortification when compared to the bulk form. The specific unique physiochemical characteristics of FeNPs can be responsible for the partly different responses of plants to nanoparticles relative to the bulk type [34; 31]. As is well known, Fe plays critical involvements in a plethora of biological processes, like respiration and photosynthesis (35). In previous studies, FeNPs have been shown to improve plant growth in rice [36], wheat [7, 37], *Arachis hypogaea* [18], *Cucumis sativus* [38], primrose [39], and soybean [40]. Marcus *et al.* [41] proposed that cellular absorption of FeNPs was influenced by two factors, including incubation time and concentration. In line with our results, the exogenous application of FeNPs was reported to increase chlorophyll and photosynthesis rate, which may result in increased growth [37]. The increase in biomass may be owing to the enhanced Fe content and the assimilation of other elements, including carbon and nitrogen. FeNPs, therefore,

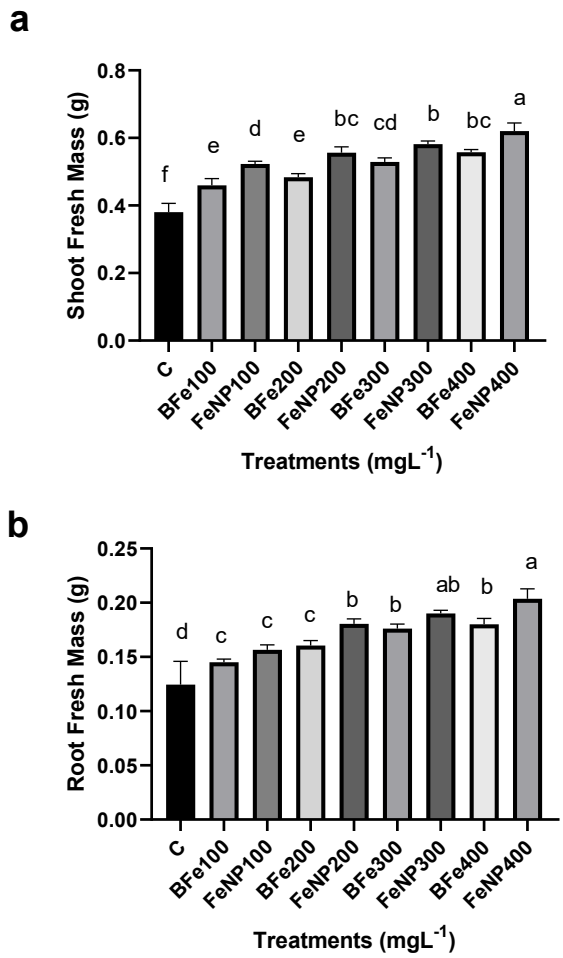

Fig. 2. Effect of FeNPs and B Fe on (a) shoot and (b) root fresh weight of Triticum aestivum. The values were shown in grams. The data indicate the mean ± SD of three replicates.

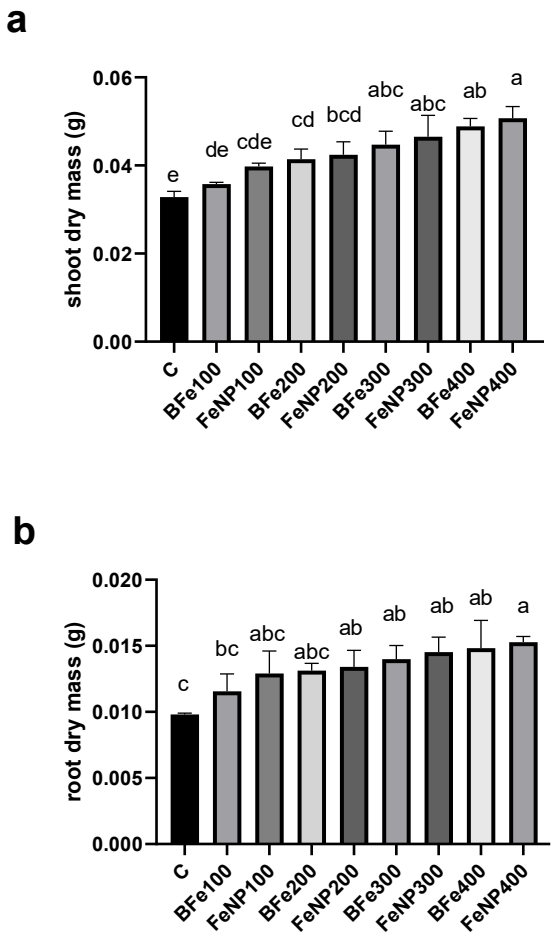

Fig. 3. Dry mass in the leaf(a) and root(b) from the plants exposed to FeNPs and BFe. The values were shown in grams. The data indicate the mean ± SD of three replicates.

may contribute to an increase in plant growth at a suitable concentration [38].

The expression patterns of *bZIP*, *DREB*, and *WRKY1* genes are shown in Fig. 5. The expression of *bZIP*, *DREB*, and *WRKY1* in all treatments was markedly up-regulated compared to the control.

FeNP treatment affected the expression of *bZIP* (Fig. 5(a)) and *WRKY1* (Fig. 5(c)) genes in a concentration-dependent manner. In FeNP200 treatment, we detected up to a threefold increase in transcript amount of *bZIP* in the leaves, while the expression level of *WRKY1* was enhanced about four-fold. Treatment with 100 and 200 mgl^{-1} FeNPs enhanced the expression of *DREB* and its transcript amount was 3-fold higher than those in the control (Fig. 5(b)). Based on the recent reports, the application of nanoparticles in plants may alter redox [31] and phytohormones [42]. The application of nanoparticles, like FeNPs, may induce reactive oxygen species (ROS) production in plants, which is also considered as a signaling molecule and induce plant growth [18]. FeNP-mediated alteration in redox may responsible for changes in these investigated genes. Transcription factors are proteins that interact with a multitude of target genes. The observed changes in *DREB*, *bZIP*, and *WRKY1* are important molecular responses to FeNPs, thereby altering the growth and physiology of plants. It has been shown that *WRKY1* [30, 31], *bZIP* [26], and *DREB* [34] transcription factors play important roles in the regulation of growth, metabolism, and defense system in plants. It seems that FeNPs mediated considerable modification in plant growth, metabolism, and stress responses through molecular changes in the expression of transcription factors.

Plants allocate a large part of their genome contents to transcription factors, for example,

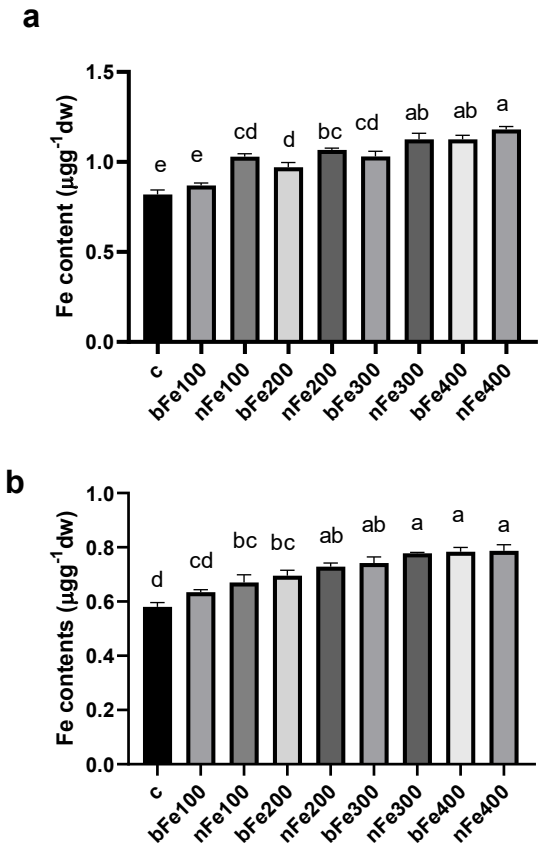

Fig. 4. Iron (Fe) content of leaf (a) and root (b) of (µg g^{-1} dw). The values were shown in grams. The data indicate the mean ± SD of three replicates.

Arabidopsis coding more than 1500 transcription factors [43, 44]. These transcription factors consist of ethylene-responsive element-binding factors, MYB, basic-domain leucine-zipper (bZIP), and WRKY proteins [44]. Such transcription factors have been involved in the expression of stress-responsive genes (SA-inducible DOF proteins) which stimulate the stress-responsive bZIP proteins and novel single-stranded DNA-binding proteins [44]. Some Dof proteins are involved in plant growth and development [45]. Modulation of stress-responsive genes, therefore, shows mechanisms involved in plant adaptation to various environmental stresses [44]. A positive close correlation was found between the expression of *WRKY1* and the expression of genes involved in the generation of secondary metabolites in hemp plants [30]. Several recent reports highlighted modification in transcription factors, including *HSFA* [46], *WRKY1* [31], and *bZIP* [26, 47], and miR172 [47] in response to nanoparticles.

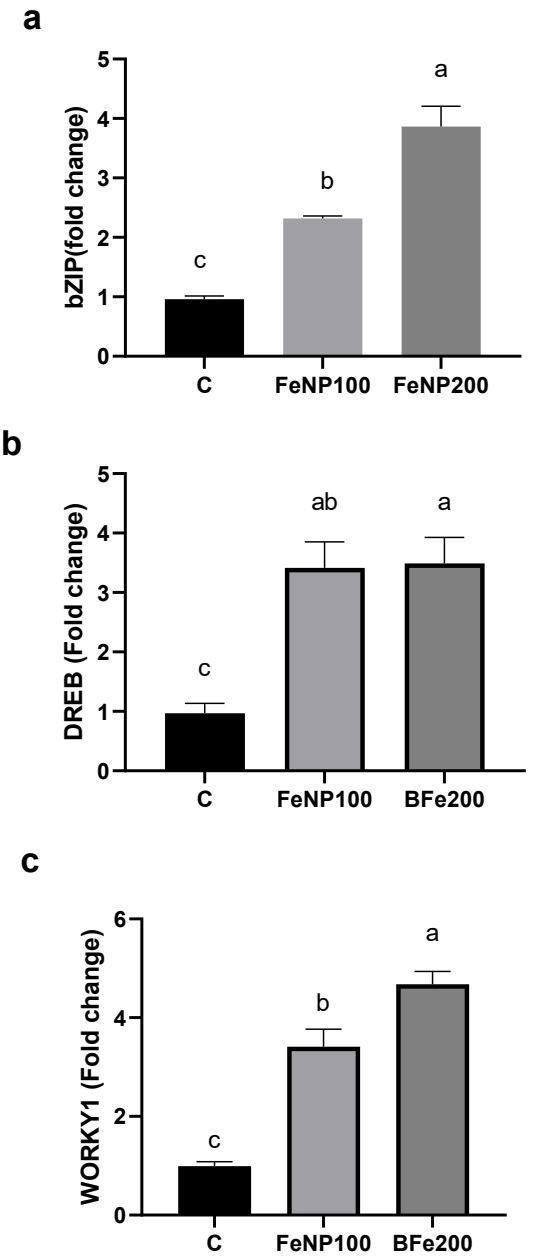

Fig. 5. Expression of bZIP (a), DREB (b), and WRKY1(c) genes expressed under FeNPs and BFe treatments. The values were shown in grams. The data indicate the mean ± SD of three replicates.

CONCLUSION

This study addressed physiological and molecular responses to FeNPs in wheat. The application of FeNPs not only improved biomass but also associated with changes in expression of three important genes, including *WRKY1*, *DREB*,

and *bZIP* in leaves. These molecular responses can be considered as a major mechanism by which utilization of FeNPs may increase growth and reinforce the defense system in crops. It could be also concluded that FeNPs could be a promising strategy to encounter iron deficiency in the human diet and to improve plant protection against biotic and abiotic stress conditions. Due to the lack of molecular studies on plant responses to nanoparticles, the need for future molecular studies is essential to identify the potentially involved mechanisms.

CONFLICT OF INTEREST

Authors have no conflict of interest.

REFERENCES

[1] Sajjadifar S., Rezayati S., Arzehgar Z., Abbaspour S., Torabi Jafroudi M., (2018), Applications of iron and nickel immobilized on hydroxyapatite-core-shell γ-Fe_2O_3 as a nanomagnetic catalyst for the chemoselective oxidation of sulfides to sulfoxides under solvent-free conditions. *J. Chin. Chem. Soc.* 65: 960-969.

[2] Arzehgar Z., Azizkhani V., Sajjadifar S., Fekri M. H., (2019), Synthesis of 2-Amino-4H-chromene derivatives under solvent-free condition using MOF-5. *Chem. Methodolog.* 3: 251-260.

[3] Sajjadifar S., Arzehgar Z., Ghayuri A., (2018), $Zn_3(BTC)_2$ as a highly efficient reusable catalyst for the synthesis of 2-Aryl-1H-Benzimidazole. *J. Chin. Chem. Soc.* 65: 205-211.

[4] Gupta S., Lakshman M., (2019), Magnetic nano Cobalt Ferrite: An efficient recoverable catalyst for synthesis of 2, 4, 5-trisubstituted imidazoles. *J. Medic. Chem. Sci.* 2: 51-54.

[5] Saidi W., Abram T., Bejjit L., Bouachrine M., (2018), New organic compounds based on biphenyl for photovoltaic devices: DFT theoretical investigation. *Chem. Methodolog.* 2: 247-259.

[6] Hameed A., Fatima G. R., Malik K., Muqadas A., Fazal-ur-Rehman M., (2019), Scope of nanotechnology in cosmetics: dermatology and skin care products. *J. Medic. Chem. Sci.* 2: 9-16.

[7] Hussain A., Ali S., Rizwan M., ur Rehman M. Z., Qayyum M. F., Wang H., Rinklebe J., (2019), Responses of wheat (Triticum aestivum) plants grown in a Cd contaminated soil to the application of iron oxide nanoparticles. *Ecotoxicol. Environ. Safety.* 173: 156-164.

[8] Ghasempour M., Iranbakhsh A., Ebadi M., Ardebili Z. O., (2019), Multi-walled carbon nanotubes improved growth, anatomy, physiology, secondary metabolism, and callus performance in Catharanthus roseus: An in vitro study. *3 Biotech.* 9: 404-409.

[9] Conte R., Calarco A., Peluso G., (2018), Nanosized biomaterials for regenerative medicine. *Int. J. Nano Dimens.* 1: 209-214.

[10] Kasote D. M., Lee J. H., Jayaprakasha G. K, Patil B. S., (2019), Seed priming with iron oxide nanoparticles modulate antioxidant potential and defense-linked hormones in watermelon seedlings. *ACS Sustainab. Chem. Eng.*7: 5142-5151.

[11] Sundaria N., Singh M., Upreti P., Chauhan R. P., Jaiswal J. P., Kumar A., (2019), Seed priming with Iron oxide nanoparticles triggers Iron acquisition and biofortification in wheat (Triticum aestivum L.) grains. *J. Plant Growth Regul.* 38: 122-131.

[12] Rui M., Ma C., Hao Y., Guo J., Rui Y., Tang X., (2016), Iron oxide nanoparticles as a potential iron fertilizer for peanut (Arachis hypogaea). *Front. Plant Sci.* 7: 815-821.

[13] Elanchezhian R., Kumar D., Ramesh K., Biswas A. K., Guhey A., Patra A. K., (2017), Morpho-physiological and biochemical response of maize (Zea mays L.) plants fertilized with nano-iron (Fe_3O_4) micronutrient. *J. Plant Nutr.* 40: 969–1977.

[14] Iannone M. F., Groppa M. D., de Sousa M. E., van Raap M. B. F., Benavides M. P., (2016), Impact of magnetite iron oxide nanoparticles on wheat (Triticum sativum L.) development: Evaluation of oxidative damage. *Environ. Exp. Bot.* 131: 77–88.

[15] Konate A., He X., Zhang Z., Ma Y., Zhang P., Alugongo G. M., Rui Y., (2017), Magnetic (Fe_3O_4) nanoparticles reduce heavy metals uptake and mitigate their toxicity in wheat seedling. *Sustainabil.* 9: 1–16.

[16] Pariona N., Martinez A. I., Hdz -García H. M., Cruz L. A., Hernandez -Valdes A., (2017), Effects of hematite and ferrihydrite nanoparticles on germination and growth of maize seedlings. *Saudi J. Biologic.* Sci. 24: 1547-1554.

[17] Li J., Chang P. R., Huang J., Wang Y., Yuan H, Ren H., (2013), Physiological effects of magnetic iron oxide nanoparticles towards watermelon. *J. Nanosc. Nanotechnol.* 13: 5561-5567.

[18] Rui M., Ma C., Hao Y., Guo J., Rui Y., Tang X., Zhao Q., Fan X., Zhang Z., Hou T., Zhu S., (2016), Iron oxide nanoparticles as a potential iron fertilizer for peanut (*Arachis hypogaea*). *Front. Plant Sci.* 7: 815-821.

[19] Ghafariyan M., Malakouti M., Dadpour M., Stroeve P., Mahmoudi M., (2013), Effects of magnetite nanoparticles on soybean chlorophyll. *Environ. Sci. Technol.* 47: 10645–10652.

[20] Delfani, M., Baradarn Firouzabadi M., Farrokhi N., Makarian H., (2014), Some physiological responses of black-eyed pea to iron and magnesium nanofertilizers. *Commun. Soil Sci. Plant Anal.* 45: 530–540.

[21] García A., Espinosa R., Delgado L., Casals E., González E., Puntes V., (2011), Acute toxicity of cerium oxide, titanium oxide and iron oxide nanoparticles using standardized tests. *Desalination.* 269: 136–141.

[22] Mushtaq Y. K., (2011), Effect of nanoscale Fe_3O_4, TiO_2 and carbon particles on cucumber seed germination. *J. Environ. Sci. Health A.* 46: 1732–1735.

[23] Ma X., Gurung A., Deng Y., (2013), Phytotoxicity and uptake of nanoscale zero-valentiron (nZVI) by two plant species. *Sci. Total Environ.* 443: 844–849.

[24] Barhoumi L., Oukarroum A., Taher L. B., Smiri L. S., Abdelmelek H., Dewez D., (2015), Effects of superparamagnetic iron oxide nanoparticles on photosynthesis and growth of the aquatic plant Lemna gibba. Arch. Environ. *Contam. Toxicol.* 68: 510–520.

[25] Tombuloglu H., Slimani Y., Tombuloglu G., Korkmaz A. D., Baykal A., Almessiere M., Ercan I., (2019), Impact of superparamagnetic iron oxide nanoparticles (SPIONs) and ionic iron on physiology of summer squash (Cucurbita pepo): A comparative study. *Plant Physiol. Biochem.* 139: 56-65.

[26] Sotoodehnia-Korani S., Iranbakhsh A., Ebadi M., Majd A., Ardebili Z. O., (2020), Selenium nanoparticles induced variations in growth, morphology, anatomy, biochemistry, gene expression, and epigenetic DNA methylation in Capsicum annuum; an in vitro study. *Environ. Pollut.* 265: 114727.

[27] Wang L., Cao H., Qian W., Yao L., Hao X., Li N., Yang Y., Wang X., (2017), Identification of a novel bZIP transcription factor in Camellia sinensis as a negative regulator of freezing tolerance in transgenic arabidopsis. *Annals Botan.* 119: 1195-209.

[28] Yang Y., Yu T. F., Ma J., Chen J., Zhou Y. B., Chen M., Ma Y. Z., Wei W. L., Xu Z. S., (2020), The soybean bZIP transcription factor gene GmbZIP2 confers drought and salt resistances in transgenic plants. *Int. J. Molec. Sci.* 21: 670-678.

[29] Ali N., Hadi F., (2018), CBF/DREB transcription factor genes play role in cadmium tolerance and phytoaccumulation in Ricinus communis under molybdenum treatments. *Chemosphere.* 208: 425-432.

[30] Iranbakhsh A., Ardebili Z. O., Molaei H., Ardebili N. O., Amini M., (2020), Cold plasma up-regulated expressions of WRKY1 transcription factor and genes involved in biosynthesis of cannabinoids in Hemp (Cannabis sativa L.). *Plasma Chem. Plasma Process.* 40: 527-537.

[31] Rajaee Behbahani S., Iranbakhsh A., Ebadi M., Majd A., Ardebili Z. O., (2020), Red elemental selenium nanoparticles mediated substantial variations in growth, tissue differentiation, metabolism, gene transcription, epigenetic cytosine DNA methylation, and callogenesis in bittermelon (Momordica charantia); an in vitro experiment. *Plos One.* 15: e0235556.

[32] Rushton P. J., Somssich I. E., Ringler P., Shen Q. J., (2010), WRKY transcription factors. *Trends in Plant Sci.* 15: 247-258.

[33] Amato A., Cavallini E., Zenoni S., Finezzo L., Begheldo M., Ruperti B., Tornielli G. B.. (2017), A grapevine TTG2-like WRKY transcription factor is involved in regulating vacuolar transport and flavonoid biosynthesis. *Front. Plant Sci.* 7: 1979-1986.

[34] Abedi S., Iranbakhsh A., Oraghi Ardebili Z., (2020), Nitric oxide and selenium nanoparticles confer changes in growth, metabolism, antioxidant machinery, gene expression, and flowering in chicory (Cichorium intybus L.): potential benefits and risk assessment. *Environ. Sci. Pollut.* Res. Int. 28: 3136-3148.

[35] Yuan J., Chen Y., Li H., Lu J., Zhao H., Liu M., Nechitaylo G. S., Glushchenko N. N., (2018), New insights into the cellular responses to iron nanoparticles in Capsicum annuum. *Scientif. Rep.* 8: 1-9.

[36] Rizwan M., Noureen S., Ali S., Anwar S., ur Rehman M. Z., Qayyum M. F., Hussain A., (2019), Influence of biochar amendment and foliar application of iron oxide nanoparticles on growth, photosynthesis, and cadmium accumulation in rice biomass. *J. Soils Sediments.* 19: 3749-3759.

[37] Adrees M., Khan Z. S., Ali S., Hafeez M., Khalid S., Ur Rehman M. Z., Hussain A., Hussain K., Chatha S. A., Rizwan M., (2020), Simultaneous mitigation of cadmium and drought stress in wheat by soil application of iron nanoparticles. *Chemosphere.* 238: 124681-124686.

[38] Konate A., Wang Y., He X., Adeel M., Zhang P., Ma Y., Ding Y., Zhang J., Yang J., Kizito S., Rui Y., (2018), Comparative effects of nano and bulk-Fe_3O_4 on the growth of cucumber (Cucumis sativus). Ecotoxicol. *Environment. Safet.* 165: 547-554.

[39] Asadi-Kavan Z., Khavari-Nejad R. A., Iranbakhsh A., Najafi F., (2020), Cooperative effects of iron oxide nanoparticle (α-Fe_2O_3) and citrate on germination and oxidative system of evening primrose (*Oenthera biennis L.*). *J. Plant Interact.* 15: 166-179.

[40] Sharma S., Malhotra H., Borah P., Meena M. K., Bindraban P., Chandra S., Pande V., Pandey R., (2019), Foliar application of organic and inorganic iron formulation induces differential detoxification response to improve growth and biofortification in soybean. *Plant Physiol. Report.* 24: 119-128.

[41] Marcus M., Karni M., Baranes K., Levy I., Alon N., Margel S., Shefi O., (2016), Iron oxide nanoparticles for neuronal cell applications: Uptake study and magnetic manipulations. *J. Nanobiotechnol.* 14: 1-12.

[42] Soleymanzadeh R., Iranbakhsh A., Habibi G., Ardebili Z. O., (2020), Selenium nanoparticle protected strawberry against salt stress through modifications in salicylic acid, ion homeostasis, antioxidant machinery, and photosynthesis performance. *Acta Biolog. Cracoviensia s. Botanic.* 62: 33-42.

[43] Riechmann J. L., Heard J., Martin G., Reuber L., Jiang C., Keddie J., Adam L., Pineda O., Ratcliffe O. J., Samaha R. R., Creelman R., Pilgrim M., Broun P., Zhang J. Z., Ghandehari D., Sherman B. K., Yu G., (2000), Arabidopsistranscription factors: Genome-wide comparative analysis among eukaryotes. *Science.* 290: e2105-e2110.

[44] Bal L. M., Satpute G. K., Srivastava A. K., (2017), Plant stress signaling through corresponding nanobiotechnology. *Int. Nanotechnol. Applic. Food.* 1: 381-391.

[45] Kang H. G., Singh K. B., (2000), Characterization of salicylic acid-responsive, arabidopsis dof domain proteins: Overexpression of OBP3 leads to growth defects. *The Plant J.* 21: 329-339.

[46] Safari M., Ardebili Z. O., Iranbakhsh A., (2018), Selenium nano-particle induced alterations in expression patterns of heat shock factor A4A (HSFA4A), and high molecular weight glutenin subunit 1Bx (Glu-1Bx) and enhanced nitrate reductase activity in wheat (*Triticum aestivum L.*). *Acta Physiolog. Plantarum.* 40: 117-125.

[47] Neysanian M., Iranbakhsh A., Ahmadvand R., Oraghi Ardebili Z., Ebadi M., (2020), Comparative efficacy of selenate and selenium nanoparticles for improving growth, productivity, fruit quality, and postharvest longevity through modifying nutrition, metabolism, and gene expression in tomato; potential benefits and risk assessment. *Plos One.* 15: e0244207-e024412.