

Can seed priming with zinc and silver NP's improve the seed germination and seedling vigour of soybean (*Glycine max L.*)

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Abstract: The laboratory experiment was carried out to study the influence of seed priming with Zinc and Silver NP's on seed germination and seedling vigour of soybean (*Glycine max L.*) at NSP, Seed Unit, Raichur & the Department of Seed Science and Technology, College of Agriculture, UAS, Raichur during 2020-21. The experiment was conducted in a completely randomized design (CRD) consisting of four different concentrations of standard nano zinc (750 ppm), green synthesized nano ZnO (500 ppm), standard nano silver (500 ppm) and green synthesized nano silver (250 ppm) particles which were standardized in the preliminary experiments along with hydro priming and control. Among the treatments imposed, standard nano zinc @ 750 ppm recorded significantly highest seed germination (84.50%), seedling vigour index - I (3769) and vigour index - II (52331) which was followed by green synthesized silver NP's (79.5%, 3069 & 49044) and green synthesized ZnO NP's (79.0%, 2891 & 48372) compared to hydro priming (75.0%, 2655 & 45548) and control (73.5%, 2528 & 44541). Similarly, standard nano zinc (750 ppm) recorded the highest enzymatic activity such as dehydrogenase (0.441 OD value), alpha amylase (24.40 mm), peroxidase (0.989 μ moles $\text{ml}^{-1} \text{min}^{-1}$) and catalase (0.058 μ moles $\text{ml}^{-1} \text{min}^{-1}$) which was followed by green synthesized silver NP's (0.389 OD, 23.60 mm, 0.709 μ moles $\text{ml}^{-1} \text{min}^{-1}$ & 0.043 μ moles $\text{ml}^{-1} \text{min}^{-1}$). But proline content was highest in control (11.848 mg g^{-1}). Hence, seed priming with standard nano zinc (750 ppm) was most effective for improving the germination and seedling vigour of soybean.

Key words: Nanoparticles, Nano zinc, Nano silver, Seed germination, Seedling vigour Soybean

Introduction

Soybean (*Glycine max L.*) is an important legume that belongs to the family Fabaceae and sub-family Papilionoidaceae. In India, it is being cultivated in an area of 12.91 million hectare with a production of 12.61 million tonnes and with a productivity of 976 kg/hectare. In Karnataka, it is cultivated in an area of 0.31 million hectares with a production of 0.37 million tonnes and with a productivity of 1212 kg/hectare (Anon., 2022).

Nanotechnology is a field of convergence among life sciences, material science and information technology which is capable of manipulating at atom level and derives solutions to unresolved field problems (Sozer and Kokini, 2009). The nanoparticle measures a dimension of 10^{-9} meter. Currently, the main thrust of research in nanotechnology is focusing its applications in the field of electronics, energy, medicine and life science.

Nano encapsulated agrochemicals could be designed in such a way that they possess all necessary properties such as effective concentration (with high solubility, stability and effectiveness), time-controlled release in response to certain stimuli, enhanced targeted activity and less eco-toxicity with safe and easy mode of delivery thus avoiding repeated application (Manjunath *et al.*, 2016). The traditional methods for producing NPs are costly, poisonous and unfriendly to the environment. The method of synthesising nanoparticles (NPs) is generally through chemical reduction. However, most of the reducing agents are highly toxic chemicals. Plant substitutes

can replace such toxic reductants because of their environmental friendliness and hence green synthesis of NPs has arisen as an emerging technology.

Seed nano priming is one of the potentially effective strategies to improve the germination performance of aged or dormant seeds which before are significant in agriculture. The technology of seed priming with green synthetic NPs is called green nano priming. This technology can promote germination of seeds effectively without poisoning seedlings. Therefore, green nano priming has huge potential for enhancing the crop productivity (Song and He, 2021).

Hence, with this background an experiment was planned to study the influence of seed priming with green synthesized zinc and silver nanoparticles on seed quality of soybean (*Glycine max L.*)

Material and methods

The laboratory experiments pertaining to present investigation was conducted at the Department of Seed Science and Technology, National Seed Project, Seed Unit and Centre for Nanotechnology, University of Agricultural Sciences, Raichur, Karnataka during 2021-22. The experiment was laid out in a Completely Randomized Design (CRD), with six treatments *viz.*, T₁- Control, T₂- Hydro priming, T₃- Standard nano zinc (750 ppm), T₄- green synthesized nano zinc (500 ppm), T₅- Standard nano silver (500 ppm) and T₆- Green synthesized

nano silver (250 ppm) which were standardized from preliminary experiments with four replications.

The seeds were primed with the respective nanoparticles and concentrations with the seed solution ratio of 1:0.3 for 3 hours (Miladinov *et al.*, 2015) and then dried back to original moisture content under shade for 24 hrs and subjected for seed germination test to assess various seed quality parameters as per ISTA rules (2013). The different seed quality parameters like seed germination (%), seedling vigour index – I and seedling vigour index – II and biochemical tests like dehydrogenase enzyme activity (OD value), alpha amylase enzyme activity (mm), peroxidase enzyme activity (μ moles $\text{ml}^{-1} \text{min}^{-1}$), catalase enzyme activity (μ moles $\text{ml}^{-1} \text{min}^{-1}$) and physiological test like proline content (mg g^{-1}) were assessed.

The standard germination test was conducted in four replications of 100 seeds each by following between paper method as per ISTA, 2013. While, the seedling vigour index was computed by adopting the formula as suggested by Abdul-Baki and Anderson (1973) and expressed in whole number.

For dehydrogenase enzyme activity (OD value), 25 primed seeds from each treatment were taken and the embryos were excised and steeped in 0.25 per cent solution of 2, 3, 5 triphenyl tetrazolium chloride solution and were kept in dark for two hours at 40°C for staining. The stained seeds were thoroughly washed with water and then soaked in 10 ml of 2 methoxy ethanol and kept overnight for extracting the red colour formazan. The intensity of red colour was measured using ELICO UV-VIS spectrophotometer using blue filter (470 nm) and methyl cellosolve as the blank. The OD values obtained were reported as dehydrogenase activity (Kittock and Law, 1968).

The α -amylase activity was analyzed as per the method suggested by Simpson and Naylor (1962), in which the diameter of halo (clear) zone formed around the seed when smeared with potassium iodide solution on half cut embryo portion intact seeds in an agar-starch gel in petri plates was measured in mm and reported as α -amylase activity.

The peroxidase activity was measured according to Fielding and Hall (1978). It was estimated by recording the increase in absorbance of oxidized guaiacol at 470 nm and was expressed as micromole of guaiacol oxidized per mili litre of reaction mixture per minute at 25°C. While, the catalase activity was assayed by

measuring the rate of disappearance of hydrogen peroxide (Maehly and Chance, 1959).

For proline content estimation 0.5 g leaves of 10 days old seedlings were homogenized in 5 mL of sulphosalicylic acid (3%) using pestle and mortar. The homogenate was filtered through Whatman No. 1 filter paper and filtrate was collected, which was used for estimation of proline content (Bates *et al.*, 1973). Then 2 mL of extract was taken in a test tube to which 2 mL of glacial acetic acid was added. The reaction mixture was heated in a boiling water bath at 100°C for 30 minutes; brick red colour was developed. After cooling the reaction mixture. 6 mL of toluene was added and then transferred to a separating funnel. After thorough mixing the chromophore containing toluene was separated and its absorbance was read at 520 in spectrophotometer against toluene as a blank which gives the proline content.

Results and discussion

Germination of seed is considered as one of the most significant step for determining the success or failure of crop establishment. In the present study, seed priming with standard nano zinc @ 750 ppm (T_3) recorded significantly highest seed germination (84.5%) (Table 1 and Plate 1), longest shoot (23.9 cm) and root (20.7 cm) length compared to all other treatments. This was followed by green synthesized silver nanoparticles @ 250 ppm (T_6 - 79.5%, 19.5 cm & 19.1 cm). Whereas the lowest seed germination, shoot and root length was observed for control (T_1 - 73.5%, 17.9 cm & 16.5 cm).



T₁ : Control T₃ : Standard nano Zn @ 750 ppm

Plate 1. Influence of seed priming with standard and green synthesized Zn and Ag NPs on seed germination of soybean

Table 1. Influence of seed priming with optimum concentration of standard and green synthesized Zn and Ag NPs on seed quality parameters of soybean

Seed priming treatments	Seed germination (%)	Shoot length (cm)	Root length (cm)	Seedling dry weight (mg)	Seedling vigour index - I	Seedling vigour index - II
T ₁ : Control	73.5 (59.0)*	17.9	16.5	606.0	2528	44541
T ₂ : Hydro priming	75.0 (60.0)	18.7	16.7	607.3	2655	45548
T ₃ : Standard nano Zn (750 ppm)	84.5 (66.8)	23.9	20.7	619.3	3769	52331
T ₄ : Green synthesized ZnO NPs (500 ppm)	79.0 (62.7)	19.1	17.5	612.3	2891	48372
T ₅ : Standard nano silver (500 ppm)	77.5 (61.7)	18.9	17.0	611.3	2782	47376
T ₆ : Green synthesized silver NPs (250 ppm)	79.5 (62.2)	19.5	19.1	616.9	3069	49044
Mean	78.2 (62.4)	19.7	17.9	612.2	2949	47868
S.Em±	1.5	0.4	0.2	2.5	64	911
C.D. @ 1%	4.5	1.2	0.7	7.4	193	2726



Legend: T₁: Control T₂: Hydro priming T₃: Standard nano zinc @ 750 ppm, T₄: Green synthesized nano zinc @ 500 ppm T₅: Standard nano silver @ 500 ppm, T₆: Green synthesized nano silver @ 250 ppm

Fig 1. Influence of seed priming with standard and green synthesized Zn and Ag NPs on seed germination of soybean

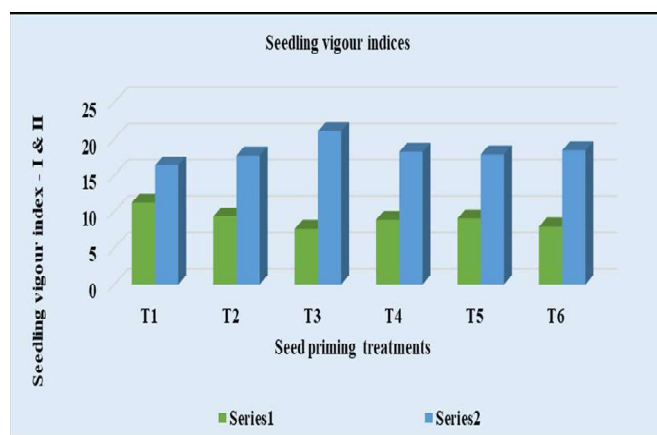
This enhanced seed germination (Fig 1) due to optimum concentration of nano Zn @ 750 ppm over absolute control might be due to the nano size of particles that might have allowed them to penetrate through the seed coat easily and thereby better absorption and utilization of these particles by seeds at a particular concentration (Savithamma *et al.*, 2012). Further, nanoparticles would induce oxidation-reduction reactions via superoxide ion radical during seed germination, resulting in quenching of free radicals from the germinating seeds. In turn, oxygen produced during such process could also be used for respiration, which would further promote seed germination (Zheng *et al.*, 2005). An increase in growth rate of plants due to seed priming with ZnO NPs (0.2%) was reported by Avinash *et al.* (2010) in *Cicer arietinum* thereby increased the level of indole acetic acid in the roots (confirmed through high pressure liquid chromatography analysis) and ultimately leading to better growth of plants at optimum concentration.

The seedling vigour index – I and II were significantly highest in standard nano zinc @ 750 ppm (T₃ – 3769 & 52331) (Fig 2) which was followed by green synthesized silver nanoparticles @ 250 ppm (T₆ – 3069 & 49044). The higher seedling vigour index – I was mainly due to better seed germination, root and shoot length. While higher seedling vigour

index – II was mainly due to better seed germination and seedling dry weight. Similar results were reported by Pradeep *et al.* (2016) in pigeon pea.

It was observed that standard nano Zn @ 750 ppm (T₃) showed the highest dehydrogenase enzyme activity (0.441) and highest α -amylase enzyme activity (24.400 mm) (Table 2) which was followed by green synthesized silver nanoparticles @ 250 ppm (T₆ – 0.389 & 23.600 mm) and the least dehydrogenase and alpha amylase enzyme activity was noticed in control (T₁ - 0.256 & 20.500 mm). The nanoparticles treated seeds were found to have higher enzymatic activity, showing positive role in improving the seed vigour and viability. It is an established fact that, Zn (acts as a micronutrient) that act as cofactor for most of the enzyme complexes involved in respiration and food mobilization in seeds (Raun, 2007). The better availability of these micronutrient at nanoscale resulted in more synthesis and activity of the enzymes or could be due to repair of damaged vital cell organelles (Raun, 2007). (Villiers, 1974; Villiers and Edgcumbe, 1975; Burgass and Powell, 1984) and counteraction of lipid peroxidation and minimization of free radical reactions (Hsu *et al.*, 2003 and Khanahmadi *et al.*, 2010).

The treatment, standard nano zinc @ 750 ppm (T₃) registered



Legend: T₁: Control T₂: Hydro priming T₃: Standard nano zinc @ 750 ppm, T₄: Green synthesized nano zinc @ 500 ppm T₅: Standard nano silver @ 500 ppm, T₆: Green synthesized nano silver @ 250 ppm

Fig 2. Influence of seed priming with standard and green synthesized Zn and Ag NPs on seedling vigour indices of soybean

Table 2. Influence of seed priming with optimum concentration of standard and green synthesized Zn and Ag NPs on biochemical parameters of soybean

Seed priming treatments	Dehydrogenase enzyme activity (OD value)	Alpha amylase enzyme activity (mm)	Peroxidase enzyme activity (μ moles ml ⁻¹ min ⁻¹)	Catalase enzyme activity (μ moles ml ⁻¹ min ⁻¹)	Proline content (mg g ⁻¹)
T ₁ : Control	0.256	20.500	0.437	0.038	11.848
T ₂ : Hydro priming	0.324	21.167	0.531	0.038	9.193
T ₃ : Standard nano Zn (750 ppm)	0.441	24.400	0.989	0.058	7.558
T ₄ : Green synthesized ZnO NPs (500 ppm)	0.349	23.083	0.663	0.041	8.963
T ₅ : Standard nano silver (500 ppm)	0.332	22.833	0.632	0.040	9.180
T ₆ : Green synthesized silver NPs (250 ppm)	0.389	23.600	0.709	0.043	7.903
Mean	0.348	22.597	0.660	0.043	9.108
S.Em \pm	0.01	0.538	0.014	0.001	0.214
C.D. @ 1%	0.031	1.611	0.042	0.003	0.641

Standard nano zinc @ 750 ppm (T₃) registered significantly higher peroxidase (0.989 μ moles ml⁻¹ min⁻¹) and catalase (0.058 μ moles ml⁻¹ min⁻¹) enzyme activity (Table 2). Whereas, the lowest peroxidase (0.437 μ moles ml⁻¹ min⁻¹) and catalase (0.038 μ moles ml⁻¹ min⁻¹) enzyme activity were recorded by control (T₁). The higher CAT activity is basically due to NPs modulating the enzymatic antioxidants in seeds; many studies observed the ability of NPs to mimic the roles of antioxidant enzymes such as CAT. Plants with better antioxidants are more resistant to the oxidative conditions and less susceptible to stress challenging environment (Wei and Wang, 2013; Khan *et al.*, 2017). During seed germination, CAT activity was increased in both the plumule and radical of the growing seedlings. The ability of growing seedlings to enhance CAT activities mediated by Zn NP during seed germination could have improved the seed germination process (Upadhyaya *et al.*, 2017).

Among the various nanoparticles seed priming, control (T₁) recorded significantly highest proline content (11.848 mg g⁻¹) (Table 2) compared to all other treatments. Whereas, the lowest proline content was observed for standard nano zinc @ 750 ppm (T₃ - 7.558 mg g⁻¹). Proline, as a multifunctional amino acid

that triggers the plant growth through signaling mechanism, upregulated and get accumulated under stressed conditions (Yang *et al.*, 2009). Our results demonstrated a high level of proline in treatment T₁ while lower in treatment T₃ that is associated with the above findings. Further, the healthy and progressive plants growth under an ideal range of NPs (T₃, T₄, T₅, T₆) by minimizing the proline content less than control because plants enhance the endogenous proline level when suffered from more stress (Szabados and Savoure, 2010).

Conclusion

Seed nano priming is one of the potentially effective strategies to improve the seed germination and seedling vigour of many crop plants. From the present study, out of the above four materials used, the seed germination and seedling vigour of soybean was improved by seed priming with standard nano Zn (750 ppm) followed by green synthesized silver NPs (250 ppm) with the seed solution ratio of 1:0.3 for 3 hours. Hence, seed priming with standard nano zinc (750 ppm) followed by green synthesized silver NPs (250 ppm) can improve the seed germination and seedling vigour of soybean.

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