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Research article

Physio-biochemical basis of iron-sulfide nanoparticle induced growth and seed yield enhancement in *B. juncea*

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ABSTRACT

Metal nanoparticles have been reported to influence plant growth and productivity. However, the molecular mechanisms underlying the effects have not been completely understood yet. Current work describes the physio-biochemical basis of iron sulfide nanoparticle induced growth and yield enhancement in *Brassica juncea*. Iron sulfide nanoparticles (0, 2, 4, 6, 8 and 10 ppm) were used for foliar treatment of *B. juncea* at 30, 45 and 60 days after sowing, under field conditions. Foliar treatment of 4 ppm iron sulfide nanoparticle solution at 30 days after sowing brought maximal enhancement in agronomic attributes of the treated plants. Results of assays *i.e.* total chlorophyll, electrolyte leakage, Malondialdehyde (MDA), proline, H_2O_2 and antioxidant enzyme activities indicated the benign effect of iron sulfide nanoparticles on plants. Consequently, improved redox status of the treated plants, enabled them to assimilate higher photosynthate. The augmentation in growth and seed yield in iron sulfide nanoparticle treated plants was amply supported by activation of RUBISCO small subunit (*rubisco S*), RUBISCO large subunit (*rubisco L*), glutamine synthetase (*gs*) and glutamate synthase (*gogat*) genes. Thus, iron sulfide nanoparticle induced growth and yield enhancement is proposed to be mediated through activation of carbon and nitrogen assimilatory pathways at specific growth stage. The iron content in the leaves and root tissues of the treated plants was also significantly improved.

1. Introduction

In the recent past nanotechnology has allowed newer applications of materials, based on the alterations in their physico-chemical properties in the nano form vis-à-vis their bulk forms. Application of nanotechnology in agriculture offers a viable alternative to GM crops, for enhancing growth and crop productivity. Nanotechnology based endeavors in agriculture sectors are still in their infancy, as several aspects are yet to be explored in detail. The impact of metal derived nanoparticles on growth and development of plants has largely been analyzed through studies based on toxicity profiling (Lin and Xing, 2007; Ma et al., 2010). Beside this, growth promotional activity of specific metal nanoparticles has also been studied by researchers globally (Salem et al., 2016; Rui et al., 2016). Both kinds of studies have produced quite fascinating results that act as a driving force to further delineate the effects of nanoparticles at cellular and molecular level, and to unravel the mechanism of action of these nanoparticles.

According to Sharma et al. (2012a) transition metal nanoparticles can actively exchange the electrons due to their incompletely filled d orbitals, thus enabling them to interact with various biomolecules inside the cells. These findings raised a quest whether nanoparticles of specific transition metal micronutrient, apart from gold, titanium oxide and silver, can also be used to enhance plant growth and yield. Essential micronutrients like Fe, Zn, Cu, Mo, Si *etc.* are required for plant growth and development. Among the micronutrients, Iron (Fe) is a crucial element involved in a number of biochemical processes in plant cell. It is one of the structural elements of organic components and is used as a cofactor in several enzymatic reactions and is present in heme- and iron–sulfur proteins participating mainly in photosynthesis, nitrogen and sulfur assimilation. (Van Hoewyk et al., 2007).

Brassica (mustard) plants have a specifically high requirement of sulfur for optimal crop yield and quality. To produce 1 tonne of rapeseed we require 16 kg of sulfur, in comparison to sulfur requirement of wheat, i.e. 2–3 kg sulfur/tonne of grain (Blake-Kalff et al., 2001). Brassica is ranked third among the most significant sources of vegetable oils in the world, *i.e.* after palm and soybean

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respectively. Although in India, Brassica has been cultivated in a large area, still its productivity (1176 kg/hectare) is significantly lower than the world average (1850 kg/hectare) (DRMR Bharatpur India, 2013). Further, a large gap exists between the domestic production and consumption of Brassica oil in India. Consequently, a significant amount of our foreign currency is being used in its import. Therefore, sincere efforts are required to improve the seed yield, in order to meet future oilseed demands. These findings encouraged us to use iron sulfide in the form of nanoparticles, for promoting growth and productivity of *Brassica juncea*.

It is well reported that the level of one nutrient influences the uptake and assimilation of other nutrients, for example, it has been observed that assimilatory pathways of carbon, sulfur and nitrogen work in a highly coordinated manner in such a way that during nitrate deficiency sulfate assimilation is reduced and the capacity of nitrate reduction is diminished in plants growing in sulfate deficient conditions (Kopriva and Rennenberg, 2004). In addition, sulfur assimilation is also regulated by carbohydrates. Such kind of regulation takes place due to interaction of theses pathways in the form of various metabolites which themselves act as signals for regulating the process in an inter-dependent manner. This coordination finally maintains the homeostasis in the metabolism of macro and micronutrients, in a synergistic manner (Kopriva et al., 2002). RUBISCO is the key regulatory enzyme for carbon fixation and GS/GOGAT pathway forms the link between carbon and nitrogen assimilation, as GS requires ATP as a source of energy while GOGAT utilizes 2-oxoglutarate and reduced ferredoxin or NADH as carbon skeletons and reductant respectively (McAllister et al., 2012). Serine acetyl transferase is the regulatory enzyme for cysteine biosynthesis in plants (Wirtz et al., 2012). Thus interplay of carbon, nitrogen and sulfur assimilating genes could be involved in regulating plant growth and development.

In the present study, experiments were designed to evaluate the overall effect of different concentrations of iron sulfide nanoparticles at different growth stages of *B. juncea* under field conditions. Alterations in various biochemical stress marker molecules, agronomic attributes, expression level of key genes of carbon, sulfur and nitrogen assimilation and endogenous iron content of treated plants were analyzed in response to foliar application of iron sulfide nanoparticles. It will help to unravel the possible molecular mechanisms behind nanoparticles mediated growth promotion in *B. juncea*.

2. Materials and methods

2.1. Plant material, field layout, sowing and fertilizer application

The seeds of *Brassica juncea* var. Pusa Jaikisan were obtained from Indian Agricultural Research Institute, New Delhi. The experiment was laid out in a Randomized Block Design (RBD) with three replicates. Before sowing, seeds were surface sterilized with $HgCl_2$ (0.01% for 2 min), and sown at a depth of 3.0–3.5 cm and with a spacing of 30 & 15 cm (row to row & plant to plant) in fields of crop research centre, Pantnagar. Fertilizers were applied as per the standard agronomic practices recommended for Brassica, *i.e.* nitrogen: phosphorus: potassium: sulfur; 80:40:40:20 (kg/hectare). All of phosphorus, potassium and sulfur fertilizers and half of nitrogen fertilizer were distributed in plots and mixed with surface soil, 24 h before seed sowing. Second dose of nitrogen (remaining half of the total quantity) fertilizer was applied immediately after first irrigation (*i.e.* 30 days after seed sowing).

2.2. Synthesis and characterization of iron sulfide nanoparticles

Iron sulfide nanoparticles were synthesized through chemical reduction method using tri sodium citrate as a reducing agent. Sodium sulfide (Na_2S) was taken as a source of sulfur. Iron sulfate ($FeSO_4 \cdot 7H_2O$) was weighed to 0.304 g and firstly dissolved in minimum volume of H_2SO_4, so as to avoid dissociation of iron via common ion effect. Then, volume was made up to 1000 ml with distilled water. Sodium carbonate (Na_2CO_3) was weighed to 0.106 g and dissolved separately in 1000 ml of distilled water. Tri sodium citrate (2.6 g) and Na_2S (0.585 g) were dissolved together in another flask and volume was made up to 200 ml with distilled water. $FeSO_4.7H_2O$ and Na_2CO_3 solutions (100 ml of each) were mixed thoroughly and the pH of the solution was adjusted to 3 and then kept on heating (55–60 °C) with constant stirring for about one hour. Solution of Tri sodium citrate and Na_2S (200 ml) was added drop wise to the heated mixture ($FeSO_4 \cdot 7H_2O$ and Na_2CO_3) with constant stirring. Thereafter, the solution was kept stirring at room temperature for one hour. Iron sulfide nanoparticles were characterized using UV-Visible spectroscopy, Dynamic Light Scattering (DLS) and Transmission Electron Microscopy.

2.3. Details of treatments

Iron sulfide nanoparticles solutions of different concentrations were prepared with double distilled water, along with *Silwet* (an adjuvant) at 3ml/1000 ml. *B. juncea* plants were treated with different concentrations of iron sulfide nanoparticles solution (2, 4, 6, 8 and 10 ppm) in the form of foliar spray at three different developmental stages of plants *i.e.* at 30DAS (Days after sowing), 45DAS and 60DAS. Biochemical stress marker molecules were estimated to study whether the applied concentrations of iron sulfide nanoparticles induce toxicity in treated plants. So, biochemical analysis was done only at vegetative and pre reproductive stages (30 DAS and 45 DAS). Agronomic attributes are the measures of growth and economic yield of treated plants, so they were studied only at maturity. The best combination of concentration of iron sulfide nanoparticles and the most responsive growth stages of *Brassica juncea*, selected on the basis of agronomic attributes, was used for understanding their effect on the expression of key regulatory genes of carbon (*rubisco* L and *rubisco* S), sulfur (*sat*) and nitrogen assimilatory (*gs* and *gogat*) pathways. Quantification of iron was also done in the best treatment in terms of agronomic attributes.

2.4. Biochemical and agronomic attributes

For Biochemical analysis leaf samples were collected after 24 h of foliar application of iron sulfide nanoparticles at 30 DAS and 45 DAS. Total chlorophyll content, electrolyte leakage, malondialdehyde content, hydrogen peroxide content, activities of ascorbate peroxidase, catalase and guaiacol peroxidase enzymes were determined according to Joshi et al. (2011). Proline was quantified according to Bates et al. (1973). Agronomic attributes related to growth and yield of *B. juncea*, like number of branches per plant, number of siliquae per plant, seed yield, harvest index and test weight were studied at maturity.

$$\% \text{ harvest index} = (\text{seed yield/biological yield}) \times 100$$

$$\text{Test weight} = 1000 \text{ seed weight}$$

2.5. Semi quantitative RT-PCR

Leaf samples were collected in liquid nitrogen after 24 and 72 h of foliar application of iron sulfide nanoparticles. For expression analysis, first strand cDNA was synthesized using Two step