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Changes in Morpho-Biochemical Characteristics of Moth Bean in Indian Thar Desert - Due to Sulphur and Iron Nutrition

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Abstract: The experiments were designed to investigate the effect of S and Fe nutrition on morphological and biochemical characters of moth bean. In the experiment four levels of S, 0, 20, 40 and 60 kg ha^{-1} were applied in the main plots. In sub plots eight levels of Fe were given. Three of the Fe levels (0, 12.5 and 25.0 kg $FeSO_4$ ha^{-1}) were given as basal and remaining five were applied as foliar though 0.5% $FeSO_4$+ 0.1% citric acid at branching, 0.5% $FeSO_4$+ 0.1% citric acid at flowering, 0.5% $FeSO_4$+ 0.1% citric acid both at branching and flowering, 0.5% $FeSO_4$ both at branching and flowering and 0.1% citric acid both at branching and flowering. Application of graded doses of S up to 40 kg ha^{-1} increased chlorophyll content and active Fe significantly in young emerged leaves at flowering. Sulphur application increased the shoot weight and root nodule weight at flowering and total plant dry matter, pod weight $plant^{-1}$ at maturity to the tune of 59.29, 7.61, 30.86 and 17.92%, respectively over So. In the experiment effect of sulphur on the concentration of S was most marked at maturity whereas on the concentration of Fe and N was at flowering. Yield attributes and harvest index also responded favourably to the application to S in soil. Method of Fe application and frequency of its foliar application had significant effect on these parameters. At flowering basal application of $FeSO_4$ at 25 kg ha^{-1} increased chlorophyll content, active Fe content, shoot weight and pods weight significantly compared to rest of the Fe treatments. At maturity, while remaining at par with foliar applied 0.5% $FeSO_4$+ 0.1% citric acid both at branching and flowering, it increased total plant dry matter, pods weight, pods $plant^{-1}$, seeds pod^{-1} and test weight by 61.42, 45.43, 31.11, 65.92 and 15.20%, respectively over Fe_0. The effect of Fe at 25 kg $FeSO_4$ ha^{-1} on the concentration of S and Fe in plant dry matter was most marked at flowering whereas on N it was at branching. Alone application of 0.1% citric acid both at branching and flowering had no significant effect on the morpho-biochemical characters of moth bean.

Key words: Active Fe · chlorophyll · sulphur · iron · morphological · biochemical · moth bean

INTRODUCTION

Vigna aconitifolia locally known as 'moth bean' is an important leguminous *kharif* crop of arid and semi-arid regions of India. The cultivation of this crop is done by poor farmers in drought prone areas. It is cultivated over a wide range of climatic conditions in the states of Maharashtra andhra Pradesh, Rajasthan and Orissa. With increasing irrigation facilities and remunerative, the area under the crop has been expanded consistently in Rajasthan. In Rajasthan productivity of moth bean is very low as compared to national average. Physico-chemical properties of the soils of the western Rajasthan besides, climatic and technological limitations restrict productivity of the crop. The loamy sand soils of the region are alkaline in reaction and calcareous in nature and thus low in overall fertility. Under such soil conditions deficiency of SO_4-S [1] and limit the growth and development of plant in addition to other plant nutrients.

Deficiency of S has been found in sandy soils of many districts of Western Rajasthan due to low native S content [2] and soil conditions which favours leaching of native S from top layers of soil [3]. Sulphur plays many important roles in the growth and development of plants. Sulphur requirements and metabolism in plants are closely related to N and Fe nutrition [4, 5], whereas N and Fe metabolism is also strongly affected by the S status of plant [6, 7]. Jat [8]; Trivedi [9] and Kasturikrishna and Ahlawat [10] have also reported the beneficial effects of S on various *kharif* pulses.

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In addition to S, Fe is also considered an essential plant nutrient for optimum plant growth. Generally plants require 10 nM of iron in the soil solution for normal growth and development. However, in calcareous soils its concentration does not reach values higher than 100 pM [11]. Bicarbonate ion is considered as the major factor limiting the availability of Fe to the dicot plants grown in such soils [12]. The physiological basis for bicarbonate induced Fe -deficiency chlorosis of plants grown on such soils is yet not completely understood [13]. Plants subjected to Fe-deficiency respond in various ways [14]. It includes low chlorophyll content, impairment of photosynthetic electron transport, low ferredoxin content, depressed regeneration of reduced ferredoxin and retarded regeneration of ribulose biphosphate [15]. Iron stressed plants show poor assimilation of S and N [5] and activities of non Fe- enzymes [16].

Among various inorganic Fe-carriers, $FeSO_4.7H_20$ is commonly used for correction of Fe- deficiency in field grown crops [17]. As a fertilizer it can be used either by soil application or through foliar application on the crop foliage. But both the methods of application have partial success. Fe applied to soil through $FeSO_4$ is susceptible to transformation into unavailable form under high pH and calcareous soils [17]. The foliar applied $FeSO_4$ alone on the other hand has usually been found relatively ineffective. Because, iron from the spray solution gets precipitated when applied on leaves, as leaves do not have an acid producing mechanism as do roots [18]. Therefore for higher fertilizer efficiency, iron solutions for foliar spray are acidified with citric acid [19]. Similarly, acidification of such soils with elemental S also result in increased availability of Fe to plants [20]. This study mainly aimed at investigating possible effects of S and Fe on morpho-biochemical characteristics of moth bean and to explore added role of S in increasing Fe nutrition to the crop.

MATERIALS AND METHODS

Experimental field: A field experiment was carried out in 2004 and 2005 on hot arid region of Indian Thar Desert, which is characterized by sandy soils with low water holding capacity, hot and arid climate, precipitation less than 300 mm. The soils of the experimental field was loamy sand having pH 8.3, organic carbon 0.31%, free $CaCO_3$ 2.55% and 122.8, 9.45 and 12.92 kg ha^{-1} of available N, P and S, respectively. The DTPA available Fe of the soil was 2.06 ppm.

Experimental design and treatments: The experiment was conducted by employing split-plot design with three replications and two factors, sulphur and iron. In main plots four levels of sulphur, 0, 20 40 and 60 kg ha^{-1} (abbreviated as S_0, S_{20}, S_{40} and S_{60}, respectively), were applied through elemental S (85% S) and gypsum (20.5% S) in 50: 50 proportion. In sub plots, eight levels of iron through $FeSO_4.7H_20$ (25.5% Fe) were applied as basal, 0, 12.5 and 25.0 kg ha^{-1} (abbreviated as Fe_0, $Fe_{12.5}$ and $Fe_{25.0}$, respectively) and foliar, 0.5% $FeSO_4$+ 0.1% citric acid at branching, 0.5% $FeSO_4$+ 0.1% citric acid at flowering, 0.5% $FeSO_4$+ 0.1% citric acid both at branching and flowering, 0.5% $FeSO_4$ both at branching and flowering and 0.1% citric acid both at branching and flowering. All the basal applied treatments of S and Fe were given at the time of sowing and mixed thoroughly in the top 10 cm layer of soil. The S supplied through soil applied $FeSO_4$ was compensated with gypsum. For foliar application, weighed quantity of $FeSO_4$ and citric acid was dissolved in the required volume of water and aqueous solution was used for spray.

Experimental Field techniques: After preparation of field a universal dose of 40 kg P and 20 kg N ha^{-1} were applied through DAP and urea in the filed just before sowing. Moth bean variety RMO-40 was used for the study. The seeds were treated with copper oxychloride as prophylactic measure against bacterial blight and were sowed in the field on 03-2004 and 03-2005 using seed rate of 20 kg ha^{-1}. The extra plants from the experiment were removed manually at 12 days after sowing (DAS) in order to maintain plant geometry of 30 x 10 cm^2. Hoeing and weeding was done manually at 22 DAS. Five irrigations were given to the crop at an interval of 10 days. Ripened pods were picked twice during the experiment to prevent shattering and were kept separately in paper bags. Crop was harvested from individual plots when plants and pods become dry.

Measurements: In the experiment, five plants from 2nd and 9th row of each plot were uprooted for the measurements of various morphological- biochemical characteristics at 20, 40 and 60 DAS corresponding the moth bean growth stages of branching, flowering and maturity, respectively. Shoots were washed in 0.1% M HCl for 30 sec and rinsed in distilled water for 60 sec to wash off any surface contamination and the young emerged leaves (YELs) and root nodules were separated from the whole shoots. The chlorophyll concentrations

Table 1: Effect of S and Fe on chlorophyll content, active Fe content, shoot weight and root nodule weight of moth bean at flowering (Average of two years, 2004 and 2005)

Treatments	Chlorophyll (mg g^{-1} fresh weight of leaf)	Active Fe (mg g^{-1} fresh weight of leaf)	Shoot weight (g $plant^{-1}$)	Root nodule weight (g $plant^{-1}$)
Sulphur				
S_0	1.90	27.56	2.41	68.06
S_{20}	2.22	40.20	2.99	70.27
S_{40}	2.63	50.78	3.64	72.70
S_{60}	2.70	57.66	3.86	73.63
SEM±	0.04	0.89	0.08	0.80
CD at 5%	0.13	2.73	0.26	2.49
Iron				
Fe_0	1.63	30.10	2.14	65.50
$Fe_{12.5}$	2.60	53.82	3.71	74.96
$Fe_{25.0}$	3.47	63.07	5.40	75.49
FeCAB	2.44	48.27	3.20	72.09
FeCAF	2.24	32.00	2.15	68.80
FeCABF	2.76	48.87	3.06	72.02
FeBF	2.27	40.20	2.93	69.74
CABF	2.10	36.10	2.60	68.68
SEM±	0.04	1.09	0.08	0.82
CD at 5%	0.10	3.08	0.21	2.30

FeCAB= Foliar spray of 0.5% $FeSO_4$ + 0.1% citric acid at branching, FeCAF = Foliar spray of 0.5% $FeSO_4$ + 0.1% citric acid at flowering, FeCABF= Foliar spray of 0.5% $FeSO_4$ + 0.1% citric acid both at branching and flowering, FeBF= Foliar spray of 0.5% FeSO both at branching and flowering, CABF= Foliar spray of 0.1% citric acid both at branching and flowering

and active Fe content were determined from the samples of YELs by following the procedures of Arnon [21] and Katyal and Sharma [22], respectively. The YELs, root nodules and shoots were dried in an oven at 70°C for 72 h to obtain constant dry weight. The combined dry weight was recorded as the shoot dry weight. For measurement of pod weight, five plants in each plot were tagged at random. Two pickings were done before the final harvest from these selected plants. The combined yield of the two pickings and finally at harvest were recorded as pod yield. The dried and ground shoot samples were analyzed for S, Fe and N using standard methods of analysis.

Data analysis: Data were subjected to analysis of variance using the software MSTATC version 2.10, 1997 (Michigan State University, USA). The critical differences were calculated to assess the significance of treatment means wherever the 'F' test was found significant at 5% level of significance.

RESULTS

Differences in morphological and biochemical characteristics of moth bean were noticed between S treatments at flowering. The chlorophyll and active Fe. Content of YELs increased significantly from 1.90 and 27.56 mg g^{-1} fresh weight of leaf in S_0 treatment to 2.70 and 57.66 mg g^{-1} fresh weight of leaf in the S_{60} treatments, respectively (Table 1). At flowering stage, dry weight of shoots increased by 62.75% with the addition of 60 kg S ha^{-1}, compared to the S_0 treatment (Table 1). The effects of S addition were most marked up to 40 kg ha^{-1} and was non significant thereafter. The significant increase in dry weight of root nodules was 7.60% higher than S_0 treatment with the application of 40 kg S ha^{-1} at this stage (Table 1). The effects of S additions on moth bean growth were even greater by crop maturity stage. Compared to the S_0 treatment, addition of 40 kg S ha^{-1} increased the total dry matter of plants by 30.86% and of pods weight by 19.90%. Yield components and harvest index is shown in Table 2. This indicates that addition of S up to 40 kg ha^{-1} increased pods $plant^{-1}$, seeds pod^{-1} and harvest index significantly. Whereas, test weight was hardly affected with the addition of 20 kg S because the effect of S on total seed weight was greater than that on the total weight of plants.

The concentrations of S, Fe and N in plant at branching, flowering and maturity are shown respectively. At early vegetative stage plants have higher S content in straw as compared to advanced stages of crop growth. When the growth advances S content in plants decreased until plants enter into anthesis. At all the stages plants responded to S addition significantly.

Gradual increase in S doses also affected the concentration of Fe and N at all the stages of plant growth. The change in Fe concentration followed one of the same trends as that was observed with S up to

Table 2: Effect of S and Fe on yield attributes and harvest index (HI) of moth bean at harvesting (Average of two years, 2004 and 2005)

Treatments	Pods plant^{-1}	Seeds pod^{-1}	Test weight (g)	HI (%)
Sulphur				
S0	20.72	5.5	38.16	22.01
S20	24.08	8.03	40.12	25.30
S40	25.94	8.60	41.17	26.88
S60	27.03	9.57	41.48	27.20
SEM ±	0.25	0.13	0.33	0.33
CD at 5%	0.80	0.40	1.01	1.01
Iron				
Fe0	20.88	6.55	36.07	22.22
Fe12.5	26.23	8.96	42.20	27.16
Fe25.0	28.91	10.48	42.70	27.80
FeCAB	23.84	7.40	40.22	25.59
FeCAF	23.70	7.20	40.05	25.03
FeCABF	28.11	9.40	43.37	27.01
FeBF	22.02	7.09	40.40	24.19
CABF	21.82	6.98	38.84	23.80
SEM ±	0.30	0.19	0.36	0.42
CD at 5%	0.88	0.49	1.01	1.16

FeCAB= Foliar spray of 0.5% $FeSO_4$ + 0.1% citric acid at branching, FeCAF = Foliar spray of 0.5% $FeSO_4$ + 0.1% citric acid at flowering, FeCABF= Foliar spray of 0.5% $FeSO_4$ + 0.1% citric acid both at branching and flowering, FeBF= Foliar spray of 0.5% $FeSO_4$ both at branching and flowering, CABF= Foliar spray of 0.1% citric acid both at branching and flowering

anthesis but the concentration of Fe continue to decrease till maturity. However, reverse trend was noticed in case of N concentration. It means concentration of N continuously increased up to flowering in plant straw.

Methods of Fe application and frequencies of foliar application had appreciable effects on the morphological and biochemical characteristics of moth bean. Throughout the experiment basal application of 25 kg $FeSO_4$ ha^{-1} remained significantly superior over rest of the treatments except at maturity with foliar application of 0.5% $FeSO_4$ + 0.1% citric acid both at branching and flowering. The chlorophyll and active Fe content in YELs at flowering increased by 61.90 and 31.92%, respectively with 25 kg $FeSO_4$ than Fe_0 treatment (Table 1). No significant improvement in these parameters were noticed with the application of 0.5% $FeSO_4$ + 0.1% citric acid at flowering and 0.1% citric acid both at branching and flowering. At flowering, plants treated with 25 kg $FeSO_4$ recorded significantly highest dry weight of shoots. It was followed by 12.5 kg $FeSO_4$, 0.5% $FeSO_4$ + 0.1% citric acid at branching and 0.5% $FeSO_4$ + 0.1% citric acid both at branching and flowering (Table 1). However, non-significant difference in shoot dry weight was noticed between Fe_0 and foliar applied 0.5% $FeSO_4$ + 0.1% citric acid at flowering. Similarly the highest dry weight of root nodules of 75.48 mg plant^{-1} was observed at 25 kg $FeSO_4$ ha^{-1} (Table 1). The array with rest of the treatments remained almost similar to dry weight of shoot plant^{-1}. However, the response of Fe treatments on moth bean growth at maturity changed slightly. Addition of 25 kg $FeSO_4$ as basal and foliar applied 0.5% $FeSO_4$ + 0.1% citric acid both at branching and flowering while remaining at par with each other produced the highest total plant dry matter and pods plant^{-1}. The magnitude of increase in total plant dry matter and pod weight with the application of basal applied 25 kg $FeSO_4$ being 61.45 and 45.32%, respectively as compared to Fe_0. Foliar application of 0.5% $FeSO_4$ + 0.1% citric acid either at branching or flowering had similar effects on these characters but were significantly superior to Fe_0. Yield attributes and harvest index data are presented in Table 2. It indicates that all these parameters are significantly higher with the basal application of 25 kg $FeSO_4$ and foliar application of 0.5% $FeSO_4$ + 0.1% citric acid both at branching and flowering. Solitary application of 0.1% citric acid both at branching and flowering hardly affected pod plant^{-1} and test weight. Rest of the treatments also influenced these parameters significantly as compared to Fe_0.

The effect of different Fe treatments on the concentrations of S, Fe and N at branching, flowering and maturity are shown respectively. The concentration of these nutrients in plant dry matter followed the same trends as that was observed with S nutrition at various growth stages. At all stages significant improvement in the concentrations of S, Fe and N were observed with 25 kg $FeSO_4$. Though effect of this treatment was noticed at all the stages of crop growth, but most remarkable effect on the concentrations of S and Fe was observed at flowering stage. It increases the concentration of S and Fe at this stage to the tune of 50.90 and 60.41% compared to Fe_0 treatment, respectively. Contrary to the concentrations of S and Fe, the effect of $FeSO_4$ @ 25 kg ha^{-1} on the concentration of N was highest at branching and maturity. The percent increase in the concentration of N over Fe_0 being 45.72 and 30.80%, respectively. Rest of the Fe treatments followed one of the same patterns as observed with other growth and biochemical parameters at flowering.

DISCUSSION

One of the aims of this article was to find out suitable dose of S to replenish the deficiency in light textured soils and to define a suitable method of iron application in calcareous soils for higher fertilizer efficiency of applied Fe-salts. The results presented here show that all the morphological and biochemical attributes increased markedly by the addition of S up to an optimal level of 40 kg ha^{-1}. As anticipated, the lowest value of chlorophyll, active Fe, shoot dry weight and root nodule weight at flowering; total plant dry matter and pods $plant^{-1}$ at maturity and concentrations of S, Fe and N at various growth stages were noticed in the S_0 treatment. This may be due to low S content (12.8 kg ha^{-1}) in top layers of soil. The decreased concentration of chlorophyll in YELs in the S_0 treatment was likely to be caused by the shortage of N as a result of decreased N_2 -fixation [23]. Furthermore, the response pattern of leaf chlorophyll content and N concentration to S were very similar, with the two measurements correlating linearly in the experiment ($r = 0.869$). In leaves most part of protein is located in chloroplasts and deficiency of S causes shortage of sulphur containing amino acids which not only inhibits protein synthesis but also decrease chlorophyll content of leaves in the similar manner [24]. The results from the study support that of De Boer and Duke [25] on alfalfa. Similarly, active Fe content of YELs increased concomitantly with the increase in S levels up to 40 kg ha^{-1}. The acidification of crop root zone with the application of elemental S changes the unavailable Fe^{+++} into plant available Fe^{++} and thereby increased active Fe content in the plant dry matter [26]. Further higher concentration of S in plants arrested the inactivation of absorbed Fe^{++} by maintaining the reduced cell sap pH [27]; hence increase active Fe content in YELs. The beneficial effects of soil acidification on active Fe have also been reported by Rao *et al.,* [28] in groundnut.

In the study, lower nodule weight in the S_0 treatment could be attributed to lower synthesis of nitrogenase and ferredoxin (S containing enzyme and protein), which play many important roles in the process of N_2 -fixation [6]. The improvement in plant dry matter, pods weight and yield attributes with increased S fertilization may be due to enhanced nodulation which in turn improved N_2-fixation, photosynthesis and other physiological parameters of the plant. The observed results are in conformity with those of Sharma and Kamath [29] and Kasturikrishna and Ahlawat [10]. S application up to 40 kg ha^{-1} had significant effect on the concentrations of S, Fe and N at various growth stages. Enhancement in the availability of Fe to the plants with the increased levels of S could be due to reduction in soil pH [7]. Similarly the concentration of N in the plant increased with the application of S to the soil as increased, S availability to the plants increased the assimilation of N within the plants by increasing the activities of nitrate reductase and nitrite reductase in the soil [30]. Consequently, increased availability of nutrients in rhizosphere coupled with increased activity at cellular level probably enhanced the nutrient uptake arid their concentrations in different plant parts.

In the study it was noted that basal application of 12.5 and 25.0 kg $FeSO_4$ and foliar application of 0.5% $FeSO_4$ + 0.1% citric acid at branching and both at branching and flowering increased the chlorophyll and active Fe content in YELs. Higher active Fe content in basal applied Fe treatments was assigned to reduction in rhizosphere pH due to the application of elemental S in the soil [7] and better absorption and translocation of reduced Fe^{++} within the plants [19]. The higher active Fe content with foliar applied Fe treatments could be due to efficient absorption and translocation of iron in citrate form (Fe -citrate) as citric acid helps in maintaining the Fe into soluble form within the plants [31]. The movement of this negatively charged Fe -citrate complex is also faster through xylem [32]. Hence, higher content of active Fe would be expected with the foliar applied Fe treatments. The significant improvement in the synthesis of chlorophyll with these treatments was associated mainly to increased availability of Fe, S and N to the plants as Fe play many vital roles in the utilization of S and N by the plants [33, 34]. The concentration of chlorophyll in leaves is also directly related to the level of Fe supply to the plants. The positive association between active Fe and chlorophyll content ($r = 0.938$) supports the findings very well. The increased availability of active Fe also favours development of root nodules markedly. The increase in dry weight of root nodules with the application of Fe has also been reported by Kishore *et al.*, [35] in cowpea. This could also be evident from significant improvement in the concentration of N in plants at all stages of crop growth. Similarly, increase in the shoot weight at flowering, total plant dry matter, pods weight and yield attributes at maturity were observed in the study at basal application of 25 kg $FeSO_4$ and foliar application of 0.5% $FeSO_4$ + 0.1% citric acid both at branching and flowering. The increase in these parameters can be attributed to the favourable effects of active Fe on the synthesis of chlorophyll, root nodules and N_2 -fixation and on the photosynthesis [28]. All these parameters might have contributed for optimum growth and finally for yield attributes and harvest index. Apart form this; increased concentration of active Fe in the plants with these treatments enhanced the concentration of N and S in plants. As physiologically active Fe play many roles in the metabolism of these nutrients within the plants by affecting the activities of nitrate reductase, sulphotransferases, sulphite reductase and organic thiosulphate reductase enzymes favourably which are directly involved in the assimilation of N and S [5]. The increased availability and assimilation of nutrients at cellular level together with higher biomass of the plants enhanced the concentration of nutrients in the plants.

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