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Improving iron content of blackgram by the application of ferrous glycinate on black calcareous soil

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Abstract
Blackgram production in alkaline or calcareous soils is frequently affected by iron (Fe) deficiency. Soil conditions such as high pH, high free calcium carbonate (lime), and low organic matter favor development of iron deficiency, which can delay crop maturity and reduce yields. The pot experiment was conducted with the blackgram variety, CO 6 at pot culture house TNAU, Coimbatore. Black in colour, sandy clay in texture and moderately calcareous. The soil was deficient in available Fe and sufficient in all other micronutrients. The experiment was laid out with nine treatments in a completely randomized design with three replications. The treatment are as follows. T_1 - NPK control, T_2 - $FeSO_4$ @ 25 kg ha^{-1} as basal soil application, T_3 - ferrous glycinate chelate @ 5 kg ha^{-1}, T_4 - ferrous citrate chelate @ 5 kg ha^{-1}, T_5 - Fe – EDTA chelate @ 5 kg ha^{-1}, T_6 - 1% $FeSO_4$ as foliar spraying on 25 and 45 DAS, T_7 - 1% ferrous glycinate as foliar spray on 25 and 45 DAS, T_8 - 1% ferrous citrate as foliar spraying on 25 and 45 DAS, and T_9 – 1% Fe – EDTA as foliar spray on 25 and 45 DAS. Iron content, Active iron content, enzymes namely catalase, peroxidase of blackgram at vegetative, flowering, and harvest stages were assessed. The highest activity of enzymes catalase, peroxidase, iron content and active iron content at all stages, were registered in treatment receiving foliar spray of 1% ferrous glycinate @ 25 and 45 DAS followed by the treatment with soil application of ferrous glycinate @ 5 kg ha^{-1}.

Keywords: Blackgram, iron content, ferrous glycinate, catalase, peroxidase

Introduction
Blackgram production in alkaline or calcareous soils is frequently affected by iron (Fe) deficiency. Soil conditions such as high pH, high free calcium carbonate (lime), and low organic matter favor development of iron deficiency, which can delay crop maturity and reduce yields. Iron is one of the most important micronutrient necessary to life and growth of plants because it helps in the formation chlorophyll and enzymes (Rivero *et al.*, 2005) [19]. Iron is a constituent of many enzymes like, cytochrome oxidase, catalase, peroxidase, acotinase, and nitrogenase. The deficiency of iron causes chlorosis leaves and exhibit in the young leaves of plants.

Iron is the third among the most limiting nutrients for plant growth primarily due to the low solubility of the oxidized ferric form in aerobic environments (Zuo and Zhang 2011, Samaranayke *et al*. 2012) [32, 21]. Susceptibility to iron deficiency is varied with varieties of same crops. Crops such as sorghum, field beans and soybeans can show severe iron chlorosis, where corn or alfalfa may appear normal. Some new varieties of soyabean can use iron efficiently than other, therefore selecting the appropriate crop and variety is important (Schulte and Kelling, 2004) [22].

Decomposition of organic matter releases some chelating agents like organic acids, amino acids, ligninsulfonates, ligninpolycarboxylates, sugar acids, derivatives, phenols, poly flavonoids, siderophores and phyto siderophores. Both classes of chelating/complexing agents increase micronutrient solubility (Sekhon, 2003) [23]. Chelating agents like citric acid and gluconic acid have the capacity to form water soluble chelates due to the presence of carboxylic and hydroxyl group combined with metal ions over coordinated covalent bonding. The hydroxyl groups are unattached complex with the metal ions under slightly acidic conditions (Clemens *et al.*, 1990) [5]. Calcium salts in soils have been found to increase the sorption of chelates (Wallace and Lunt, 1956) [28]. With increased concentrations of Ca-salts, there is a suppression of electrical double layer around the negatively charged surfaces, which permit anionic species to adsorb to sorption sites (Bolt and Warkentin, 1958) [2].

Carboxylate is one of the constituent in the root exudates which act as a chelating agent to alleviate nutrient deficiencies (Marschner and Römheld, 1994) [13]; (Ström *et al.*, 2002) [27]. Goos and Johnson, (2000) studied that the foliar application chelated iron fertilizer correcting iron deficiency in soybean and increase yield (Penas *et al*., 1990) [17], but have also had no effect in soybeans yields (Lingerfelser *et al*., 2005) [12] and corn yields (Godsey *et al*., 2003) [7]. Yunta *et al*. (2012) [30] reported that on comparing with other metal chelates Fe-EDTA was the best treatment for Fe uptake and translocation. Effectiveness of Fe fertilizers may be overestimated when only Fe concentration from sprayed leaf is analyzed, because both active physiological and precipitated Fe could be measured.

Application of soil or foliar treatments may also increase the yield and quality of citrus fruits. However, when compare to foliar treatment, soil application is advantage especially in terms of fruit yield and quality, is good information for growers to managing citrus on calcareous soils (Fang Chen and Jianwei Lu., 2006) [4]. Fe-sulfate and Fe-EDTA treatments are applied through foliar application that will more provide to the Fe to the plant than other Fe sources, if only leaf Fe concentration (mg kg^{-1} DW) is taken into account. However, Fe uptake and Fe immobilization did not allow to the plants (Yunta *et al*., 2012) [30].

With these facts in mind a pot culture experiment was conducted to improve the iron content by the application of ferrous glycinate on black calcareous soil.

Material and Methods

Pot experiment

The pot experiment was conducted at Tamil Nadu Agricultural University, Coimbatore to find out the effect of amino acid and organic acid chelated iron on growth and productivity of blackgram. The seeds of blackgram were obtained from Department of Pulses, TNAU, Coimbatore and soils were collected from farmer's field of Thondamuthur, Coimbatore. Seeds were sown in the pots at three seeds $pots^{-1}$ with nine treatments involving T_1 - NPK control, T_2 – $FeSO_4$ 25 kg ha^{-1} as basal soil application, T_3 - Ferrous glycinate chelate @ 5 kg ha^{-1}, T_4 - Ferrous citrate chelate @ 5 kg ha^{-1}, T_5 - Fe – EDTA chelate @ 5 kg ha^{-1}, T_6 - 1% $FeSO_4$ as foliar spraying on 25 & 45 DAS, T_7 - 1% Ferrous glycinate as foliar spray on 25 & 45 DAS, T_8 - 1% Ferrous citrate as foliar spraying on 25 and 45 DAS, and T_9 – 1% Fe – EDTA as foliar spray on 25 and 45 DAS. Five randomly selected plants from net plot area were used to record the dry matter production at different growth stages.

Elemental Analysis

Plant samples were collected at three stages *viz*., vegetative, flowering and harvest stages, shade dried and then kept in a hot air oven at 65°C and ground in a Willey Mill. The processed plant samples were analyzed for total nutrient content of Fe by adopting standard procedure (Hessey, 1971) [9].

Active iron content

Two grams of chopped plant sample was weighed and transferred to 100 ml glass bottles. Twenty ml of o-phenanthroline solution was added and the content of the bottles were stirred gently in order to embathe the plant sample with the extractant. The bottles were stoppered and allowed to stand for 16 hrs at room temperature. The contents were filtered through Whatman No. 1 filter paper. The active iron was estimated directly in the filtrate by measuring the transmittance at 510 nm in spectrophotometer (Katyal and Sharma, 1980) [11].

Estimation of catalase activity

Catalase activity was determined by following titration method using potassium permanganate (NC, 1963) [15] and expressed as μg H_2O_2 g^{-1} min^{-1}. 250 mg of leaf sample was macerated with 10 ml of sodium phosphate buffer (0.2 M, pH 6.8) and the content was centrifuged at 10,000 rpm at 4°C for 20 minutes. The supernatant solution of 1 ml was taken in four beakers, 5 ml of 1.5 per cent sodium perborate, 1.5 ml of sodium phosphate buffer (0.2 M, pH 6.8) and 10 ml of sulphuric acid was added in a first four beakers at the time interval of 1, 2, 3, 4 minutes respectively. In 5th beaker, 10 ml of sulphuric acid was added first, then 5 ml of sodium perborate (1.5%), 1.5 ml of sodium phosphate buffer (0.2 M, pH 6.8) and 1 ml of enzyme extract was taken and kept as blank. The solution was titrated against with 0.05 per cent potassium permanganate and the development of pink colour, which persists for 30 seconds, is the end point.

Estimation of peroxidase activity

Peroxidase activity (change in absorbance value at 430 nm g^{-1} min^{-1}) was determined according to Perur (1962) [18] and Angelini *et al.* (1990) [1]. 100 mg of leaf sample was extracted using 0.1M phosphate buffer (pH 7.0) and a known volume of the extract was added to a cuvette containing 3 ml phosphate buffer and 3 ml pyrogallol and increase in absorbance at 430 nm was recorded. The change in absorbance in minutes was used to calculate the enzyme activity.

Statistical Analysis

The data obtained from different experiments was analysed statistically to find out the effects of various treatments and their interactions. Analysis of variance was calculated as suggested by Panse and Sukhatme (1985) [16]. Simple correlation and regression co-efficient were also worked out between certain inter-related parameters to observe their degree of dependence as suggested by Snedecor and Cochran (1967) [25].

Results

Shoot iron content

The gradual increase in iron content of above ground bio mass with advancement of growth has observed (Table 1). Iron content in black gram tented is also to fluctuating at different growth stages. The highest mean shoot iron content of 325, 351 and 347 mg kg^{-1} at vegetative, flowering and harvest stages respectively with 1% ferrous glycinate as foliar spraying on 25 and 45 DAS which was on par with foliar spraying of 1% ferrous citrate (T_8 318, 346 and 341 mg kg^{-1} at vegetative, flowering and harvest stages respectively), while control treatment recorded lower iron content of 283, 311 and 308 mg kg^{-1} at vegetative, flowering and harvest stages respectively, in the black calcareous soil.

Root Fe content

The highest root Fe content of 145, 159 and 153 mg kg^{-1} at vegetative, flowering and harvest stages respectively was recorded in the treatment that received 1% ferrous glycinate as foliar spraying on 25 and 45 DAS which has followed by soil application of ferrous glycinate at 5kg ha^{-1} (T_3 138, 151 and 146 mg ha^{-1}) while control recorded lower iron content of 128, 141 and 136 mg kg^{-1} at vegetative, flowering and

harvesting stages respectively in black calcareous soil (Table 1).

Leaf active iron content

Increase in leaf active iron content was observed up to flowering stage and thereafter it declined at harvest stage (Table 2). The mean leaf active iron content of 39.2, 48.5 and 42.9 mg kg^{-1} was recorded for the foliar spraying of 1% ferrous glycinate twice at 25 and 45 DAS at vegetative, flowering and harvest stages respectively, which on par with soil application of ferrous glycinate at 5 kg ha^{-1} (T_3) and foliar spraying 1% ferrous citrate at 25 and 45 DAS as compared to control (no iron chelate) which recorded 18.8, 28.5 and 21.1 mg kg^{-1} at vegetative, flowering and harvest stages respectively in black calcareous soil.

Enzymes Activity

The data on catalase activity revealed an increase in all the treatment for the application of iron chelates (fig. 1). Foliar spraying of 1% ferrous glycinate registered the highest mean catalase activity of 11.4 and 14.8 µg of H_2O_2 g^{-1} min^{-1} at vegetative and flowering stages and on par with soil application ferrous glycinate at 5 kg ha^{-1} (10.6 and 13.1 µg of H_2O_2 g^{-1} min^{-1} at vegetative and flowering stages), while control registered the lower mean catalase activity of 2.83 and 6.80 µg of H_2O_2 g^{-1} min^{-1} at both the stages.

The enzyme peroxidase activity showed an upheaval trend for the application of iron chelates. Foliar spraying of 1% ferrous glycinate (T_7) registered the highest mean peroxidase activity of 6.90 and 7.40 Δ 430 nm min^{-1} g^{-1} at vegetative and flowering stages and on par with foliar spraying of 1% ferrous citrate (T_8) which registered the highest mean catalase activity of 6.74 and 7.16 Δ 430 nm min^{-1} g^{-1} at vegetative and flowering stages (fig. 2). The lower mean peroxidase activity of 5.44 and 5.92 Δ 430 nm min^{-1} g^{-1} at vegetative and flowering stages has recorded in control.

Table 1: Effect of ferrous glycinate on iron content at different stages of black gram (mg kg^{-1}) on Black calcareous soil

Treatments	Shoot				Root			
	Vegetative	Flowering	Harvest	Mean	Vegetative	Flowering	Harvest	Mean
T_1	283	311	308	301	128	141	136	135
T_2	305	332	327	321	131	146	141	139
T_3	315	343	339	332	138	151	146	145
T_4	311	340	336	329	134	148	142	141
T_5	307	338	331	325	135	147	144	142
T_6	306	336	329	324	137	149	145	144
T_7	325	351	347	341	145	159	153	152
T_8	318	346	341	335	136	145	139	140
T_9	312	341	338	330	130	143	138	137
SEd	6.28	6.33	7.81		2.46	3.30	3.36	
CD (P=0.05)	13.2	13.3	16.4		5.17	6.94	7.06	

T_1 - NPK control; T_2 –$FeSO_4$ 25 kg ha^{-1} as basal soil application; T_3 - Ferrous glycinate chelate @ 5kg ha^{-1}; T_4 - Ferrous citrate chelate @ 5kg ha^{-1}; T_5 - Fe – EDTA chelate @ 5kg ha^{-1}; T_6 - 1% $FeSO_4$ as foliar spraying on 25 & 45 DAS; T_7 - 1% Ferrous glycinate as foliar spray on 25 & 45 DAS; T_8 - 1% Ferrous citrate as foliar spraying on 25 & 45 DAS; T_9 – 1% Fe – EDTA as foliar spray on 25 and 45 DAS

Table 2: Effect of ferrous glycinate on leaf active Fe content (mg kg^{-1}) of Black gram in Black calcareous soil

Treatments	Leaf active iron content		
	Vegetative	Flowering	Harvest
T_1 - NPK control	18.8	28.5	21.1
T_2 –$FeSO_4$ @ 25 kg ha^{-1} as basal soil application	35.5	45.1	39.5
T_3 - Ferrous glycinate chelate @ 5kg ha^{-1}	38.7	47.9	41.2
T_4 - Ferrous citrate chelate @ 5kg ha^{-1}	36.2	46.1	40.9
T_5 - Fe – EDTA chelate @ 5kg ha^{-1}	34.8	43.2	38.5
T_6 - 1% $FeSO_4$ as foliar spraying on 25 & 45 DAS	35.6	44.7	37.5
T_7 - 1% Ferrous glycinate as foliar spraying on 25 & 45 DAS	39.2	48.5	42.9
T_8 - 1% Ferrous citrate as foliar spraying on 25 & 45 DAS	38.5	47.2	40.3
T_9 – 1% Fe – EDTA as foliar spraying on 25 & 45 DAS	36.5	45.8	39.7
Mean	34.9	44.1	38.0
SEd	0.61	0.88	0.67
CD (P=0.05)	1.29	1.84	1.41

T_1 - NPK control; T_2 –$FeSO_4$ 25 kg ha^{-1} as basal soil application; T_3 - Ferrous glycinate chelate @ 5kg ha^{-1}; T_4 - Ferrous citrate chelate @ 5kg ha^{-1}; T_5 - Fe – EDTA chelate @ 5kg ha^{-1}; T_6 - 1% $FeSO_4$ as foliar spraying on 25 & 45 DAS; T_7 - 1% Ferrous glycinate as foliar spray on 25 & 45 DAS; T_8 - 1% Ferrous citrate as foliar spraying on 25 & 45 DAS; T_9 – 1% Fe – EDTA as foliar spray on 25 and 45 DAS

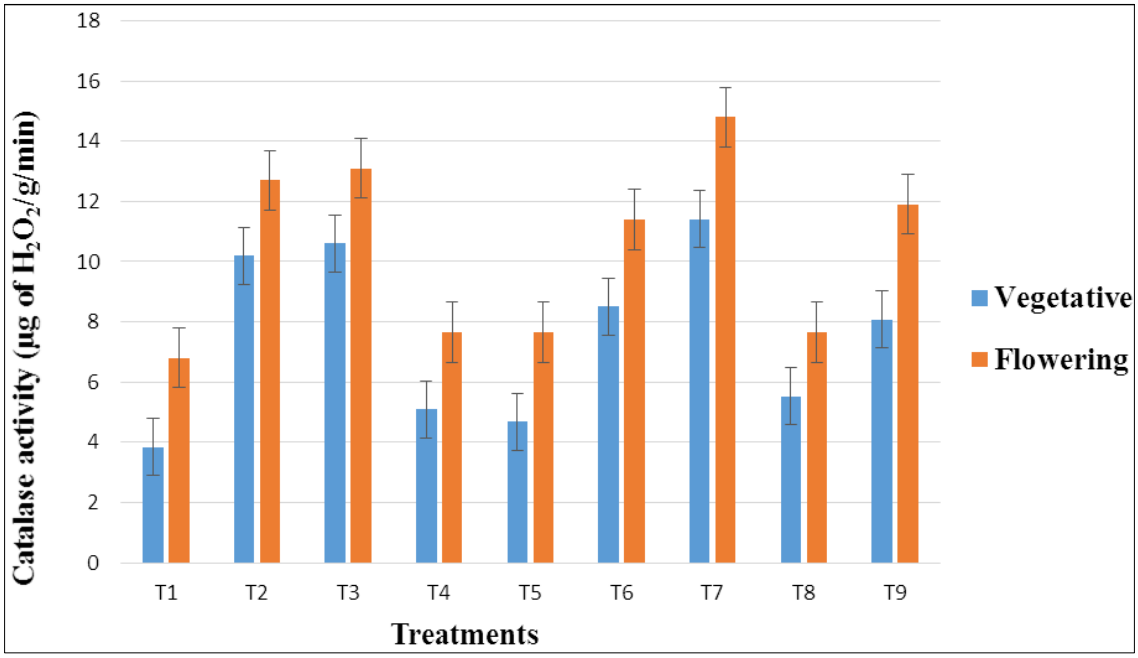

Fig 1: Effect of ferrous glycinate on catalase activity at different stages of black gram (µg of H_2O_2/g/min)

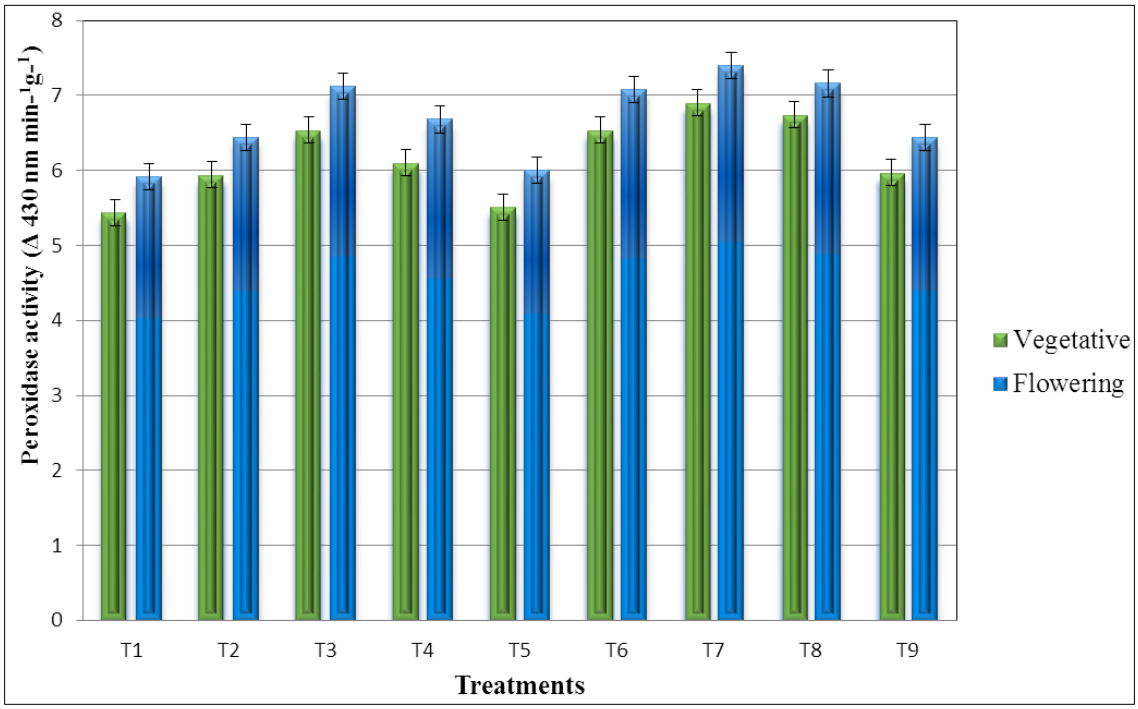

Fig 2: Effect of ferrous glycinate on peroxidase activity at different stages of black gram (Δ 430 nm $min^{-1}g^{-1}$)

Discussion

Foliar application of 1% ferrous glycinate (T_7) and soil application of ferrous glycinate (T_3) increased iron content in the subsequent growth stages. This influence is due to the application of higher concentration of iron at different stages and thereby increased the absorption of iron in black gram. Similar results were reported by Shuedzhen (1991) [24] where the higher active iron content in the foliar application of 1% ferrous glycinate treatment could be due to the fact that plant absorbs and translocates iron efficiently in citrate form. The results are in accordance with those obtained by Kalbasl *et al.* (1986) [10].

The results obtained from our study indicated that using Fe – amino acid chelates, sufficient amount of Fe can be supplied for plant uptake. Increase in leaf active iron content was observed up to flowering stage and thereafter it declined at harvest stage. The mean leaf active iron content of 39.2, 48.5 and 48.5 mg kg^{-1} was recorded for the foliar spraying of 1% ferrous glycinate (T_7) at 25 and 45 DAS at vegetative, flowering and harvest stages respectively. The high leaf active iron content in flowering stage is due to higher uptake of iron in leaves. Amino acids act as a constituent in translocation, re-translocation, and internal compartmentation of many nutrient elements, especially micronutrients. They are also important in better N uptake and play a greater role in iron and zinc content of bean leaves. Higher iron concentration in leaves due to the application of ferrous glycinate improves the nitrogen content in blackgram plants which can rise the expression and activity of iron transporters in root cell membrane. These results are corroborating with the findings of Causin (1996) [3]; Souri and Yarahmadi (2016) [26];

Ghasemi *et al.* (2014) [6]; Murata *et al.* (2008) [14] and Souri *et al.* (2016) [26].

Foliar spraying of 1% ferrous glycinate (T_7) registered the highest shoot activity of catalase followed by soil application ferrous glycinate (T_3). Higher activity in the presence of ferrous glycinate is due to the role of glycine expression of genes encoding the catalase activity.The enzyme peroxidase activity showed on increaseing trend with the under application of iron chelates. Foliar spraying of 1% ferrous glycinate at 25 and 45 DAS registered the highest mean peroxidase activity of 6.90 and 7.40 Δ 430 nm min $^{-1}$ g^{-1} at vegetative and flowering stages. Activity of peroxidase (APX) was affected by ferrous glycinate, but compared to iron EDTA and control significant differences was observed. The activity of these enzyme increased with decreasing iron rates. Iron is a constituent of enzyme system and so it helps for carrying out different enzymatic reactions in plant like, cytochrome oxidase, catalase, peroxidase, acotinase, and nitrogenase. This has been proved by Zahra Karimi (2014) [31] who ascribed that comparison effect of nano-iron chelate and iron chelate on growth parameters and antioxidant enzymes activity of green gram.

Conclusion

Results obtained from the present study showed that foliar spraying of 1% ferrous glycinate was effective in increasing the iron content, active iron content, catalase, and peroxidase of blackgram in black calcareous soil. Further these iron chelates were superior in increasing the uptake and translocation of Fe in blackgram. Higher activity of Catalase and Peroxidase confirmed the improvement in plant Fe status. Between the soil and foliar application foliar spraying is better in improving the plant micronutrient content which was evidenced from higher enzyme activities.

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