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Effect of seed priming with iron and zinc on yield contributing parameters as well as the nutrient uptake of the soybean (*Glycine max*) in calcareous soil

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Abstract
An investigation was undertaken to study the effect of seed priming with iron and zinc on yield contributing parameters as well as the nutrient uptake of the Soybean (*Glycine max*). The field experiment was conducted at PGI, Research Farm. Department of Soil Science and Agril. Chemistry, M.P.K.V., Rahuri, during the kharif 2016-17. The effect of seed priming, or soaking seeds in nutrient solution for 12 hours before drying and sowing them, was done for soybean (*Glycine max*) during the *kharif* season of 2016 i.e. from 30-6-2016 to 10-9-2016 in M.P.K.V, Rahuri, Maharashtra. The experiment was laid out in a randomized block design with three replication and seven treatments. The treatment comprised of absolute control, GRDF (general recommended dose of fertilizer) (50:75:45 kg ha-1 N : P_2O_5:K_2O + 5 t FYM ha-1), GRDF with seed priming with distilled water, GRDF with seed priming with Zn EDTA, GRDF with seed priming with ZnSO4, GRDF with seed priming with Fe EDTA, GRDF with seed priming with $FeSO_4$. Better results were obtained from priming the seeds compared to the controlled one. There were significant differences in the germination percentage among the treatments. The highest was shown in the seed priming with Zn EDTA @ 0.2%. Vigour index too showed significant difference due to seed priming. Germination index was highest in the treatment $FeSO_4.7H_2O$ @ 0.02%. Chlorophyll content showed non-significant difference among the treatments with highest being in treatment $FeSO_4.7H_2O$ @ 0.02%. The nodule count of soybean plants was highest in treatment $FeSO_4.7H_2O$ @ 0.02%. The major nutrient (NPK) uptake was comparatively higher in the treated plot over the non-treated controlled plot.

Keywords: seed priming, GRDF (general recommended dose of fertilizer), nutrient solution, vigour index, Germination index

Introduction
Soybean is the richest, cheapest and easiest source of best quality protein and fat. It is also called "Gold of soil". It is the second most important oil seed crop in India. Soybean was introduced in India during 1880s. It is major source of edible oil and protein. Hence, the crop is called as vegetarian meat and wonder crop. Calcareous soils are frequently characterized by their low bioavailability of plant nutrients due to high base status and pH between 7.5 to 8.5 and the presence of carbonate minerals (Marschner, 1995) [7]. The presence of calcium carbonate aggravates the problem of nutritional disorders, particularly Fe and Zn in plants growing on them. Iron chlorosis on rice in varying degree of intensity was reported in calcareous soils of north Bihar (Sakal, 1976) [10]. A substantial amount of soluble iron in such soils gets converted into unavailable form. Otzurk *et al.* (2006) [9] found that, Zn in newly-developed radicles and coleoptiles during seed germination was much higher (up to 200 mg kg^{-1}) thus, highlighting the involvement of Zn in physiological process during early seedling development, possibly in protein synthesis, cell elongation membrane function and resistance to abiotic stresses (Cakmak, 2000) [3]. In addition, higher seed Zn contents may better resist invasion of soil- borne pathogens during germination and seedling development thus, ensuring good crop stands (Marschner, 1995) [7] and ultimately better yield. Among the micronutrients, iron is needed in the greatest quantity and its availability is dependent on the pH of the growing medium. Iron is a constituent of several enzymes and some pigments, and assists in nitrate and sulphate reduction and energy production within the plant.

Although iron is not used in the synthesis of chlorophyll (the green pigment in leaves), it is essential for its formation. This explains why plants deficient in iron show chlorosis in the new leaves. In present study, an attempt has been done to supply the Fe and Zn through seed priming treatment which is a cost effective and farmer friendly method. Seed priming is a low cost and attractive alternative to the conventional soil or foliar application. Calcareous soil is especially deficient in Fe and Zn which can lead to complete failure of crop if not taken care of. Soil and foliar application are the most prevalent methods of micronutrient addition but the cost involved and difficulty in obtaining high quality micronutrient fertilisers are major concerns with these in developing countries. Seed priming is a simple method of soaking the seeds in a nutrient solution prior to the sowing.

Materials and Methods

The trial was carried out at post Graduate Research Farm, Department of Soil Science and Agril. Chemistry, M.P.K.V., Rahuri during the *kharif* 2016-17. The experiment was conducted in randomized block design with 3 replications. The soil was medium black calcareous soil (*Typic Haplustept*) and had the following characteristics: pH 8.13 (1:2.5), organic carbon (OC) 0.55%, electrical conductivity (EC) 0.26 dS m^{-1}, calcium carbonate 10.29%, DTPA- Fe 4.30 mg kg^{-1}, DTPA-Zn 0.43 mg kg^{-1}, available N 246.11 kg ha^{-1}, available P 12.49 kg ha^{-1} and available K 335.72 kg ha^{-1}. The treatments comprised of *viz.*, T_1- control, T_2- GRDF (50:75:45 $N:P_2O_5:K_2O$ kg ha^{-1} + 5 t ha^{-1} FYM), T_3- T_2 + seed priming with distilled water, T_4- T_2 + seed priming with Zn EDTA @ 0.2%, T_5- T_2 + seed priming with $ZnSO_4.7H_2O$ @ 0.02%, T_6- T_2 + seed priming with Fe EDTA @ 0.1% and T_7- T_2 + seed priming with $FeSO_4.7H_2O$ @ 0.02%. Full dose of N, P, and K were applied at the time of sowing soybean seeds. Seed treatment with *Rhizobium* @ 25 g kg^{-1} seeds were common to all except treatment T_1 after priming. Recommended agronomic practices were followed. Fresh mature leaves samples were collected at 45 days for estimation of chlorophyll content by SPAD. Nodule count at 45 days after sowing was also taken. Germination percentage, germination index, vigour index and seedling vigour index were calculated by recording shoot length, root length and dry weight. Fresh plant samples were collected and processed with following standard procedure of washing, drying and grinding. Ground material (0.2 g) was digested with 5 ml of di-acid mixture ($HNO_3:HClO_4$ in 9:4). It was kept in digestion chamber till complete digestion of the sample. The residue was dissolved in double-distilled water and after filtration, final volume was made to 50 ml. Total uptake of phosphorus and potassium were determined with the standard procedure (Chapman and Pratt, 1961). To determine total uptake of nitrogen, plant samples were digested with di-acid mixture of $H_2SO_4:H_2O_2$ (1:1) and final volume made to 50 ml. Total uptake of nitrogen was determined following the procedure of Jackson (1958) [6].

Results and Discussion

Effect of seed priming with iron and zinc on yield contributing parameters of soybean Germination percentage in soybean showed significant differences among the treatments with the highest being in T_4 (T_2 + seed priming with Zn EDTA @ 0.2%) which was significantly higher than all the treatments except T_6 (T_2 + seed priming with Fe EDTA @ 0.1%) and T_7 (T_2 + seed priming with $FeSO_4.7H_2O$ @ 0.02%) which were at par with T_4. Similar results were also reported by Basra *et al.* (2003) [2] in canola that germination percentage as well as germination rate were increased in response to priming. Subedi and Ma (2005) [11] also noted that one of the most important conditions for crop potential performance is fast and uniform germination which is a resultant of priming. Similar results were also obtained by Mirshekari (2010) [8] who showed that seed priming with concentrations of 1% and 1.5% Fe and 1% B, solely or in combination, and 1.5% Fe and 1% B for 12 hrs. increased the rate of germination of dill (*Anethum graveolens*) seeds.

Table 1: Yield contributing parameters of soybean as influenced by seed priming with iron and zinc in soybean seeds on calcareous soil

Tr. No.	Treatment	Germination (%)	Vigor index	Germination index	Total chlorophyll (mg 100 g^{-1} fresh plant tissue)	Nodule count (45 DAS)
T_1	Absolute control	81.33	12.19	16.00	44.67	14.66
T_2	GRDF (50:75:45 $N:P_2O_5:K_2O$ kg ha^{-1} + 5t ha^{-1} FYM).	82.33	13.99	16.00	45.33	16.00
T_3	T_2 + seed priming with distilled water	82.67	14.8	16.67	45.67	16.33
T_4	T_2 + seed priming with Zn EDTA @ 0.2%	84.67	16.08	17.67	45.80	16.66
T_5	T_2 + seed priming with $ZnSO_4.7H_2O$ @ 0.02%	83.00	15.77	17.33	45.43	16.33
T_6	T_2 + seed priming with Fe EDTA @ 0.1%	84.00	15.12	17.33	47.00	16.00
T_7	T_2 + seed priming with $FeSO_4.7H_2O$ @ 0.02%	83.33	14.99	20.00	47.33	17.00
	SE±	0.49	0.05	1.12	0.62	0.62
	CD at 5%	1.53	1.23	N.S.	N.S.	N.S.

Vigour index too showed significant difference due to seed priming with iron and zinc in soybean seeds on calcareous soil. Treatment T_4 (T_2 + seed priming with Zn EDTA @ 0.2%) was significantly highest among all the treatments. Similar results were obtained by Hossein *et al.* (2011) [5] who stated that vigour index decreased as a result of artificial drying and increase of initial moisture content. But after osmopriming treatment the difference between initial moisture was minimized. Faster seed germination made them use better the environmental condition and increase of seedling length and vigour index as a result.

Germination index showed non-significant difference among the treatments with highest in treatment T_7 (T_2 + seed priming with $FeSO_4.7H_2O$ @ 0.02%). Chlorophyll content showed non-significant difference among the treatments with highest being in treatment T_7 (T_2 + seed priming with $FeSO_4.7H_2O$ @ 0.02%) followed by treatment T_6 (T_2 + seed priming with Fe EDTA @ 0.1%). This may be because iron is essential for chlorophyll synthesis, which is why an iron deficiency results to chlorosis in calcareous soil. The nodule count of soybean plants too showed non-significant difference with highest in treatment T_7 (T_2 + seed priming with $FeSO_4.7H_2O$ @ 0.02%)

followed by treatment T_4 (T_2 + seed priming with Zn EDTA @ 0.2%).

Effect of seed priming with iron and zinc on total uptake of major nutrients in soybean grown on calcareous soil at harvest stage

The data in respect of total nitrogen uptake by soybean was found non-significant. However, the seed priming treatment with micronutrients showed numerically higher total uptake of N by soybean plants compared to all other treatments under study.

Table 2: Effect of seed priming with iron and zinc on total uptake of N, P and K by Soybean crop at harvest stage

Tr. No.	Treatment	Total uptake of nutrient (kg ha^{-1})		
		N	P	K
T_1	Absolute control	134.88	12.63	65.94
T_2	GRDF (50:75:45 N:P_2O_5:K_2O kg ha^{-1} + 5 t ha^{-1} FYM).	158.98	17.06	83.48
T_3	T_2 + seed priming with distilled water	162.12	16.48	85.69
T_4	T_2 + seed priming with Zn EDTA @ 0.2%	173.49	19.17	90.92
T_5	T_2 + seed priming with$ZnSO_4$. $7H_2O$ @ 0.02%	171.19	19.91	89.41
T_6	T_2 + seed priming with Fe EDTA @ 0.1%	170.55	21.11	97.39
T_7	T_2 + seed priming with $FeSO_4.7H_2O$ @ 0.02%	195.55	23.12	105.92
	SE±	11.73	1.32	6.74
	CD at 5%	N.S.	4.13	21.00

The highest total P uptake by soybean plant was significantly observed in treatment of T_7 (T_2 + seed priming with $FeSO_4.7H_2O$ @ 0.02%) over treatment T_1 (Absolute control) and T_3 (T_2 + seed priming with distilled water), however treatment T_7 was at par with treatment T_4 (T_2 + seed priming with Zn EDTA @ 0.2%) and T_6 (T_2 + seed priming with Fe EDTA @ 0.1%). This can be because of seed priming with ferrous sulphate @ 0.02% might have increased the availability of P in soil. Similar type of results were also reported by Vyas *et al.*, (2003) [12] in soybean and Aziza *et al.* (2004) [1] too stated that addition of 10 mM Zn and 50 mM P to the priming solution increased the P and Zn uptake by 4-week-old seedlings and improved the water use efficiency of drought stressed plants by 44% above that of unprimed seeds.

Data on total potassium uptake by soybean revealed that, the significant increase in total K uptake was observed in treatment T_7 (T_2 + seed priming with $FeSO_4.7H_2O$ @ 0.02%) over treatment T_1 (Absolute control) and T_2 (GRDF (50:75:45 N:P_2O_5:K_2O kg ha^{-1} + 5 t ha^{-1} FYM).). However, the rest treatments were at par with treatment T_7 (T_2 + seed priming with $FeSO_4.7H_2O$ @ 0.02%).

The higher total uptake of nutrients (N, P and K) might be due to increase in grain and stover yield of soybean and availability of micronutrients through its application with seed priming in soybean seed.

Conclusions

Seed priming has been found effective in supplying the micronutrient requirement of seeds at the time of sowing as well as during the entire life cycle of plant and in total uptake of nitrogen, phosphorus and potassium, germination and vigour index in soybean on iron and zinc deficient medium deep black calcareous soil. Also there was no requirement for extra supplement of micronutrients through other method. This method is cost effective since very less micronutrient is required for soaking of the seeds.

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