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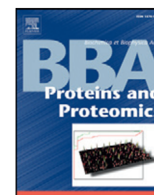

Proteomic and physiological analyses of wheat seeds exposed to copper and iron nanoparticles

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ABSTRACT

To elucidate the role of Cu and Fe NPs on the yield of wheat varieties, a gel-free proteomic technique was used. NPs were synthesized and characterized through zeta potential, EDX, and SEM. Spike length, number of grains per spike, and 1000 grain weight were increased in wheat varieties treated with 25 ppm Cu and Fe NPs. On treatment with 25 ppm Cu and Fe NPs, a total of 58, 121, and 25 proteins were changed in abundance in wheat seeds of galaxy-13, Pakistan-13, and NARC-11, respectively. In galaxy-13, exposure to Cu NPs increased proteins involved in starch degradation and glycolysis. Furthermore, the number of proteins related to starch degradation, glycolysis, and tricarboxylic acid cycle was increased in galaxy-13 on Fe NPs exposure. Proteins related to glycolysis and the tricarboxylic acid cycle was increased in Pakistan-13 and NARC-11 by Fe NPs exposure. The sugar content and SOD activity was increased in wheat seeds treated with Cu and Fe NPs. The Cu content was increased at 25 ppm Cu NPs exposure in seeds of wheat varieties. These results suggest that Cu NPs improved stress tolerance in wheat varieties by mediating the process of starch degradation, glycolysis, and tricarboxylic acid cycle through NPs uptake.

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1. Introduction

Wheat, which belongs to the family Poaceae, is one of the most important cereal crops worldwide, as more calories and proteins in the world diet are derived from wheat than any other cereal crop [1,2]. The forecasts demand of wheat to meet the nutritional requirements of the growing population of world needs 70% increase in grain production for 2050 [3]. Abiotic stresses, particularly salinity and drought, are the primary causes of loss in wheat yield worldwide [4]. Plant adaptation to environmental stresses is reliant upon the initiation of cascades of molecular networks for stress adaptation, signal transduction, and expression of specific stress-related genes and metabolites [5]. Wheat varieties with high yield and an appropriate end-use quality are the primary objective of all breeding programs around the world [6]. To meet the nutritional requirements of growing population, improvement in the yield of wheat is utmost requirement.

Nanotechnology is growing scientific field with the potential to bring radical change in society [7]. In the agricultural and food industry, advances in nanotechnology provide novel tools for the molecular management of diseases and enhanced the ability of plants to absorb nutrients, thereby increased crop yield and nutritional value [8]. The expansion of nanotechnology research has increased the release and accumulation of engineered nanoparticles (NPs) into the environment [9]. NPs have both detrimental and beneficial effects on agronomic traits, yield, and productivity of plants including modification in the nutritional value of food crops [10]. For these reasons, further studies on the effects of NPs in the environment and the plant growth are needed.

Accumulation of NPs in edible parts of plants is an important aspect of NPs fate and has implications for human health and agricultural productivity [10]. Titanium and cesium NPs accumulated in significant amounts in the tissues and edible portions of soybean, tomato, cucumber, and rice [11–14]. However, titanium NPs increased in the grains of soil-grown wheat [15]. Iron (Fe) NPs improved the leaf and pod dry weights and yield of soybean [16]; whereas cesium NPs increased the pod weight of peanut [17]. In contrast, zinc and titanium NPs reduced plant biomass, but did not affect grain production in wheat [15]. Copper (Cu) NPs reduced the growth of wheat plant and accumulated in wheat seed [18]. Exposure to NPs, such as silver, titanium, silicon, and zinc modulated the protein levels and nutrient content of beans and corn

Abbreviations: LC, liquid chromatography; MS, mass spectrometry; NPs, nanoparticles; Cu, copper; Fe, iron; EDX, energy dispersive x-ray spectroscopy; SEM, scanning electron microscopy; SOD, superoxide dismutase.

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[19,20]. Silver NPs altered the carbohydrate contents in bean, corn, rice, and mustard [19,21,22]. Cu NPs-coated vetch seeds showed an approximately three- to four-folds increase in the ascorbic acid and carotene content [23]. Despite these findings, the molecular mechanisms underlying the changes in the nutrient content and productivity of plants exposed to NPs remain unclear.

Fe is the first rare earth element recognized as being essential for plant and animal growth and development. Fe plays an important role in a number of biochemical and physiological processes [24] and a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions [25]. Cu is a redox-active transition metal that is essential for plants as well as for all living organisms as a micronutrient. Within plant cells, Cu is required in at least six subcellular locations: cytosol, endoplasmic reticulum, mitochondrial inner membrane, chloroplast stroma, thylakoid lumen, and apoplasts [26]. At the cellular level, Cu is a structural and catalytic component of many proteins involved in various metabolic pathways [27]. Due to the importance of Fe and Cu in plant cellular processes, the effects of Fe and Cu NPs on the quality of wheat grain warrant investigation.

Fe NPs stimulated the growth of peanut seedling [28] and increased the germination ratio of wheat seeds [29]. Cu NPs enhanced the root and shoot growth of mung bean [30], but reduced shoot growth in wheat [18]. On the other hand, drought and salinity reduces the growth and yield of wheat [4]. Although the effects of Cu and Fe NPs on the growth of various plants have been evaluated, seed productivity and quality have not been analyzed. In the present study, the effects of Cu and Fe NPs on the nutritional quality of seeds from the wheat varieties galaxy-13, Pakistan-13, and NARC-11 were investigated using proteomic and physiological analyses.

2. Materials and methods

2.1. Synthesis of Cu and Fe NPs

Cu and Fe NPs were synthesized by the reduction of $CuSO_4\ 5H_2O$ and $FeCl_3\ 6H_2O$ with an organic extract from onion. For preparation of the onion extract, small chopped pieces of onion were boiled in distilled water for 30 min and the mixture was then strained and filtered to obtain a clear organic onion extract. For the preparation of Cu NPs, $CuSO_4\ 5H_2O$ (200 mg) was dissolved in 900 ml of distilled water under stirring with a magnetic stirrer and was gradually reduced by the stepwise addition of 100 ml of onion extract, which resulted in the formation of solution of light green. For the preparation of Fe NPs, $FeCl_3\ 6H_2O$ (242.1 mg) was dissolved in 990 ml of distilled water and the resulting mixture was heated in an oven for 4 min and 10 s. $FeCl_3\ 6H_2O$ was reduced by the stepwise addition of 10 ml of onion extract. The solution was continuously boiled until the color of the solution changed to reddish brown [31]. The resulting solutions were stocked as 50 ppm Cu and Fe NPs solutions. The freshly prepared Cu and Fe NPs were characterized through Zeta potential, energy dispersive x-ray spectroscopy (EDX), and scanning electron microscopy (SEM).

2.2. Plant material and NPs treatment

For determination of the effects of NPs exposure on the yield of wheat, seeds of the high-yielding variety galaxy-13, drought-tolerant variety Pakistan-13, and salinity-tolerant variety NARC-11 (National Agriculture Research Centre, Islamabad, Pakistan) were sown into earthen pots (20 cm diameter) filled with homogenized soil (15 kg) and water was supplied according to field capacity (2.5 L). The soil used for the study was collected from the research area of PMAS Arid Agriculture University Pakistan, air dried at room temperature, and sieved through a 2 mm mesh prior to characterization. The soil characters were described in Table 1. Growth conditions include 25 °C temperature, photoperiod is 16 h light and 8 h dark and humidity is 20%. Prior to sowing, seeds were sterilized in 3% sodium hypochlorite solution and were rinsed twice in water. After seedling emergence, 20, 25, 30, 35, and 40 ppm Cu and Fe NPs were added once to the soil according to the field capacity of soil at the tillering and anthesis stages of growth. For making different concentrations of NPs, dilutions were made and stirred properly from 50 ppm Cu and Fe NPs stock solution. For control, water without NPs was supplied to plants according to the field capacity. NPs were supplied in the morning at the time of active absorption to avoid chance of accumulation in soil. For morphological analysis, fully ripened plants were used, and spike length, number of grains per spike, and 1000 grain weight were measured. 1000 grain weight was measured by randomly calculating the weight of 100 grains and then it was multiplied with 10. Five plants were grown in a single pot for each treatment in each replication and three independent experiments were performed as biological replicates for all experiments.

Table 1
Properties of soil at the time of sowing of wheat varieties.

Texture	Loam
pH	7.3
EC (dS/m)	0.93
Organic matter (%)	1.1
Available P (mg/Kg)	8
Available K (mg/Kg)	120
Cu content (mg/Kg)	0.1
Fe content (mg/Kg)	3

2.3. Protein extraction

A portion (500 mg) of each plant sample was ground to powder in liquid nitrogen using a mortar and pestle in an extraction buffer containing 20 mM Tris–HCl (pH 8.0), 4% CHAPS, 5 mM EDTA, and 2 mM phenylmethylsulfonyl fluoride. The solution was transferred to a 2-ml tube and centrifuged at 20,000 $\times g$ for 20 min at 4 °C, and the supernatant was then collected. Proteins in the supernatant were precipitated by the addition of 4 volumes of ice-cold acetone solution containing 10% trichloroacetic acid and 0.07% 2-mercaptoethanol [32]. The resulting mixture was vortexed, sonicated for 10 min, and then incubated for 60 min at −20 °C. The suspension was centrifuged at 9000 $\times g$ for 20 min at 4 °C, the supernatant was discarded, and the obtained pellet was washed twice with 0.07% 2-mercaptoethanol in acetone. The final pellet was dried using a Speed-Vac concentrator (Savant Instruments, Hickville, NY, USA) and resuspended in lysis buffer consisting of 7 M urea, 2 M thiourea, 5% CHAPS, and 2 mM tributylphosphine by vortexing for 1 h at 25 °C. The resulting suspension was centrifuged twice at 20,000 $\times g$ for 20 min at 25 °C, and the supernatant was collected as total protein. Protein concentrations were determined using the Bradford assay with bovine serum albumin as the standard [33].

2.4. Protein purification and digestion for mass spectrometry analysis

Proteins (150 μg) were purified with methanol and chloroform to remove any detergent from the total protein sample solutions, as previously described [34]. Briefly, 600 μL methanol was added to each sample and the resulting solution was mixed before the further addition of 150 μL chloroform and 450 μL water. After further mixing, the samples were centrifuged at 20,000 $\times g$ for 10 min to achieve phase separation. The upper aqueous phase was discarded without disturbing the white protein disk. Methanol (450 μL) was then added slowly to the lower phase. The samples were further centrifuged at 20,000 $\times g$ for 10 min, supernatants were discarded, and the obtained pellets were dried and resuspended in 50 mM NH_4HCO_3. Proteins in the samples were reduced with 50 mM dithiothreitol for 30 min at 56 °C, and were then alkylated with 50 mM iodoacetamide for 30 min at 37 °C in the dark. Alkylated proteins were digested with trypsin and lysyl endopeptidase (Wako, Osaka, Japan) at 1:100 enzyme/protein concentrations at 37 °C for 16 h. The resulting tryptic peptides were acidified by mixing

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