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EFFECT OF IRON AND SULPHUR APPLICATION ON MORPHO-PHYSIOLOGICAL ATTRIBUTES OF PEARL MILLET GROWN HYDROPONICALLY UNDER IRON DEFICIENCY

SUNIL[1], VINOD KUMAR[1,2]*, PUNESH SANGWAN[1], VISHAL CHUGH[3], SUSHIL NAGAR[1] AND NEERAJ KHAROR[1]

[1]Department of Biochemistry, College of Basic Sciences & Humanities, CCS Haryana Agricultural University, Hisar-125 004 (Haryana), India
[2]Division of Biochemistry, FBSc, SKUAST-Jammu, Main Campus Chatha-180 009 (J&K), India
[3]Department of Basic and Social Sciences, College of Horticulture, Banda University of Agriculture and Technology, Banda-210 001 (Uttar Pradesh), India
**(e-mail: sangwan.vinod@yahoo.com)*
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SUMMARY

Iron (Fe) is crucial for various biological functions, including plants. The deficiency of Fe is a vital problem in calcareous soils due to poor Fe^{3+} solubility, which limits plant growth and yield. In India, 13% of soils are affected by Fe deficiency. The present study was conducted under hydroponic conditions on contrasting Fe-containing Pearl millet hybrids to evaluate their performance under Fe deficiency and the effect of Sulphur (S) supplementation on its growth characteristics under such treatments. Significant changes in the shoot length, root length, leaf area, and chlorophyll content were observed in both genotypes. The shoot length, root length, leaf area, and chlorophyll were highest in treatment with Fe and S (+Fe+S), whereas the lowest values were obtained in the absence of Fe and S (-Fe-S) in both genotypes. Compared to both Fe and S absence, the application of S decreased chlorosis in the absence of Fe, which reveals mimicking of Fe deficiency symptoms by S application. Comparatively, leaf area was significantly higher in Fe-biofortified hybrid HHB-299 over non-biofortified hybrid HHB-67 (Improved). Overall, Fe-biofortified hybrid HHB-299 performed better over non-biofortified hybrid HHB-67 (Improved) under Fe deficient conditions and exhibited better tolerance to deficiency of Fe.

Key words: Hydroponic, Fe deficiency, sulphur application, pearl millet, biofortification

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a highly nutritious and multipurpose millet crop primarily cultivated on more than 27 million ha in Africa and Asia's arid and semi-arid tropical regions. It is a chief source of food for the rural people. The Fe and S are crucial for adequately functioning metabolic processes such as respiration and photosynthesis. These are kingpins for plant growth and development, being required as a redox-active metal involved in physiological and metabolic processes, including photosynthesis, respiration, nitrogen assimilation, sulphur metabolism, hormone biosynthesis, and production and scavenging of reactive oxygen species (ROS), osmoprotection, and pathogen defense (Hänsch & Mendel, 2009; Gupta *et al*., 2021). The redox properties of Fe make it an essential element for all life forms, and its availability in the soil is pH dependent, with the ferric form (Fe^{3+}) particularly insoluble in calcareous soils. Calcareous soils account for 1/3rd of the world's farmed soils. Therefore, frequent Fe shortage in these soils reduces plant growth (Fourcroy *et al.*, 2016). About 21.6% of Haryana soils have already been recorded as Fe-deficient. Its deficiency is expected to increase rapidly (Shukla *et al.*, 2014). The incapability of the plant to acquire Fe from the rhizosphere results in Fe deficiency chlorosis visible in the interveinal tissues of young leaves. Iron deficiency results in developmental defects at different plant growth stages, including chlorosis and growth retardation, leading to nutritional loss of the crop and the overall reduction of the yield (Briat *et al.*, 2015; Sangwan *et al.*, 2023). Sulphur deficiency also results in uniform pale green chlorosis throughout the plant. Interactions between S and Fe indicate that S-deficiency could limit Fe-deficiency response through a decrease in the production and release of

phytosiderophores (Kuwajima and Kawai, 1997; Astolfi *et al.*, 2006; Bouranis *et al.*, 2003). When plants are under Fe deficiency, their younger leaves develop a light greenish-yellow color, often called 'iron chlorosis.' This occurs because leaf xanthophyll content decreases less than Chlorophyll and carotene content with Fe deficiency (Boue-Jones & Notton 1953; Terry 1980). Compared with other compounds in root exudates, phenolics are particularly interesting because of their multiple chemical and biological functions. These functions include Fe chelation and reduction, radical scavenging, antimicrobial activity, and serving as a carbon source for microbial growth (Rice-Evans and Miller, 1996; Cao *et al.*, 1997; Blum *et al.*, 2000). Nutrition stresses generally result in an imbalance of cell redox status due to the overproduction of oxidative radicals. This, in turn, leads to an increase in the synthesis of antioxidants, such as glutathione (GSH) and ascorbic acid, and to an increase in the activity of antioxidant enzymes (Noctor and Foyer, 1998). Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), and hydroxyl radical ($OH^{\cdot}$) can be detoxified by oxidizing glutathione, ascorbic acid, antioxidant enzyme (Foyer & Noctor, 2011). The protective effect of antioxidant enzymes and their metabolite correlated with decreased levels of ROS. Therefore, it is postulated that Fe and S treatments contribute towards keeping cell redox homeostasis in plants growing under iron-deficient conditions.

MATERIALS AND METHODS

Plant material, growth conditions and treatments

Two contrasting grain Fe-containing pearl millet hybrids viz. HHB-299 as biofortified and HHB-67 (Improved) as non-biofortified hybrid were used in this study. The genotype seeds were obtained from the Bajra Section, Department of Genetics & Plant Breeding, CCSHAU, Hisar. Briefly, seeds were treated with 1% NaOCl for 10 min, followed by three rinses with distilled water. The sterilized seeds were subjected to cold stratification at 4°C overnight in the dark to break dormancy, followed by germination for four days in Petri dishes containing three layers of moist and sterile Whatman filter paper. Subsequently, the seedlings were transferred to a synthetic hydroponic chamber. The hydroponic chamber was designed to accommodate 21 seedlings at an equidistant position. Nutrient deficiencies treatments were provided at the five-leaf stage, where the following four treatments of Fe and S were provided in nutrient solution;

Treatments	Fe concentration (μM) in nutrient solution	S concentration (mM) in nutrient solution
Control (T_0): (-Fe-S)	-	-
T_1: (+Fe-S)	10	-
T_2: (-Fe+S)	-	1
T_3: (+Fe+S)	10	1

Nutrient solution was composed of 0.2mM KH_2PO_4, 2.0mM Ca $(NO_3)_2$, 1.0 mM $MgCl_2$, 0.2μM $CuCl_2$, 1.0μM H_3BO_3, 1.0 μM $MnCl_2$, 1.0 μM $ZnCl_2$, 0.02 μM $(NH_4)_6MO_7O_{24}$, 0.1 mM KCl, 1.0 mM $(NH_4)_2SO_4$ and 10 μM Fe-EDTA. It was replaced on every alternative day. Seedlings were grown at the panicle initiation stage in a growth chamber maintained at a 10 h day/14 h night cycle and 30 ± 8°C, with relative humidity of 60–70%. Root and shoot tissues were independently harvested and frozen in liquid nitrogen for morpho-physiological analysis and stored at 80°C until further use.

Measurement of seedling growth parameters

The length of the shoot and root was measured using a standardized scale. The leaf area was measured using the LI 3000C Portable Area Meter in combination with the LI-3050C Transparent Belt Conveyor Accessory. The chlorophyll content was measured using the DMSO method and calculated according to Arnon (1956). The data was analysed using OPSTAT online statistical analysis tools.

RESULTS AND DISCUSSION

Effect of differential Fe and S treatments on shoot length

The effect of the Fe and S treatment combination on shoot length is shown in Table 1. In pearl millet, compared to the control (T_0) (-Fe-S), shoot length increased by 4.95%, 12.43%, and 24.39% at the panicle initiation stage under treatments T_1, T_2, and T_3, respectively, in genotype HHB-67 (Improved). Similarly, compared to control (T_0), shoot length increased by 8.62%, 16.06%, and 21% at the

TABLE 1
Effect of Fe and S applications on pearl millet genotypes' shoot length (cm).

Treatments \ Genotypes	Shoot length (cm)	
	Panicle initiation stage	
	HHB-67(I)	HHB-299
T_0 (Fe 0µM, S 0mM)	47.91	52.03
T_1 (Fe 10µM, S 0mM)	52.43	54.74
T_2 (Fe 0µM, S 0mM)	57.08	59.41
T_3 (Fe 10µM, S 1mM)	60.65	68.81
Overall Mean	54.52	58.75
CD Value	V=2.26, T=3.20	

panicle initiation stage under treatments T_1, T_2, and T_3, respectively, in genotype HHB-299. Shoot length is low in the absence of iron and sulphur and Highest in the presence of iron and sulphur. Shoot length increase was more prominent in the hybrid HHB-299 than HHB-67 (Improved) under similar treatments, which may be one reason for its greater tolerance to Fe deficiency. The role of S in the growth of shoot length is significantly high compared to Fe. Gupta *et al.* (2021) observed that shoot length loss was more pronounced in genotype PBW 502, with declines of 44% and 51% noted, respectively, compared to 38% and 40% in Narmada 195 for T_1 and T_2. Zuchi *et al.* (2009) reported that S shortage led to a notable reduction in the fresh weight of the roots and shoots, with drops of 40% and 20%, respectively. Shoot length is low in the absence of Fe and S and higher in their presence. Cakmak *et al.* (2008) also reported that Fe deficiency decreases shoot growth in wheat genotypes. Shoot length increase was more prominent in the biofortified hybrid HHB-299, compared to HHB-67 (Improved) under similar treatments. It might contribute to its higher Fe uptake potential and, in turn, possibly tolerance to Fe deficiency conditions.

Effect of differential Fe and S treatments on root length

It was observed that, compared to the control (T_0) (-Fe-S), the root length increased at the panicle initiation stage under treatments T_1, T_3, and T_2, respectively, in both genotypes, including HHB-67 (Improved) and HHB-299 (Table 2). The maximum increase in root length was observed in the presence of S and the absence of Fe in both genotypes, likely due to Fe deficiency. Under only Fe absence, root system architecture (RSA) grows well for iron

TABLE 2
Effect of Fe and S application on pearl millet genotypes' root length (cm).

Treatments \ Genotypes	Root length (cm)	
	HHB-67(I) Panicle initiation stage	HHB-299 Panicle initiation stage
T_0	38.78	40.94
T_1	43.44	45.45
T_2	47.44	50.58
T_3	45.51	48.55
Overall Mean	43.80	46.38
CD Value	V=0.732, T=1.035	

absorption. Root length in the absence of Fe and the presence of S was slightly higher than in the presence of both Fe and S. Root length was slightly higher in the HHB-299 genotype compared to HHB-67 (Improved). In research by Gupta *et al.* (2021), under Fe and Zn deficit conditions, root length was reduced in PBW 502, whereas it increased somewhat in Narmada 195. Root characteristics, such as root length, exhibit a reduction under Fe-deficient situations, according to Hua *et al.* (2022). Cakmak *et al.* (2008) also reported reduced root growth in wheat genotypes under Fe deficiency.

Effect of Fe and S treatments on leaf area and chlorophyll content

The effect of the Fe and S treatment combination on leaf area and chlorophyll content is shown in Tables 3 and 4. As compared to control, leaf area in both genotypes increased at the panicle initiation stage under treatments T_1, T_2, and T_3, respectively. Leaf area was highest in the presence of Fe and S (+Fe+S), and lowest in the absence of Fe and S (-Fe-S) in both genotypes. The leaf area was

TABLE 3
Effect of Fe and S application on leaf area (cm2) of pearl millet genotypes.

Treatments \ Genotypes	Leaf area (cm^2)	
	Panicle initiation stage	
	HHB-67(I)	HHB-299
T_0 (Fe 0µM, S 0mM)	20.77	26.66
T_1 (Fe 10µM, S 0mM)	25.63	28.06
T_2 (Fe 0µM, S 1mM)	26.20	38.54
T_3 (Fe 10µM, S 1mM)	35.99	43.15
Overall Mean	27.14	34.10
CD Value	V=0.88, T= 1.24	

significantly higher in HHB-299 than in the HHB-67 (Improved) genotype. Leaf area in both genotypes increased at the panicle initiation stage under treatments T_1, T_2, and T_3, respectively, as compared to control (Table 3). Gupta *et al.* (2021) reported that under Fe and Zn deficiency, both genotypes exhibited a significant decrease in total leaf area. However, the decline was more pronounced in the inefficient genotype PBW 502 (51% and 49% for T_1 and T_2, respectively) than in the efficient Narmada 195 (33% and 35% for T_1 and T_2, respectively). Leaf area was highest in the presence of iron and sulphur (+Fe+S) and lowest in absence of Fe and S (-Fe-S) in both genotypes. Leaf area was significantly higher in HHB-299 compared to HHB-67 (Improved) genotypes. It has also been hypothesized that these morphological changes enhance the absorption surface area, which would naturally help in plants' ability to develop when they are starved of Fe (Schmidt *et al.*, 2000; Curie *et al.*, 2009; Garcia *et al.*, 2015). Significant changes in the morphological and biochemical attributes were observed amongst both the genotypes at the stage of growth after treating the plant with different combinations of Fe and S.

Compared to control (T_0) (-Fe-S), the chlorophyll content increased at the panicle initiation stage under treatments T_1, T_2, and T_3, respectively, in genotypes HHB-67 (Improved) and HHB-299. Chlorophyll content was highest in the presence of Fe and S and lowest in (T0) without Fe and S conditions. The photosynthetic cells of plants, primarily responsible for synthesizing heme molecules such as chlorophyll, cytochromes, and core proteins in the stroma, contain approximately 80% of the iron in plants. Compared to control (T_0) (-Fe-S), the chlorophyll content increased at the panicle initiation stage under treatments T_1, T_2, and T_3, respectively, in genotypes HHB-67 (Improved) and HHB-299 (Table 4). According to Zamboni *et al.* (2017), leaf chlorosis developed similarly in both Fe deficiency and combined Fe/S deficiency, consistent with the decreased Fe concentrations in the roots of seedlings with Fe and combined Fe/S deficiency. According to Zuchi *et al.* (2009), based on visual screening of the plants, there were no noticeable differences between plants grown under S-deficit and control conditions. Chlorophyll content was higher in the presence of Fe and S and lower in its absence (T_0). Compared to both Fe and S absence, chlorosis was decreased in the absence of Fe and the presence of S. Therefore, it is clear that the presence of S decreased chlorosis. Khobra *et al.* (2014) also reported that plants grown under Fe-deficient conditions clearly exhibited a chlorosis phenotype. Hence, when S availability was limited (-Fe + S vs. -Fe -S), the chlorosis phenotype observed in plants grown under Fe deprivation was less pronounced, especially in the youngest leaves that formed during the treatment period. Chlorosis was less in Fe biofortified genotype HHB-299 compared to non-Fe biofortified HHB-67 (Improved).

TABLE 4
Effect of Fe and S applications on chlorophyll content (mg/gFW) in the leaf of pearl millet genotypes.

Genotypes / Treatments	Chlorophyll content (mg/g FW)	
	Panicle initiation stage	
	HHB-67(I)	HHB-299
T_0 (Fe 0µM, S 0mM)	3.33	4.74
T_1 (Fe 10µM, S 0mM)	3.72	5.18
T_2 (Fe 0µM, S 1mM)	3.83	5.46
T_3 (Fe 10µM, S 1mM)	4.94	6.64
Overall Mean	3.96	5.50
CD Value	V=0.086, T=0.12	

CONCLUSION

The study demonstrated that iron (Fe) and sulphur (S) applications significantly improved the morpho-physiological attributes of pearl millet under hydroponic Fe-deficient conditions. The combined treatment (+Fe+S) resulted in the highest shoot length, root length, leaf area, and chlorophyll content, while the absence of both nutrients (-Fe-S) led to the poorest performance. Sulphur supplementation mitigated Fe deficiency symptoms, reducing chlorosis and enhancing growth. The Fe-biofortified hybrid HHB-299 outperformed the non-biofortified HHB-67 (Improved), exhibiting greater tolerance to Fe deficiency. These findings highlight the synergistic role of Fe and S in improving pearl millet growth and suggest that biofortified hybrids are more resilient under nutrient stress, offering potential for cultivation in Fe-deficient soils.

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