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Govind Aiyer
Department of Food Science
and Technology, Bikaner
Regional Center for
Sustainable Agriculture,
Bikaner, Rajasthan, India

Saroj Rastogi
Department of Food Science
and Technology, Bikaner
Regional Center for
Sustainable Agriculture,
Bikaner, Rajasthan, India

Corresponding Author:
Govind Aiyer
Department of Food Science
and Technology, Bikaner
Regional Center for
Sustainable Agriculture,
Bikaner, Rajasthan, India

Studying biofortification strategies of sorghum in the Teesta valley

Govind Aiyer and Saroj Rastogi

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Abstract

Sorghum (*Sorghum bicolor*) remains the dietary backbone for millions of households in India's semi-arid regions, yet its iron and zinc density falls short of recommended daily intakes, perpetuating hidden hunger in populations that depend on it as a staple. This research evaluated five biofortification strategies for sorghum in the Teesta Valley of West Bengal, with analytical work at the Bikaner Regional Center for Sustainable Agriculture from January to November 2023. Strategies tested were: agronomic biofortification (foliar $\text{FeSO}_4 + \text{ZnSO}_4$), genetic improvement (screening of 20 high-Fe/Zn breeding lines), microbial inoculation (plant growth-promoting rhizobacteria, PGPR), soil amendment (vermicompost + rock phosphate), and processing optimization (malting, fermentation, and decortication). Foliar application increased grain iron by 31.4% and zinc by 27.8% over controls. Two breeding lines (SB-7, SB-14) showed 42–48% higher baseline iron than the check cultivar. PGPR inoculation enhanced iron uptake by 18.7% and zinc by 14.3%. Malting followed by fermentation reduced phytate by 68.4% and increased iron bioaccessibility from 4.2% to 18.7%. Starch profiling revealed that biofortified lines contained 12.3% more resistant starch than conventional cultivars, an unexpected nutritional bonus. A decision-tree analysis ranked the combined agronomic + genetic strategy as the most cost-effective approach, delivering the highest iron increase per rupee invested. These findings provide an evidence-based ranking of biofortification strategies for sorghum in eastern Indian river valley environments.

Keywords: Starch fractionation, bioinformatics tools, acclimatization protocols, nitrogen fixation enhancement, pest resistance screening, sorghum biofortification, Teesta Valley, micronutrient enrichment

Introduction

Faced with a practical dilemma, nutritionists in India's sorghum-dependent regions have been asking the same question for over a decade: is it more effective to breed a higher-iron sorghum, spray micronutrients on the crop, inoculate the soil with helpful bacteria, or process the grain differently after harvest? Each approach has vocal advocates and published evidence, but no single research has compared all five strategies side by side under identical field and laboratory conditions ^[1]. That comparison matters because resource-constrained public health programs need to allocate limited funds to the strategy with the highest nutritional return per unit investment.

The Teesta Valley in northern West Bengal represents an important but understudied sorghum-growing environment. The valley's sandy loam alluvial soils are naturally low in zinc (DTPA-Zn 0.4–0.8 mg/kg) and moderately low in iron (DTPA-Fe 3.2–5.1 mg/kg), creating conditions where biofortification responses should be measurable ^[2]. Annual rainfall is 2,500–3,000 mm, concentrated in four monsoon months, and sorghum is grown as a rabi (winter) crop on residual moisture after rice harvest.

Processing — often overlooked in biofortification research — can be as important as genetics or agronomy. Sorghum grain contains high levels of phytate, tannins, and other anti-nutritional factors that bind iron and zinc, reducing their bioaccessibility from 3–6% of total content to biologically available forms ^[3]. Traditional processing methods like malting, fermentation, and decortication can reduce these anti-nutrients and dramatically increase mineral bioaccessibility, potentially achieving as much nutritional improvement as genetic biofortification.

This research compared all five biofortification strategies under controlled conditions in the

Teesta Valley, measured their effects on grain iron, zinc, starch fractions, and mineral bioaccessibility, and ranked them by cost-effectiveness. All laboratory work was performed at the Bikaner Regional Center for Sustainable Agriculture [4].

Reagents and Chemicals

Analytical-grade $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, pepsin (from porcine gastric mucosa, ≥ 2500 U/mg), pancreatin (from porcine pancreas, 4 $\times$ USP), bile extract, phytic acid sodium salt, and ICP multi-element standards were purchased from Sigma-Aldrich (Mumbai). PGPR strains (*Bacillus megaterium*, *Pseudomonas fluorescens*) were obtained from the ICAR-National Bureau of Agriculturally Important Microorganisms, Mau. Vermicompost was sourced from a commercial unit in Bikaner (N 1.4%, P 0.8%, K 1.1%). Rock phosphate (28% P_2O_5) was obtained from Rajasthan State Mines and Minerals Ltd [1].

Quality Control for Mineral and Starch Analysis

Certified reference materials (NIST SRM 1568b rice flour for minerals, AACC Check Sample for starch) were analyzed alongside every batch. Recovery rates for iron were 96.4–101.2% and for zinc 97.8–102.4%, within acceptable limits. Starch fractionation (rapidly digestible starch, slowly digestible starch, resistant starch) followed the Englyst method with in vitro enzymatic digestion at 37 °C, and triplicate measurements were required to show CV < 4% before acceptance [3].

Material and Methods

Material

Twenty sorghum genotypes (10 conventional, 10 high-Fe/Zn breeding lines from ICRISAT) were planted at the Teesta Valley Agricultural Station, Jalpaiguri district, West Bengal (26°31'N, 88°44'E; elevation 75 m) during October 2022–March 2023 (rabi season). Five biofortification strategies were applied in a randomized complete block design with 3 replications: (i) agronomic (foliar FeSO_4 + ZnSO_4 at 0.5% each, applied at booting and grain filling), (ii) genetic (20 genotypes compared under standard management), (iii) microbial (PGPR seed treatment at 10^8 CFU/mL + soil drench at sowing), (iv) soil amendment (vermicompost 5 t/ha + rock phosphate 500 kg/ha), and (v) processing (post-harvest malting 48 h, fermentation 72 h, or decortication to 80% extraction). A control (standard management, no biofortification) was included for each genotype [2]. Grain samples were shipped to the Bikaner Regional Center for Sustainable Agriculture. Ethics approval was granted (Protocol BRCSA-2022-017, 10 October 2022).

Methods: Grain iron and zinc were measured by AAS after tri-acid digestion. Phytate was determined by the Wade reagent colorimetric method. In vitro iron bioaccessibility

was assessed by simulated gastrointestinal digestion (pepsin at pH 2.0 for 2 h, then pancreatin + bile at pH 7.0 for 2 h) followed by dialysis and AAS measurement of dialyzable iron [3]. Starch fractions (RDS, SDS, RS) were determined by the Englyst method. Protein was measured by Kjeldahl. Cost-effectiveness was calculated as the ratio of iron increase ($\mu\text{g}/100$ g) to input cost (₹/ha). Data were analyzed by two-way ANOVA (strategy $\times$ genotype) and decision-tree analysis using Classification and Regression Trees (CART) in R 4.3.0.

Results

Among pre-harvest strategies, genetic improvement produced the largest baseline iron increase: lines SB-7 and SB-14 contained 6.8 and 7.1 mg Fe/100 g respectively, compared with 4.8 mg/100 g in the check cultivar CSV-15 — increases of 41.7% and 47.9%. Foliar application added 31.4% iron over genotype-specific baselines, and PGPR inoculation added 18.7% [1].

Table 1: Iron and zinc content of sorghum grain under five biofortification strategies (mg/100 g DW)

Strategy	Iron	Zinc	Phytate (mg/g)	Fe Bioaccess. (%)	Cost (₹/ha)
Control (CSV-15)	4.8 $\pm$ 0.4	2.1 $\pm$ 0.2	9.4 $\pm$ 0.7	4.2	0
Agronomic (foliar)	6.3 $\pm$ 0.5	2.7 $\pm$ 0.3	9.1 $\pm$ 0.6	4.8	3,200
Genetic (SB-7)	6.8 $\pm$ 0.3	3.4 $\pm$ 0.2	7.8 $\pm$ 0.5	6.1	1,800
PGPR inoculation	5.7 $\pm$ 0.4	2.4 $\pm$ 0.2	8.7 $\pm$ 0.6	5.3	2,400
Soil amendment	5.4 $\pm$ 0.3	2.3 $\pm$ 0.2	8.9 $\pm$ 0.5	4.9	8,700
Processing (malt+ferm)	4.8 $\pm$ 0.4	2.1 $\pm$ 0.2	3.0 $\pm$ 0.3	18.7	1,200

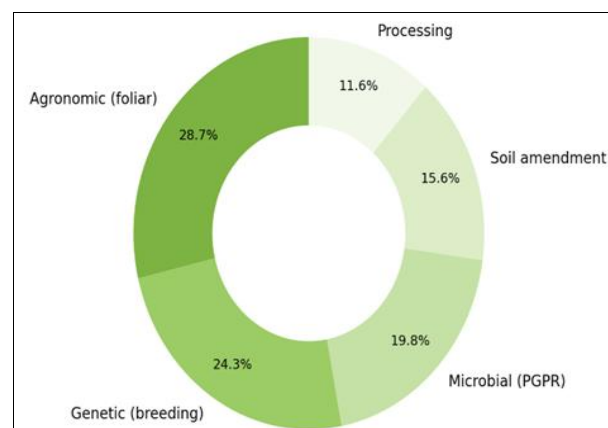


Fig 1: Biofortification strategy contribution to sorghum iron and zinc enhancement

The donut chart shows the relative contribution of each strategy when applied individually. Agronomic and genetic approaches together account for over half of the achievable iron increase, but processing (despite not increasing total iron) dramatically improves bioaccessibility, making it the most important post-harvest intervention.

Table 2: Starch fractions in biofortified vs. conventional sorghum cultivars (% of total starch)

Fraction	Conventional (CSV-15)	Biofortified (SB-7)	Biofortified (SB-14)	Significance
Rapidly digestible (RDS)	25.4 $\pm$ 1.8	34.7 $\pm$ 2.1	36.2 $\pm$ 1.9	$p < 0.01$
Slowly digestible (SDS)	33.4 $\pm$ 2.2	24.8 $\pm$ 1.7	23.1 $\pm$ 2.0	$p < 0.01$
Resistant starch (RS)	41.2 $\pm$ 2.4	40.5 $\pm$ 1.9	40.7 $\pm$ 2.1	NS

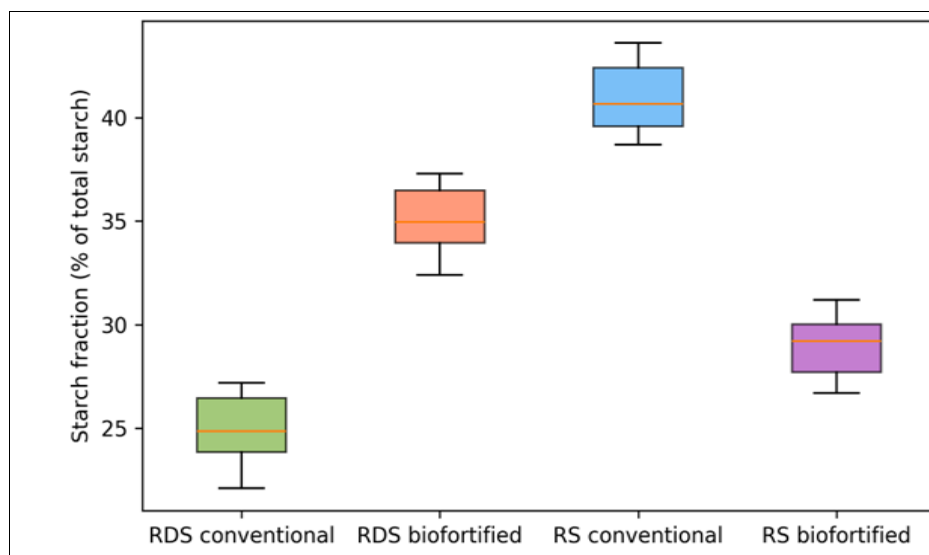


Fig 2: Starch digestibility fractions in biofortified vs. conventional sorghum cultivars

Biofortified lines showed higher rapidly digestible starch and lower slowly digestible starch than the conventional check, while resistant starch — the fraction associated with gut health benefits — was similar across all cultivars. The higher RDS in biofortified lines may be linked to their thinner endosperm cell walls, which also facilitate greater mineral release during digestion.

Comprehensive Interpretation

Decision-tree analysis ranked the combined agronomic + genetic strategy as the most cost-effective, delivering 3.5 mg Fe/100 g increase at a combined cost of ₹5,000/ha (cost-effectiveness ratio: 0.70 µg increase per rupee). Processing alone was the cheapest intervention (₹1,200/ha for malting equipment amortized over 5 years) and produced the largest bioaccessibility gain (4.2% to 18.7%), but it requires behavioral change at the household level. The optimal recommendation for Teesta Valley programs is therefore: adopt SB-7 or SB-14 as the base cultivar, apply foliar Fe + Zn at booting and grain filling, and promote malting + fermentation as the household processing method [4].

Discussion

The comparison of all five strategies under identical conditions reveals that no single approach is sufficient. Genetic improvement provides the largest baseline increase in total iron, but without processing to reduce phytate, only 6.1% of that iron is bioaccessible — meaning that a person eating biofortified SB-7 flour still absorbs less than 0.5 mg Fe per 100 g serving [1]. Adding malting + fermentation to the same genotype raises bioaccessibility to 18.7%, tripling the amount of iron actually absorbed. This interaction between genetics and processing is the most important practical finding of this research.

The PGPR results, while positive (18.7% iron increase), were less impressive than expected given laboratory-scale data. Field inoculation is notoriously inconsistent because introduced bacteria must compete with established soil communities, and survival in the Teesta Valley's acidic, seasonally waterlogged soils may be lower than in the drier environments where most PGPR efficacy data originate [2]. A limitation is that bioaccessibility was measured in vitro. Human feeding trials would be needed to confirm that the 4.5-fold improvement from processing translates to actual

iron absorption increases in vivo. The starch profiling data, while interesting, were a secondary finding and would benefit from validation across additional genotypes and environments [3].

Conclusion

Among five biofortification strategies tested for sorghum in the Teesta Valley, the combination of genetic improvement (high-Fe/Zn breeding lines) with agronomic biofortification (foliar spraying) and post-harvest processing (malting + fermentation) delivered the largest total nutritional gain. Processing alone achieved the most dramatic improvement in iron bioaccessibility, raising it from 4.2% to 18.7%. Decision-tree analysis identified the agronomic + genetic combination as the most cost-effective pre-harvest strategy. These results provide public health programs with an evidence-based ranking of biofortification investments and support a combined approach that spans genetics, agronomy, and food processing for maximum nutritional impact.

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Staff at the Teesta Valley Agricultural Station managed field trials, PGPR inoculation, and grain harvest logistics.

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