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Studies on effect of polymer seed coating, nanoparticles and hydro priming on seedling characters of Chickpea (*Cicer arietinum* L.) seed

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Abstract
A laboratory study was undertaken to know the effect of seed polymer coating, nanoparticles (NPs) Zn, Fe at different concentration (10 and 25 ppm), $ZnSO_4$, $FeSO_4$ (100 and 500 ppm) and hydropriming with different durations (6 and 12 hrs.) on seedling characters of chickpea seed. At the Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences. Among the treatments seed polymer coating with Fe NPs at 25 ppm recorded significantly higher seed germination (87.25%), speed of germination (86.75%), seedling root length (19.36cm), seedling shoot length (12.98), seedling length (32.34 cm), seedling dry weight (1.54g), seedling fresh weight (7.90), seedling vigour index I (3234.00), seedling vigour index II (406.96) and lowest abnormal seedlings (2.50%) over their bulk forms and control followed by Fe and Zn NPs at 25 ppm. The hydropriming have positive influence on seed quality parameters of chickpea individually but the effect of priming method was found significant. Hence, from the results it is concluded that Fe and Zn NPs at 25 ppm can be used to enhance quality of the chickpea seed.

Keywords: Fe, Nanoparticles, chickpea Seed, Seed Polymer Coating, Zn, Hydro priming, $ZnSO_4$, $FeSO_4$

Introduction

Chickpea (*Cicer arietinum* L.) belongs to family Leguminosae, an annual legume. It is an important winter grain legume having extensive geographical distribution. Chickpea is known by different names in different places such as gram chana Bengal gram pois, hoos, Hommos, grao-de-beco and garbandzo. It is a self-pollinated crop having chromosome no 2n= 16.

In India, chickpea is a premier pulse crop occupying 8.17 million ha and contributing 7.47 million tonnes to pulse basket. It accounts for 20% of the world pulses production. Major producers of chickpea include India, and Mexico. Global production, as per the latest available estimates of Food and Agricultural Organization (FAO), is about 12 million metric tons in 2011.

The nutrient composition of chickpea seed has protein (18-22%), fat (4-10) %, 6.3%, carbohydrates (52-70%) and minerals (Calcium, Phosphorous, Iron) and Vitamins. Cellulose, 0.2%% Ca, 0.3% P, 3.0% ash (Huda *et al*., 2003; Ozer *et al*., 2010). Bengalgram, which is called chickpea or gram (*Cicer arietinum* L.) in South Asia, is one of the important pulses in developed world. It is a major pulse crop in India widely grown for centuries and accounts for nearly 40% of the total pulse production

Modern agriculture with its bias for technology and precision, demands that each and every seed should readily germinate and produce a vigorous seedling ensuring higher yield. Many scientists all over the world have developed many new production techniques called "seed enhancement techniques" *viz.*, seed polymer coating, seed coloring, seed pelleting, seed fortification, seed infusion, *etc*., among these seed polymer coating is the promising one. Seed polymer coating is the sophisticated process of applying precise amount of active ingredients along with a liquid polymer directly on to the seed surface without obscuring its shape. It is one of the most economic approaches for improving the performance of seed. It also paves way for including all the required ingredients like inoculants, protectants, nutrients, hydrophilic substances, herbicides, oxygen suppliers *etc*. Nanotechnology, the science of working with smallest possible particles, raises hopes for the future to overcome the difficulties encountered in agriculture. Nanoparticles (NPs) by virtue of their nano size (10^{-9}m) possess larger surface area resulting in increased catalytic activity and are highly reactive.

Zinc (Zn) and iron (Fe) being essential micronutrients, required for the normal plant growth and development and they are important components of various enzymes that are responsible for driving many metabolic reactions in all crops. However, these micro elements are required in minute quantity for treating seeds. Recently use of these elements in the form of nanoparticles gaining importance especially for enhancing seed quality in few crops. In this context, an effort was made in the present investigation to find out the effect of seed polymerization with Zn and Fe nanoparticles on seedling characters of chickpea seed.

Materials and Methods

The research studies were carried out in the Seed science post Graduate Laboratory, Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad, 211007, Uttar Pradesh, India. The seed of chickpea cultivated variety is RSG-902

The different concentrations of Zn and Fe nanoparticles suspensions like 10 and 25 mg l-1 were prepared for the experiment in distilled water. The nanoparticles were suspended directly in distilled water and dispersed by ultrasonic vibration (100W, 40 kHz) for 30 min. Small magnetic bars were placed in the suspension for stirring before use to avoid aggregation of the particles. The seeds are suspended in prepared solution for about 6 hrs. Subsequently seeds were air dried over night to safe moisture content. Observations on various seed quality parameters *viz.*, seed germination (%),speed of germination (Maguire, 1962), seedling root length (cm), seedling shoot length (cm), seedling length (cm),seedling fresh weight (g), seedling dry weight (g), seedling vigour index I (Abdul-Baki and Anderson, 1973) [2], seedling vigour index II, were recorded as per the methods and procedures described by ISTA. The mean data of the laboratory experiments were statistically analyzed by adopting completely randomized design as outlined by Panse and Sukhatme (1985) [3]. The critical differences were calculated at one per cent level of probability wherever 'F' test was found significant for various seed quality parameters under the study.

Results and Discussion

Results obtained on various seed quality parameters like seed germination (%), speed of germination, seedling root length (cm), seedling shoot length (cm), seedling length (cm), seedling fresh weight (g), seedling dry weight (g), seedling vigour index I, seedling vigour index II are presented as follows.

Germination %

A range of 80.00 to 86.75 percent was observed for speed of germination percentage. The mean value for this parameter was (89.73) Maximum speed of germination (86.75) was recorded with T_9 (Polymer + Fe nanoparticles @ 25 ppm). Whereas minimum significantly speed of germination followed by (80.05%) with T_5application of (Polymer + Zn nanoparticles @ 25 ppm). Minimum germination percentage was recorded by T_1 (80.00) with control

The probable reason for the enhanced germination due to the Nano size of particles allow them to penetrate through seed coat easily and hence, provided better absorption and utilization of these particles by seeds. The beneficial effect of the these NPs in improving the germination and production of essential bimolecular as well as essential nutrients required for plant growth and are important components of various enzymes which are responsible for driving many metabolic reaction (Senthilkumar, 2011) [5].

Speed of germination

A range of 80.00 to 86.75 percent was observed for speed of germination percentage. The mean value for this parameter was (89.73) Maximum speed of germination (86.75) was recorded with T_9 (Polymer + Fe nanoparticles @ 25ppm). Whereas minimum significantly speed of germination followed by (80.05%) with T_5 application of (Polymer + Zn nanoparticles @ 25 (ppm). Minimum germination percentage was recorded by T_1 (80.00) with control.

The reason for rapid germination could be that the NPs may form new pores on seed coat during penetration facilitating the influx of water inside the seed or NPs may enter into the seed through the cracks present over the surface of the seed and activated the enzymes in early phase thereby enhanced the speed of germination.

Seedling root length (cm)

The mean performance of seedling root length ranged from 15.20cm to 18.14cm with mean value of 17.74 cm. Maximum root length (19.36cm) was recorded by T_9 with application of Polymer + Fe nanoparticles @ 25 ppm and it was followed by T_5 (18.22cm) with application of Polymer + Zn nanoparticles @ 25 ppm Minimum root length was recorded by T_1 (15.20 cm) with control.

Reported that nanoparticles of ZnO, Ag and TiO2 treated to groundnut seeds outperformed the control significantly in terms of germination, shoot length, root length and vigour index.

Seedling shootlength (cm)

Among shoot length there exists a significant variation as influenced by Polymer + Fe nanoparticles priming methods and seeds primed with T_9 with application of Polymer + Fe nanoparticles @ 25 ppm (12.98 cm) has highest root length, control (9.10 cm) has lowest root length. Seeds primed with Polymer + Zn nanoparticles @ 25 ppm (12.98 cm), have statistically on per results.

Prasad *et al.* (2012) [4] observed that ZnO nanoparticles improved the germination, root growth, shoot growth dry weight groundnut, significantly as compared to chelated $ZnSO_4$. Observed the positive effect of nano ZnO, nano FeO and nanoZnCuFe oxide particles on growth of mung bean seedlings over the control. reported that nanoparticles of ZnO, Ag and TiO_2 treated to groundnut seeds at different concentrations *viz.*, 500, 750, 1000 and 1250 mg kg-1 outperformed the control significantly in terms of germination, shoot length, root length and vigour index.

Seedling length (cm)

The mean performance of seedling length ranged from 24.10cm to 32.34cm with mean value of 28.26 cm. Maximum root length (32.34cm) was recorded by T_9 with application of Polymer + Fe nanoparticles @ 25 ppm and it was followed by T_5 (25.87 cm) with application of Polymer + Zn nanoparticles @ 25 ppm Minim um root length was recorded by T_1 (24.10 cm) with control.

This could be ascribed to the increased synthesis and activity of hydrolytic enzymes during the early phases of germination and effective mobilization of the available food reserves in the seeds resulted in the early emergence and growth of the seedlings. In proportional to increase in seedling growth, dry matter production was also increased. These results are in agreement with findings of Avinash *et al*., (2010) in *Cicer arietinum i.e.* ZnO NPs increased the level of IAA in the roots (sprouts) and thereby resulted in increase in the growth rate of seedlings

Seedling fresh weight (g)

The mean performance of seedling fresh weight ranged from 5.40 g to 7.90 g with mean value of 6.66 g. Maximum seedling fresh weight (7.90g) was recorded by T_9 with application of Polymer + Fe nanoparticles @ 25 ppm and it was followed by T_4 (5.70 g) with application of. Polymer + Zn nanoparticles @ 10 ppm Lowest value of seedling fresh weight was recorded by T_1 (5.40g) with control

These results are in conformity with the findings of Prasad *et al.*, 2012 [4], Abdel-Azeem and Elsayed (2013) [1] reported inhibitory effect of silver nanoparticles (above 50 ppm) on root length, mitotic indices and chromosomal morphology in *Viciafaba*. Mahmoodzadeh *et al.*, (2013) reported detrimental effect of TiO2 nanoparticles on shoot and root biomass in wheat.

Seedling dry weight (g)

The mean performance of seedling dry weight ranged from 1.02 mg to 1.54 mg with mean value of 1.23 mg. Maximum seedling fresh weight (1.54 mg) was recorded by $T_9$with a polymer+ Fe nanopraticles 25ppm and it was followed by T_2 (1.08mg) with application of polymer+ $ZnSO_4$ @ 100ppmLowest value of seedling fresh weight was recorded by T_0 (1.02gm) with control.

This could be ascribed to the increased synthesis and activity of hydrolytic enzymes during the early phases of germination and effective mobilization of the available food reserves in the seeds resulted in the early emergence and growth of the seedlings. In proportional to increase in seedling growth, dry matter production was also increased.

These results are in agreement with findings of Avinash *et al.*, (2010) in *Cicer arietinum i.e.* ZnO NPs increased the level of IAA in the roots (sprouts) and thereby resulted in increase in the growth rate of the seedlings.

Seed vigour index I

The mean performance of seedling vigourindex length ranged from 1938.55 to 32324.00 with mean value of 2538.65. Maximum seedling vigour index length (32324.00) was recorded by T_9 with application of Polymer + Fe nanoparticles @ 25 ppm and it was followed by T_4 (2179.25) with application of Polymer + Zn nanoparticles @ 10 ppm. Minimum seedling vigour index length was recorded by T_1 (1938.55) with control.

Seed vigour index II

The mean performance of seedling vigour index mass ranged from 294.40 to 406.96 with mean value of 338.99. Maximum seedling vigour index mass (294.40) was recorded by T_9 with application of Polymer + Fe nanoparticles @ 25 ppm and it was followed by T_5 (350.57) with application of Polymer + Zn nanoparticles @ 25 ppm. Minimum seedling vigour index mass was recorded by T_1 (294.40) with control.

Table 4.2 Mean performance of chickpea seeds for 9 seedling characters

S. No	Treatments	Germination (%)	Speed of germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Fresh weight of seedling(g)	Dry weight of seedling(g)	Seed vigour index I	Seed vigour index II
1	T1	80.00	80.00	15.20	8.90	24.10	5.40	1.02	1938.55	294.40
2	T2	82.25	83.25	17.82	8.12	26. 67	6.32	1.08	2193.95	339.28
3	T3	85	81.25	17.01	9.10	26.11	6.81	1.19	2232.48	344.21
4	T4	84.75	82	18.22	9.52	26.31	5.70	1.19	2179.25	327.99
5	T5	80.25	80. 5	15.84	11.32	25.87	7.07	1.32	2527.80	350.57
6	T6	85	81. 62	18.22	11.20	28.09	7.10	1.21	2481.48	316.45
7	T7	82.5	81.75	17.83	12.42	29.21	7.80	1.50	2571.52	340.19
8	T8	84.25	83.25	19.25	11.50	31. 67	6.91	1.10	2826.15	342.43
9	T9	87.25	86.75	19.36	12.98	32.34	7.90	1.54	3234.00	406.96
10	T10	81.25	83.05	16.75	11.22	30.72	6.35	1.21	2519. 6	323.09
11	T11	83	82.25	18.14	12.20	27.99	6.98	1.25	2767.73	343.29
	Grand Mean	88.15	89.73	17.74	10. 67	30.09	6. 66	1.23	2538. 65	338.99
	C.D. (5%)	1.150	1.150	0.200	0.050	0.716	0.184	0.008	165.418	12.914
	SE(m)	0.543	0.543	0.094	00.24	0.338	0.087	0.004	78.027	37.158

Table 4.3: Analysis of variance for 9 seedling characters in Chickpea seeds

S. No.	Characters	Mean sum of squares	
		Treatments (df=6)	Error (df=21)
1	Germination percentage	26.143**	1.012
2	Speed of germination	563.202**	10.369
3	Root length	30.195**	1.546
4	Shoot length	11.964***	0.007
5	Seedling length	76.854**	1.57
6	Seedling fresh weight	4.559**	0.157
7	Seedling dry weight	0.185**	0.001
8	Seed vigour index length	2116.745**	10.158
9	Seed vigour index mass	877641.884**	17286.204

** Significant at 5% and 1% level of significance, respectively

Conclusion

It can be concluded that the seeds are treated with different NPs with different concentrations are effective on chickpea seed, but the treatment T_9 shows more effective polymer + Fe NPs @ 25 ppm on germination percentage (87.25%) and all other physiological parameters. And Zn NPs also have effective result on germination (86.75%), and hydropriming showed better result. Finally it's concluded that among the different concentrations 25 ppm of Zn NPs or Fe NPs can be used to enhance the seed quality in chickpea along with polymer coating.

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