International Scholars Journals

African Journal of Agronomy ISSN 2375-1185 Vol. 9 (2), pp. 001-005, December, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Research Paper

Seed nanopriming with thiamine encapsulated chitosan nanoparticle induce defense enzymes in chickpea seedlings

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Accepted 16 December, 2021

Seed nanopriming with thiamine encapsulated chitosan nanoparticle, enhanced the growth of chickpea seedlings. Increase in number of leaves, plant fresh and dry weight was observed in treated chickpea seedlings when compared to untreated control. Increase in PR proteins viz, chitinase, β-1, 3 glucanase, peroxidase, polyphenol oxidase in leaves and roots of treated seedlings were observed. Treated and control seedlings were challenged with Fusarium oxysporum f. sp. ciceri and allowed to grow further. In comparison to control, treatment suppressed the wilt pathogen attack on plants and nearly 81% wilt disease incidence was observed after 10 days in control plants. There is a correlation between the accumulation of defense enzymes and suppression of wilt disease incidence in chickpea seedlings.

Key words: Chickpea, seed nanopriming, chitinase, peroxidase, wilt incidence, nanotechnology.

INTRODUCTION

The chickpea (*Cicer arietinum L.*) is a legume crop that ranks world's third most important pulse crop after beans (*Phaseolus vulgaris L.*) and peas (*Pisum sativum L.*). It is a good source of protein, carbohydrates and minerals. Despite its importance, its production is affected by several diseases. Conventional method of disease control involves the application of chemical fungicides. Indiscriminate and excessive use of fungicides affects soil health and the environment. Therefore, an alternate method to manage the disease is necessary. Inducing plant innate immunity enhances protection from biotic stress [10].

Recently, agricultural nanotechnology has emerged as a novel method in crop management techniques including disease control. Several nanomaterials, nanocomposites have been developed to manage agricultural diseases to increase the food production [2].

Nanoparticles alter the nutritional status of the host and activate the defense mechanisms. Nanomaterials prepared from biopolymers encapsulated with vitamins, growth regulators play a major role in plant growth promotion as well as control of disease caused by phytopathogens. The application of these engineered nanomaterials enhances protection in plants by inducing defense responses. Elicitor application enhances the innate immunity in higher plants. Chitinases, β-1, 3 glucanases are hydrolytic enzymes which hydrolyze the cell wall of invading fungal pathogens [3]. Peroxidases, polyphenol oxidases are enzymes which play a major role in the control of phytopathogens by reinforcing the lignin in the cell wall. These enzymes are grouped under PR proteins since they are induced in

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plants

during microbial infection.

Fusarium oxysporum f. sp. ciceri (FOC) is a seed borne as well as soil borne pathogen which affects chickpea at all growing stages, including seedling stage. It causes mortality in young seedlings, brown to black discoloration, drooping of leaves etc. Nearly 60-70% loss in crop production was reported, when the fungus affects during seedling stage. Seed priming technology enhances seed quality, crop yield and stress tolerance. Hence, this study involves priming of chickpea seeds with thiamine encapsulated chitosan nanoparticle (CTNP), to check whether it induce any defense enzymes in chickpea seedlings to control this pathogen.

MATERIALS AND METHODS

Biological material

Chickpea seeds were procured from ICRISAT, Hyderabad. FOC obtained from IARI, New Delhi and stored at 4ºC.

Seed priming of TCNP

TCNP was synthesized as described previously. Chickpea seeds were surface sterilized by 0.1% sodium hypochlorite solution for 30 minutes and washed thoroughly sterile with distilled water. TCNP (0.1%) was dissolved in water and added to the petri plate containing seeds (1 ml/seed) [4]. For control, the seeds were added to water. All the plates were placed in rocker overnight. The seeds were then placed in plastic bags (3-4 seeds/bag) containing sterilized soil, manure and allowed to grow under greenhouse condition. For each experiment, 30 plants were used and replicated thrice.

Growth and challenge inoculation

The growth of control and treated seedlings were monitored for number of leaves, plant fresh weight and dry weight up to 25 days. Another set of seedlings were challenge infected with FOC. The rhizospere soil of 14 day old chickpea seedlings were artificially inoculated with 10 mm disc of 10 day old FOC. The seedlings were monitored for disease incidence for next 10 days. Wilt disease incidence was calculated based on the score, an estimate of the area affected using a scale (0-5) as follows: 0=no symptoms on the leaves/root, 1=less than 20% affected tissue, 2=20-40% affected tissue, 3=40-60% affected tissue, 4=60-80% affected tissue, 5=80-100% affected tissue. The % of wilt disease incidence was calculated using the formula: Wilt disease incidence=(scale x number of plants infected)/ (highest scale x total number of plants) x 100.

Defense enzyme assays

Leaves and roots of control and treated seedlings were removed at different intervals. They were extracted (1 g/2 ml) with 0.01M Tris-HCl buffer pH 7.2 using a pre-chilled mortar and pestle. The extract was centrifuged at 10,000G for 10 minutes at 4ºC (Eppendorf, Germany) and the supernatant was used for enzyme assay. Chitinase, β-1, 3 glucanase, peroxidase, polyphenol oxidase were assayed as described previously.

Statistical analysis

All data were subjected to one-way analysis of variance to determine the significance of individual differences at $p<0.01$ and 0.05 levels. All statistical analysis was conducted using SPSS 16 software support [5].

RESULTS AND DISCUSSION

The significant interest of using nanotechnology in agriculture is to increase the productivity without decontamination of soil, water and protection against microbial diseases. Application of TCNP to chickpea seeds showed positive impact. Increase in number of leaves, plant fresh and dry weight (Figure 1) was observed when compared to untreated control. This may be due to its size, solubility and surface charge. Since the synthesized nanoparticle is soluble, it is easy to absorb by the seeds. Also, the small size and a high surface to volume ratio made it possible to take up by the seeds. The high surface area and penetrability of engineered nanomaterials make them potentially more efficient product in terms of nutrient use relative to conventional fertilizers. The results implicated that TCNP application to chickpea seeds influence the metabolism and enhance the growth. Ability of metal nanoparticles to penetrate the seeds and translocate in to seedlings has been reported. It is reported that positively charged nanoparticles were taken up faster by the plants due to negative charge present in the plant cell wall. Coffee seedlings treated with chitosan nanoparticle showed enhanced growth parameters (Figure 1) [6].

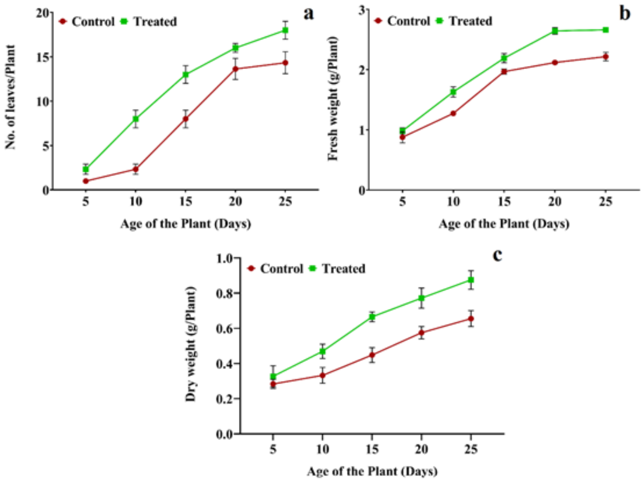

Figure 1. Effect of chickpea seed nanopriming with thiamine encapsulated Chitosan nanoparticle on growth parameters.

Plant chitinase, β-1, 3 glucanase are hydrolytic enzymes which are reported to hydrolyze the cell wall of fungal plant pathogens. Chitinase is constitutively expressed at low levels in plants and can be induced by application of various elicitors. In this study, chitinase activity was increased in the leaves and roots of treated plants when compared to untreated control plants (Figure 2). No endogeneous substrate for chitinase has been found in plants and chitin is a major component of the cell walls of many fungi. Chitinase functions as a defense against chitin containing pathogens. Several studies supported a major role for chitinase in plant defense response to several biotic and abiotic stresses. β-1, 3 glucan nanoparticle application induced chitinase activity in turmeric plants. Nanopriming of chickpea seeds with TCNP also enhanced β-1, 3 glucanase activity in leaves and roots of chickpea seedlings (Figure 2). However, β-1,3 glucanase activity was higher in leaves than root. β-1, 3 glucan, the substrate for β-1,3 glucanase is a major cell wall component of fungi in addition to chitin. Increase in chitinase, β-1, 3 glucanase activity in leaves and roots of chickpea plants treated with TCNP suggesting a role for these enzymes in restriction of the pathogen. Chitinase and β-1, 3 glucanase act synergistically to destruct the cell wall of plant pathogens and provide effective control of plant pathogenic fungi.

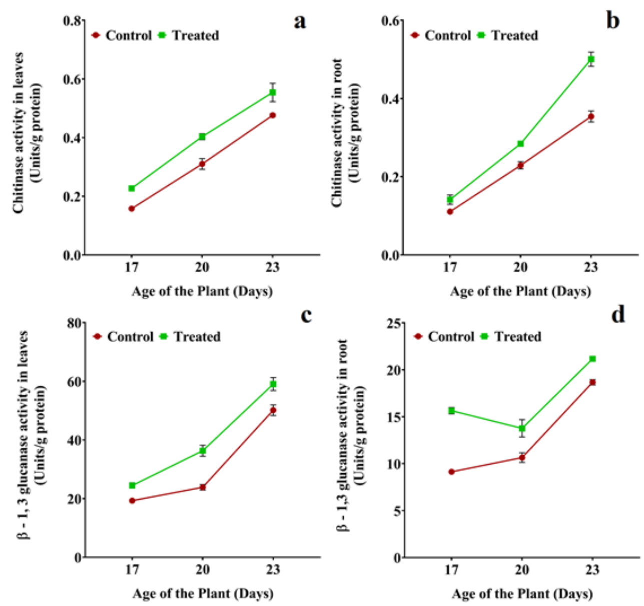

Figure 2. Chitinase and β-1, 3 glucanase activity in control and treated chickpea seedlings.

Apart from these two PR Proteins, Peroxidase (PO) and Polyphenol Oxidase (PPO) was increased in the leaves and roots of nanoprimed chickpea seedlings (Figure 3) [7]. Induction of PO, PPO in banana leaves and roots treated with Trichoderma harzianum has been reported. Camellia sinensis treated with chitosan nanoparticle showed increased peroxidase, polyphenol oxidase, β-1,3 glucanase acitivies.

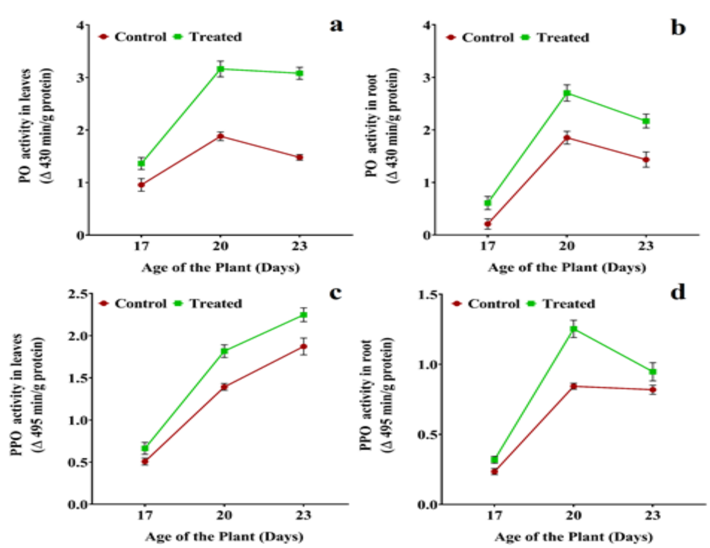

Figure 3. Peroxidase (PO), Polyphenol oxidase (PPO) activity in

control and treated chickpea seedlings.

Nanoprimed seedlings challenged with FOC showed no wilt disease incidence, whereas untreated control plants showed nearly 81% disease incidence after 10 days (Figure 4). There is a correlation between the enhanced activity of defense enzymes and suppression of wilt incidences in nanoprimed chickpea seedlings. Many soil borne fungal diseases have been successfully controlled by inducing defense enzymes in plants. Accumulation of PO has been correlated with Induced Systemic Resistance in several plants by the deposition of phenolic materials into the plant cell wall during resistance interactions. PO, PPO activities are associated with disease resistance in plants which catalyze the condensation of phenolic compounds into lignin [8]. Phenolic compounds enhance the mechanical strength of the host cell wall and also inhibit the invading pathogens. All these results imply that nanopriming of chickpea seeds with TCNP protected chickpea seedlings against wilt pathogen by reinforcing the defense mechanism through elaboration of defense enzyme (Figure 4).

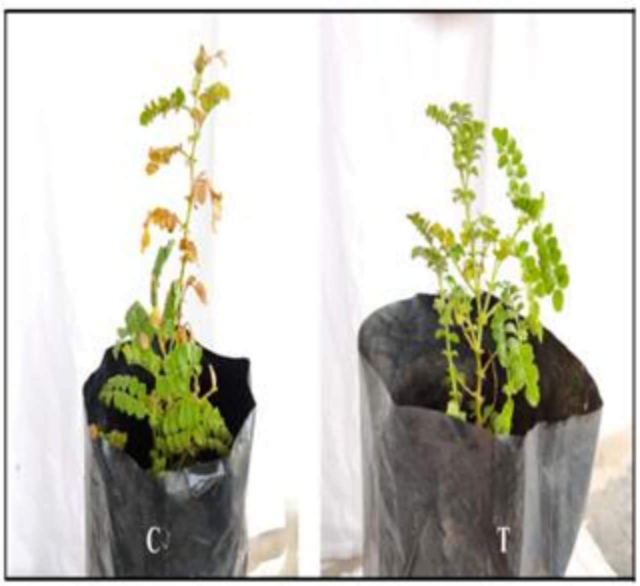

Figure 4. Wilt disease incidence in control (C) and treated (T) Chickpea seedlings.

CONCLUSIONS

Seed nanopriming with thiamine encapsulated chitosan nanoparticle enhances growth and induce defense enzymes in chickpea their by protecting the seedlings from wilt pathogen. This method of inducing disease resistance by strengthening the plants innate immune system represents a sustainable approach to protect chickpea plants from invading phytopathgens. Given the large active surface area of nanoparticles, smaller amount is required to enhance protection in plants when compared to conventional pesticides [9]. Hence, this biopolymer based nanoparticle encapsulated with vitamin, has great potential in agriculture as agrochemical with high specificity and improved function.

AUTHOR STATEMENT

This work was carried out in collaboration between all authors. MS conceptualized, designed the study, analyzed the data and wrote the manuscript [10]. MI performed the experiments, SM carried statistical analysis. All authors read and approve the final manuscript.

ACKNOWLEDGEMENT

The authors thank the University Authorities for providing the facilities. MI thanks DST-PURSE for providing Fellowship. SM thanks the University for providing fellowship.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

REFERENCES

1. do Espirito Santo Pereira A, Caixeta Oliveira H, Fernandes Fraceto L, Santaella C (2021). Nanotechnology potential in seed priming for sustainable agriculture. Nanomaterials. 11: 267.

2. Bartolucci C, Antonacci A, Arduini F, Moscone D, Fraceto L, Campos E et al(2020). Green nanomaterials fostering agrifood sustainability. Trends Analyt Chem. 125: 115840.

3. Singh N, Bhuker A, Jeevanadam J (2021). Effects of metal nanoparticle-mediated treatment on seed quality parameters of different crops. Naunyn Schmiedebergs Arch Pharmacol. 1-23.

4. Eyidogan F, Öz MT (2007). Effect of salinity on antioxidant responses of chickpea seedlings. Acta Physiologiae Plantarum. 29: 485-493.

5. Burman U, Saini M, Kumar P (2013). Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. Toxicol Environ Chem. 95: 605-612.

6. Yousef F, Shafique F, Ali Q, Malik A (2020). Effects of salt stress on the growth traits of chickpea (*Cicer arietinum L.*) and pea (*Pisum sativum L.*) seedlings. J Biol Res.

7. El-Soud WA, Hegab MM, AbdElgawad H, Zinta G, Asard H (2013). Ability of ellagic acid to alleviate osmotic stress on chickpea seedlings. Plant Physiol Biochem. 71: 173-183.

8. Grewal HS, Maheshwari BL (2011). Magnetic treatment of irrigation water and snow pea and chickpea seeds enhances early growth and nutrient contents of seedlings. Bioelectromagnetics. 32:

58-65.

9. Kaur S, Gupta AK., Kaur N (2000). Effect of GA3, kinetin and indole acetic acid on carbohydrate metabolism in chickpea seedlings germinating under water stress. Plant Growth Regul. 30: 61-70.

10. Nayyar H, Bains TS, Kumar S (2005). Chilling stressed chickpea seedlings: effect of cold acclimation, calcium and abscisic acid on cryoprotective solutes and oxidative damage. Environ Exp Bot. 54: 275-285.