



OPEN ACCESS

EDITED BY

Kathleen L. Hefferon,
Cornell University, United States

REVIEWED BY

Sheeja K. Raj,
Kerala Agricultural University, India
Thavaprakash Nallasamy,
Tamil Nadu Horticulture University, India

*CORRESPONDENCE

Pradeep Rajput
✉ pradeeprajput.soag@itmuniversity.ac.in
Rajan Bhatt
✉ rajansoils@pau.edu
Saud Alamri
✉ saualamri@ksu.edu.sa

RECEIVED 21 February 2024

ACCEPTED 23 April 2024

PUBLISHED 14 May 2024

CITATION

Rajput P, Singh A, Rajput RK, Bhatt R, Alamri S,
Kanaujiya PK and Gautam SK (2024)
Agronomic biofortification of basmati rice
(*Oryza sativa* L.) through iron and boron
under varying seedling densities.
Front. Sustain. Food Syst. 8:1388807.
doi: 10.3389/fsufs.2024.1388807

COPYRIGHT

© 2024 Rajput, Singh, Rajput, Bhatt, Alamri,
Kanaujiya and Gautam. This is an
open-access article distributed under the
terms of the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or reproduction
is permitted which does not comply with
these terms.

Agronomic biofortification of basmati rice (*Oryza sativa* L.) through iron and boron under varying seedling densities

Pradeep Rajput^{1*}, Adesh Singh², Ravindra Kumar Rajput³,
Rajan Bhatt^{4*}, Saud Alamri^{5*}, Pradeep Kr. Kanaujiya⁶ and
Sandip Kumar Gautam⁷

¹School of Agriculture, ITM University, Gwalior, India, ²Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India, ³Krishi Vigyan Kendra, UP Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura, India, ⁴PAU-Krishi Vigyan Kendra Amritsar, Punjab, India, ⁵Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia, ⁶School of Agriculture, ITM University, Gwalior, India, ⁷Chhatrapati Shahu Ji Maharaj University, Kanpur, India

At present, we have adequate production of rice to fulfill the needs of humans. Now it is time to produce biofortified rice, whose grains contain enough amounts of nutrients like iron and boron. Two field experiments were carried out in Kharif 2019 and 2020 at Meerut (Uttar Pradesh), India, to find the effect of iron and boron nutrition on rice with different planting densities. The main plot treatment consists of three planting densities, and the subplot treatment consists of five foliar applications of micronutrients. They were tested in a split-plot design (SPD) with three replications. The results revealed that the rice transplanted with one seedling/hill gave the highest iron and boron content in grains and/or the crop fortified with iron and boron at 0.1 and 0.04% applied at the maximum tillering (MT) and panicle initiation (PI) stages of rice, respectively. The higher values of yield-contributing characters such as effective tillers/m², panicle length (cm), grain weight/panicle (g), and test weight (g), as well as quality parameters such as nutrient content in grain (mg/kg), volume expansion ratio, protein content in grains (%), and amylose content (%) of rice were noticed in one seedling/hill except effective tillers/m² during the first and second years. Application of Fe and B at the MT and PI stages of rice improved almost all the yield attributes and quality parameters. Planting density of two and three seedlings/hill was recorded at par values of grain yield, followed by one seedling/hill. Rice transplanted with three seedlings/hill obtained an average of 10.1 and 10.6% more grain yield than one seedling/hill during the first and second years, respectively. However, the application of Fe at 0.1% and B at 0.04% during both stages through foliar spray resulted in the highest seed yield and showed parity with the application of iron at 0.1% at the MT stage and boron at 0.04% at the PI stage. Economics revealed that the planting density of three seedlings/hill gave the maximum net returns of 61,585 and 66,752 ₹/ha with a benefit–cost ratio of 2.12 and 2.19 during 2019 and 2020, respectively. Furthermore, the highest net returns (62,188 and 67,938 ₹/ha) and benefit–cost ratio (2.15 and 2.23) were observed from the treatment of iron at 0.1% and boron at 0.04% at the MT and PI stages during the first and second years, respectively.

KEYWORDS

biofortification, seedlings/hill, micronutrients, rice, yields and quality

1 Introduction

Rice is a major cereal crop that is grown as a staple food by millions of people around the world. Worldwide, rice is cultivated in an area of about 167.1 million hectares, with a periodic production of about 782.0 metric tons and a productivity of 4,679 kg/hectare. About 90 percent of the entire rice grown in the world is produced and consumed in the Asian region. It accounts for 43 percent of India's total food grain production and 55 percent of its cereal production. In India, rice is grown on an area of about 43.8 million hectares, producing 118.9 million tons with an average productivity of 2,715 kg/ha.

Nowadays, we need methods of crop production that are eco-friendly, cost-effective, and require fewer inputs, like reducing the number of seedlings. What we have to do is change the number of scattered plants without peeping into other operations and artistic practices. Both qualitatively and quantitatively, values are affected by the number of seedlings/hill. The optimal planting hill ensures the stores grow both in their elevated and underground corridors through effective appliances of solar energy, water, and nutrients. All of these factors can be skillfully employed, and if proper care is taken, they will eventually form a cushion crop product. The number of seedlings/hill was an important aspect as it told the optimum population/unit area, proper use of sunlight, photosynthesis, respiration, and nutrients, which finally told the yield attributes and yield of rice (Barua et al., 2014).

Densely populated rice fields have greater inter-specific competition between plants, sometimes resulting in gradual shading and accommodation, hence increasing the production of straw rather than grain. For the purpose of delivering targeted micronutrients to populations that do not have or do not have access to differentiated diets and other interventions such as fortified foods and supplementation, biofortification of rice is intended to be a sustainable, cost-effective, and food-based method of delivering these micronutrients. Food security goes beyond hunger—it includes having consistent access to food that is not only safe but also nutritious and economical. About 1.3 billion people are affected by moderate conditions of nutritional insecurity all over the world because they do not have access to food that is both safe and nutritious. This is despite the fact that the number of people suffering from hunger around the world has surpassed 820 million. More than half of the world's population, as well as many who live in poverty, rely on rice crops for their daily calories because they cannot access nutritious foods like fruits, vegetables, meat, and dairy—or they cannot afford them.

As a result, malnutrition is a real problem, causing immediate and long-term health problems including depression, cardiac complaints, diabetes, and putrefaction. People in both developed and developing countries suffer from mineral deficiencies. In addition, approximately one-third of the world's population is at high risk due to the consumption of low-nutrient diets (Stein, 2010). At present, considering that we have enough rice for consumption, the time has come to cultivate high-quality rice, the grains of which contain an adequate quantity of nutrients such as iron and vitamin B. Micronutrient deficiencies are becoming more prevalent in some countries. This global extreme in micronutrient deficiency is the result of insufficient food systems that do not deliver adequate amounts of micronutrients to meet the nutritional supplies of individuals and their nutritional status. Biofortification has been planned as an

indispensable long-term approach to enriching mineral nutrition (Zhu et al., 2007).

2 Materials and methods

2.1 Site of experiment

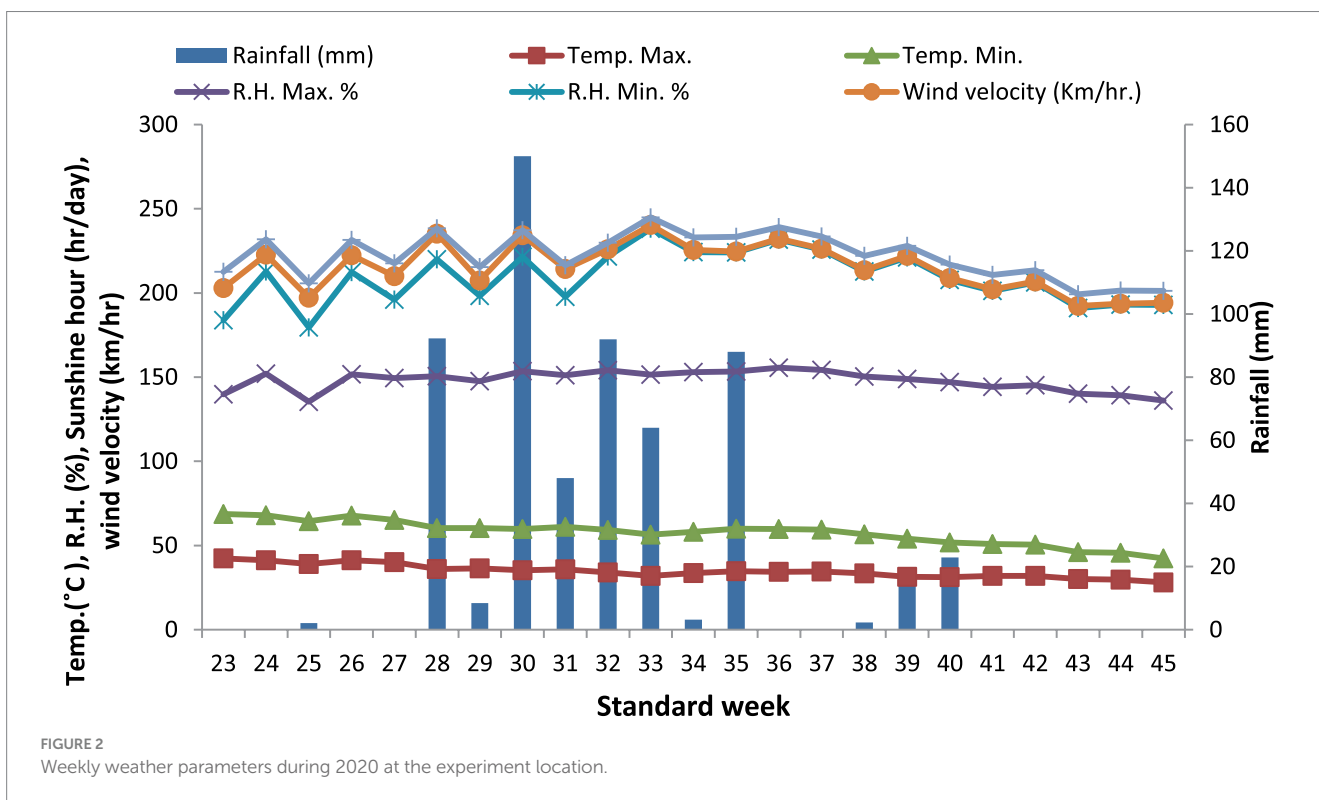
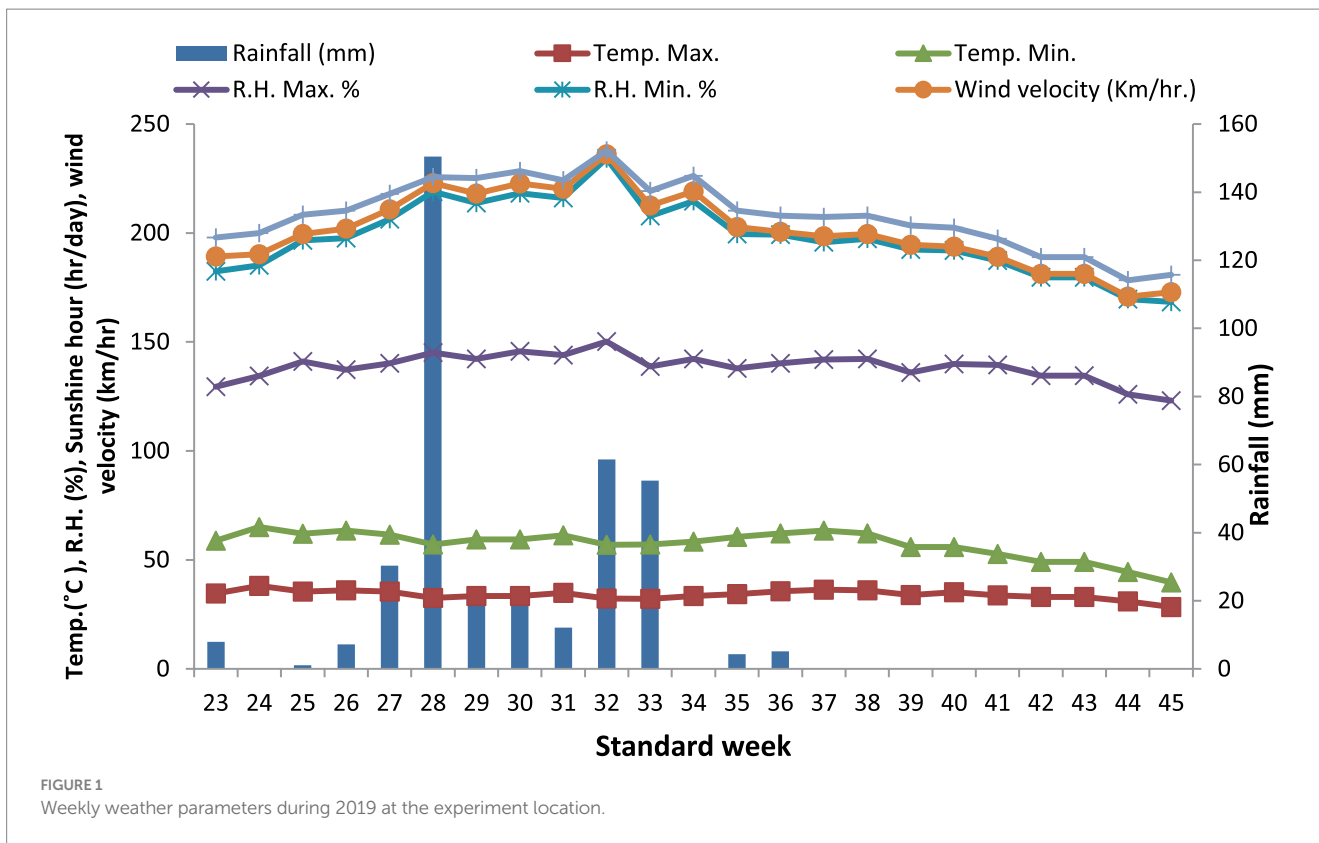
The field experiment was carried out at the Crop Research Centre (CRC), located on the Main Campus of Sardar Vallabhbhai Patel University of Agriculture and Technology in Meerut, Uttar Pradesh, India. The site is situated at 29° 13' 96" N latitude and 77° 68' 43" E longitude, with an elevation of 237 m above mean sea level. The experimental area has a uniform topography and an effective drainage system. The texture of the soil was sandy loam, exhibiting a slightly alkaline pH (8.67). It was characterized by low levels of organic carbon (0.323%) and available nitrogen (190.4 kg/ha) and boron (1.57 kg/ha), but moderate levels of available phosphorus (13.3 kg/ha) and potassium (163.7 kg/ha). Additionally, the soil contained high levels of available iron (41.7 kg/ha).

2.2 Climate and weather conditions

Meerut lies within the semi-arid and sub-tropical climatic zones, characterized by scorching hot and arid summers and chilly winters. A distinctive trait of this climate is moderate rainfall and significant temperature fluctuations. The average annual rainfall in Meerut is 733 mm, with approximately 80–90% occurring between June and September. The data reveal that during the years 2019 and 2020, the average maximum weekly temperature ranged from 28.1°C to 28.4°C in the second week of November, peaking at 42.3°C in the first week of June, with a slight decrease to 38.1°C in the second week of June. Meanwhile, the average minimum weekly temperature fluctuated between 14.2°C and 11.3°C in the second week of November, rising to 26.4°C and 24.2°C in the first week of June, respectively. The mean weekly relative humidity ranged from 71.0 to 70.7% at its lowest to 95.8 to 86.7% at its highest during the years 2019 and 2020, respectively. Additionally, the total rainfall received during the crop periods was 587.6 mm and 377.7 mm in 2019 and 2020, respectively. Wind velocity varied significantly, with speeds ranging from 0.50 km/h in the second week of September to 19.10 km/h in the first week of June during the first year, and from 1.10 km/h in the first week of November to 6.90 km/h in the first week of June during the second year (Figures 1, 2).

2.3 Description of treatments

The experiment employed a split-plot design (SPD) comprising three replicates and 15 treatment combinations. The main plot treatments involved three planting densities: one, two, and three seedlings/hill. The subplot treatments encompassed five foliar applications of micronutrients (Fe and B), specifically: control (MN₀), iron 0.1% and boron 0.04% at the maximum tillering (MT) stage (MN₁), iron 0.1% and boron 0.04% at the panicle initiation (PI) stage (MN₂), iron 0.1% at the MT stage and boron 0.04% at the PI stage (MN₃), and iron 0.1% and boron 0.04% at both stages (MN₄). A total



volume of 400 and 600 liters of water was utilized for foliar spraying of FeSO_4 and was soluble at the maximum tillering and panicle initiating stages, respectively, in both years. For neutralizing the acidic effect of sulfate ions, 0.25% of $\text{Ca}(\text{OH})_2$ was used.

2.4 Cultural practices

High-quality, robust, and vigorous seeds were chosen for sowing. Subsequently, the seeds underwent a soaking process in

water for 24 h, followed by an incubation period of 48 h within a moist gunny bag to facilitate sprouting. The sprouted seeds were spread in the puddled seed beds in rows, 10 cm apart, manually on 23 and 27 June during 2019 and 2020, respectively. Adequate care was taken for water, nutrients, weeding, etc., and thus seedlings were raised for up to 25 days in the nursery. Following this, seedlings aged 25 days were manually transplanted on 22 July 2019, and 18 July 2020, on a puddled soil with a spacing of 20 cm × 15 cm.

2.5 Management of fertilizers and crops

The recommended nitrogen rate of 100 kg/ha was applied in three equal splits: at the time of transplanting (during the last puddling), maximum tillering, and at the panicle initiation stage of the crop, utilizing urea (46% N). Phosphorus and potassium were administered at the time of the last puddling, following the recommended doses of 60 kg P₂O₅ and 40 kg K₂O per hectare, respectively, in the form of DAP (18:46 N:P₂O₅) and MOP (60% K₂O). Additionally, 5 kg of zinc per hectare through zinc sulfate monohydrate (ZnSO₄·H₂O, 33% Zn) was applied at the time of transplanting. Pretilachlor 30% EC herbicide, at 0.75 kg/ha, was applied 2 days after transplanting.

2.6 Harvesting and threshing

The crop reached full physiological maturity before harvesting. Initially, a section measuring 0.45 m in length along both sides of the rows and two border rows (0.40 m) on each side of the experimental plots were harvested and set aside. Subsequently, the crop from the remaining plot area was harvested and subjected to sun drying. Harvesting occurred during the first week of November 2019 and the last week of October 2020. Each harvested portion from the net plot was bundled, tagged, and prepared for threshing. The grain weight was measured at 14% moisture content after threshing, cleaning, and drying. The grain, straw, and biological yield were quantified in quintals per hectare.

The benefit-to-cost ratio was computed using the following formula:

$$\text{Benefit : Cost} = \frac{\text{Gross return (₹/ha)}}{\text{Cultivation cost (₹/ha)}}$$

2.7 Grain quality analysis

An estimation of Fe was done on an atomic absorption spectrophotometer (AAS) using an iron cathode. Wet digestion using a di-acid mixture (HClO₄ + HNO₃, 4:10) was followed for the digestion of the plant sample for determining the B and iron in the plant sample, and estimation of B in grains and straw was done by the Azomethine H method. Azomethine H forms a colored complex with H₃BO₄ in aqueous media, and finally, the boron was determined by using the spectrophotometer. To compute the protein content in rice grains, the nitrogen percentage in the grains

was multiplied by a factor of 5.95 (Prasad et al., 2006). Thus, the formula used was: grain protein content (%) = nitrogen content in grains (%) × 5.95. For determining the volume expansion ratio, the standard method was used (Verghese, 1950; Murthy and Govindaswamy, 1967). At first, 5 g of milled rice grains were submerged in 15 mL of water for 15 min in a centrifuge tube, with the initial measurement of rice volume taken using the volume displacement technique. Then, for cooking, the tubes were positioned in a pressure cooker, and the cooked rice was strained on filter paper to remove excess water. Finally, the volumes of cooked rice grains were determined using the water displacement method. The volume expansion ratio was then calculated using the following formula:

$$\text{Volume expansion ratio} = \frac{\text{Volume of cooked rice (ml)}}{\text{Volume of uncooked rice (ml)}} \times 100$$

A 1 g portion of milled rice grains underwent gentle crushing and fine powdering with a glass pestle and mortar. The powdered samples were then stored to attain a consistent moisture level of 12%. Following this, a precise 100 mg sample was weighed using an electronic balance and transferred into a 100 mL volumetric flask. Ten milliliters of freshly prepared 1 N NaOH solution was carefully pipetted into the conical flask to immerse the flour, allowing it to form a clump, followed by the addition of 1 mL of distilled ethanol and mixing thoroughly. After undergoing gelatinization for an hour, the sample suspension was heated for 10 min in a boiling water bath. Subsequently, the volume was adjusted to 100 mL by adding distilled water. After thorough shaking, a 2.5 mL aliquot was pipetted into a 50 mL volumetric flask, followed by the addition of approximately 20 mL of water. Three drops of phenolphthalein indicator were introduced and mixed well. After that, the substance was acidified by adding 0.1 N HCl drop by drop until the pink tint was almost completely gone. Following that, 1 mL of iodine reagent was added in order to produce a blue color, and the volume was brought up to 50 mL. A spectrophotometer was employed to measure absorbance at a wavelength of 590 nm. To construct a standard curve, absorbance values of known quantities of pure amylose were utilized as reference points. The amylose content in the sample was then calculated using the standard curve generated from pure amylose (ranging from 0.2 to 1.0 mg) against a blank, which involved diluting 1 mL of iodine reagent to 50 mL with distilled water (Thimmaiah, 1999). Absorbance corresponds to 2.5 mL of test solution = 'x' mg amylose in test solution.

$$100\text{mL} = \frac{X}{2.5} \times 100\% \text{ amylose}$$

2.8 Statistical analysis

Statistical analysis was investigated by the OPSTAT program. The significance of differences between treatment means was indomitable using the least significant difference (LSD) values at the 5% significance level.

3 Results

3.1 Yield attributes of rice

Data reveal that the yield attributes of each treatment were higher in the second year than the first year (Table 1). Significantly longest panicle (27.5 and 28.0 cm), higher grain weight/panicle (1.70 and 1.80 g), and test weight (25.8 and 26.3 g) were recorded with one seedling/hill, while the highest number of productive tillers/m² were found in three seedlings/hill during the first and second years of the experiment (Table 1).

Crops fortified with Fe and B had a significant effect on all the yield attributes except the length of the panicle (Table 1). A higher number of productive tillers (433 and 437), grain weight/panicle (1.61 and 1.77 g), panicle length (27.5 and 28.2 cm), and test weight (26.2 and 26.5) were found in the treatment and foliar application of iron and boron at MT and PI stages (MN₄) over control (MN₀) during the first and second years, respectively.

3.2 Grain yield

Economic yield was significantly higher at three seedlings/hill as compared to one and two seedlings/hill. in account of more tillering causative to the maximum biomass and effective tillers/m² during both years. However, rice transplanted with three seedlings/hill obtained an average of 10.1 and 10.6% more grain yield than the one seedling/hill during the first and second years, respectively. The crop fortified with iron and boron had a higher economic yield with the treatment of MN₄, while the lowest was found in control (MN₀) during both years. Moreover, the treatment MN₄ produced 1.0, 2.4, 7.7, and 9.6% higher grain yield/ha than MN₃ and control (MN₀) during the first and second years, respectively (Table 2).

3.3 Economics

The rice crop grown with two seedlings/hill fetched 7,988 and 8,458 more net returns/ha as compared to one seedling/hill during 2019 and 2020, respectively. The B:C ratio did not show any significant variation during the first and second years. A higher B:C ratio of 2.16 and 2.23 was recorded in two seedlings/hill (Table 2). The slightly higher net profit/ha (₹ 61,999 and 66,797) was recorded with two seedlings/hill as compared to three seedlings/hill, which was mainly due to less expenditure incurred on seedlings and the cost of transplanting under two seedlings/hill. Similarly, net profit and the return/rupee invested were also higher with two seedlings/hill than with three seedlings/hill. The higher gross and net returns/ha were recorded with the foliar application of iron at 0.1% and boron at 0.04% at both stages. This treatment also gave additional net returns of ₹8,471 and 8,823/ha with a benefit–cost ratio of 2.15 and 2.23 over unfertilized control during 2019 and 2020, respectively.

3.4 Concentration of iron and boron in grain

The maximum Fe (37.3 and 37.6 mg/kg) and B (36.6 and 37.2 mg/kg) content in grain was observed under one seedling/hill (Table 3). Whereas, three seedlings/hill exhibited minimum nutrient content during the first and second years, respectively. Foliar application of micronutrients resulted in higher nutrient content in grain. As regards Fe and B nutrition, the highest Fe (38.9 and 39.0%) and B (36.0 and 36.3 mg/kg) content in grains were recorded with foliar application of Fe and B at both stages (MN₄) during the first and second years, respectively. Foliar-applied substances (Fe and B) can go into leaves either by penetration or via the stomatal pathway and enter directly into the plant.

TABLE 1 Effect of planting density and micronutrient application on yield attributes of basmati rice.

Treatment	Productive tillers/m ²		Panicle length (cm)		Grains weight/panicle (g)		Test weight (g)	
	2019	2020	2019	2020	2019	2020	2019	2020
Seedling density								
1 Seedling/hill	368	371	27.5	28.0	1.70	1.80	25.8	26.3
2 Seedlings/hill	413	416	26.8	27.6	1.54	1.63	24.8	25.4
3 Seedlings/hill	441	446	26.4	27.2	1.50	1.57	24.3	24.9
SEm±	10	8	0.3	0.1	0.02	0.04	0.3	0.2
C.D. (p=0.05)	42	31	1.0	0.5	0.08	0.16	1.2	1.0
Micronutrient application (Fe and B)								
MN ₀	380	381	26.2	26.7	1.54	1.58	23.7	24.7
MN ₁	398	400	26.6	27.1	1.57	1.59	24.2	25.1
MN ₂	410	412	26.9	27.6	1.58	1.64	25.0	25.6
MN ₃	418	421	27.1	27.7	1.60	1.73	25.7	26.2
MN ₄	433	437	27.5	28.2	1.61	1.77	26.2	26.5
SEm±	8	10	0.6	0.4	0.05	0.03	0.3	0.3
C.D. (P=0.05)	23	30	NS	NS	NS	NS	0.9	1.0

TABLE 2 Effect of planting density and micronutrient application on grain yield and the economics of basmati rice.

Treatment	Grain Yield (t/ha)		Gross return (₹/ha)		Net return (₹/ha)		B:C ratio	
	2019	2020	2019	2020	2019	2020	2019	2020
Seedling density								
1 Seedling/hill	4.56	4.62	105,705	110,853	54,011	58,339	2.05	2.11
2 Seedlings/hill	4.97	5.04	115,523	121,123	61,999	66,797	2.16	2.23
3 Seedlings/hill	5.02	5.11	116,839	122,848	61,585	66,752	2.12	2.19
SEm±	0.08	0.07	1,548	1,572	1,548	1,572	0.03	0.03
C.D. (P=0.05)	0.32	0.30	6,242	6,338	6,242	6,338	NS	NS
Micronutrient application (Fe and B)								
MN ₀	4.66	4.71	106,813	112,992	53,717	59,115	2.01	2.10
MN ₁	4.80	4.89	112,244	116,760	59,037	62,917	2.11	2.17
MN ₂	4.83	4.91	112,916	117,955	59,509	63,668	2.12	2.17
MN ₃	4.97	5.04	115,188	120,653	61,541	66,176	2.15	2.21
MN ₄	5.02	5.16	116,285	123,015	62,188	67,938	2.15	2.23
SEm±	0.06	0.05	1,220	974	1,220	974	0.02	0.02
C.D. (P=0.05)	0.18	0.15	3,581	2,861	3,581	2,861	0.07	0.05

TABLE 3 Effect of planting density and micronutrient application on quality parameters of basmati rice.

Treatment	Nutrient content in grain (mg/kg)				Volume expansion ratio		Protein content in grains (%)		Amylose content (%)	
	Iron		Boron		2019	2020	2019	2020	2019	2020
	2019	2020	2019	2020						
Seedling density										
1 Seedling/hill	37.3	37.6	36.6	37.2	0.376	0.380	7.64	7.67	24.4	24.7
2 Seedlings/hill	33.6	33.7	32.5	32.5	0.364	0.368	7.57	7.60	23.6	23.8
3 Seedlings/hill	30.5	30.6	25.7	25.8	0.357	0.360	7.62	7.63	21.7	21.8
SEm±	1.2	1.2	1.3	1.2	0.005	0.006	0.05	0.16	0.2	0.3
C.D. (P=0.05)	4.8	4.8	5.0	4.7	NS	NS	NS	NS	0.9	1.3
Micronutrient application (Fe and B)										
MN ₀	28.3	28.4	26.7	26.8	0.361	0.364	7.50	7.52	22.1	22.4
MN ₁	33.3	33.4	31.0	31.2	0.366	0.369	7.66	7.68	23.8	23.9
MN ₂	35.3	35.5	32.8	33.1	0.369	0.372	7.56	7.60	23.0	23.2
MN ₃	33.3	33.5	31.0	31.3	0.376	0.379	7.74	7.77	24.1	24.3
MN ₄	38.9	39.0	36.0	36.3	0.378	0.380	7.60	7.62	23.1	23.4
SEm±	1.2	1.1	1.3	1.1	0.007	0.006	0.18	0.16	0.3	0.32
C.D. (P=0.05)	3.4	3.1	3.7	3.3	NS	NS	NS	NS	1.0	0.9

3.5 Quality parameter

The maximum amylose content (24.4 and 24.7%) was recorded in one seedling/hill in comparison to three seedlings/hill (21.7 and 21.8%) during 2019 and 2020, respectively (Table 3). There was no significant variation among different planting densities with regards to volume expansion ratio and protein content in grain (%) during the first and second years. A higher volume expansion ratio of 0.376 and 0.380 and protein content in grains (7.64 and 7.67%) were recorded with one seedling/hill during the first and second years,

respectively. A higher volume expansion ratio and protein content in grains (%) were recorded in one seedling/hill due to the higher nutrient contents of the grains. Micronutrients application, the highest volume expansion ratio of 0.378 and 0.380, amylose content (23.1 and 23.4), and protein content in grains (7.74 and 7.77%) were recorded with iron and boron applied at both stages (MN₄), which was on par with MN₃ during the first and second years, respectively.

Rice transplanted with one seedling/hill recorded higher kernel length (11.1 and 11.3 mm) and breadth (2.24 and 2.25 mm) during

2019 and 2020, respectively. Length and breadth ratio was higher in three seedlings/hill, followed by two seedlings/hill, during both years. It might be due to the higher nutrient contents of grains and straw and the more food material supplied by the plants.

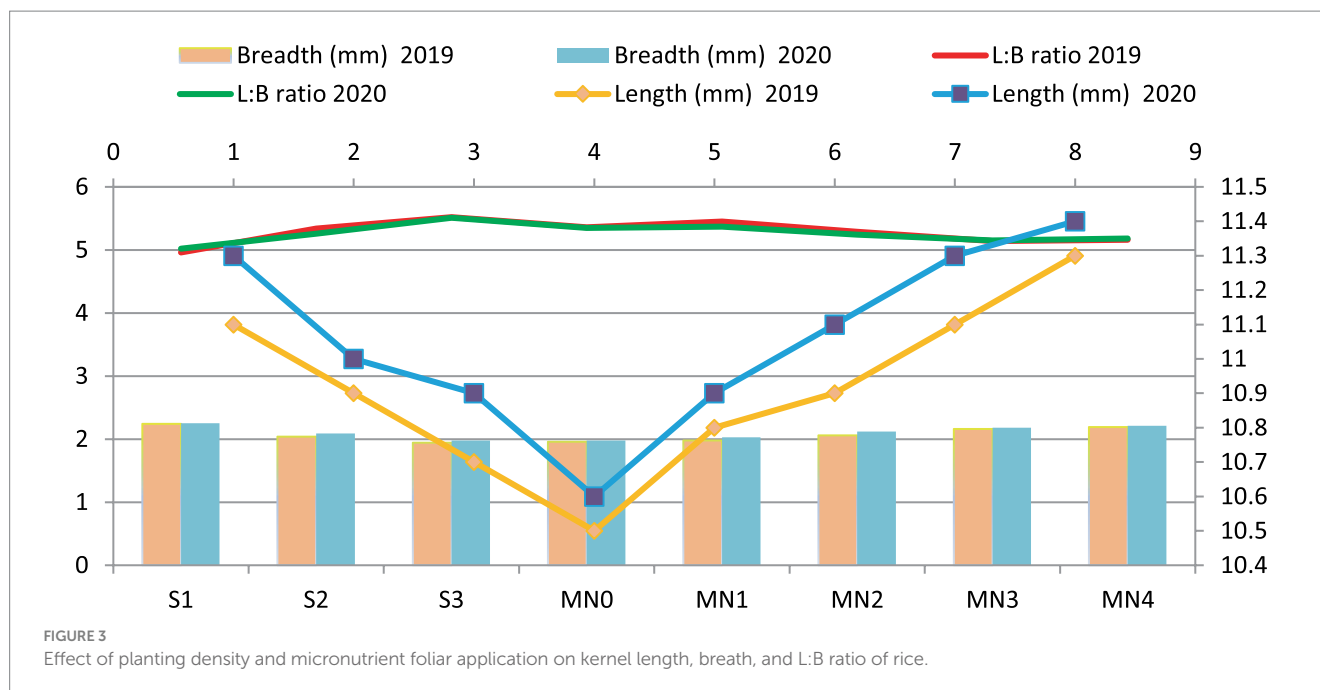
4 Discussion

The data revealed that there was comparatively higher rice production in 2020 than in 2019, which might be due to favorable weather conditions and a low incidence of pests and diseases. Rainfall at the tillering and stem elongation stages positively impacts the rice plants, resulting in an increase in the rate of tillering, which ultimately improves rice production. Agronomic practices involving fertilizer application have been proposed as a promising approach to enhance both productivity and grain quality in rice cultivation. However, the effectiveness of this management strategy depends on several variables, including the type of rice variety, soil fertility, and the method employed for fertilizer application (Khampuang et al., 2021). In conducive environments, plant development relies not solely on photosynthesis, which limits grain size, but also on the compatibility between physiological sinks and sources, which influences grain weight. Improvement in yield attributes was probably due to proper utilization of all the available and terrestrial growth resources, which may be influenced by the good translocation of photosynthates to sink from the source, and finally expressed the maximum values of yield attributes under one seedling/hill (Islam et al., 2013; Rajput et al., 2020; Dhungana et al., 2021). Crop fortified with Fe and B gave better results. It might be due to more growth and development of rice with better plant height and more periodic DMA and LAI. It also enhanced metabolic activity, which improved floral primordial development and the conversion of vegetative tillers into reproductive tillers. Boron plays a key role in the flowering and grain-setting processes in rice. Good seed setting, better pollination, reduced spike sterility, and increased grain formation were observed in various rice varieties due to boron nutrition (Aslam et al., 2002). A sufficient amount of iron in the soil provided different nutrient uptake, leading to enhanced plant growth and photosynthetic rates, thereby increasing the translocation of dry matter and ultimately boosting straw yield (Reddy et al., 2012; Kannan et al., 2015). The increased economic yield could be attributed to a higher number of effective tillers/m², enhanced dry matter accumulation, and other yield-contributing factors (Table 1), in addition to the translocation of photosynthates toward the sink (Singh et al., 2013; Negalur et al., 2016; Promsomboon et al., 2019).

The rice crop grown with two and three seedlings/hill fetched 7,988 and 8,458 and 7,574 and 8,413 more net returns/ha as compared to one seedling/hill during 2019 and 2020, respectively. It might be due to reduced production cost and increased yield of the crop, which ultimately increased net return, whereas the lowest B:C ratio was observed in one seedling/hill. The slightly higher net profit/ha (₹ 61,999 and 66,797) was recorded with two seedlings/hill than with three seedlings/hill, which was mainly due to less expenditure incurred on seedlings and the cost of transplanting under two seedlings/hill. Similarly, net profit and the return/rupee

invested were also higher with two seedlings/hill than with three seedlings/hill (Bommayasamy and Durairaj, 2018; Devi et al., 2019). The highest gross and net returns/ha were recorded with the application of iron at 0.1% and boron at 0.04%. This treatment also gave an additional net return of ₹8,471 and 8,823/ha with a B:C ratio of 2.15 and 2.23 over unfertilized control during 2019 and 2020, respectively. The increased grain yield resulting from iron nutrition primarily stemmed from enhanced crop growth, characterized by a greater number of productive tillers/m², increased the filled grains per panicle, higher panicle weight, and 1,000-grain weight, along with an augmented supply of photosynthates from source to sink (Yadav et al., 2013; Kumar et al., 2015). Higher nutrient content (%) in grains and straw was recorded under one seedling/hill as compared to two and three seedlings/hill. Intense competition among plants for growth factors, including nutrients, under higher plant density and the dilution effect could have led to lower nitrogen (N), phosphorus (P), potassium (K), iron (Fe), and boron (B) content in sinks (Pradhan and Dixit, 2021). More nutrient content in grains and straw and their uptake were recorded due to the application of micronutrients iron and boron. As regards iron and boron nutrition, the highest iron and boron content in grain was recorded with foliar application of Fe and B nutrition during 2019 and 2020, respectively. Increased iron and boron concentrations in the grain could be attributed to greater transfer of these nutrients from the source to the sink (grain), particularly when higher doses of iron and boron were administered as foliar sprays during the later stages of growth (Kumar et al., 2018; Patel et al., 2019).

The quality of grains increased with the transplanting of one seedling/hill as compared to the two seedlings/hill. The protein content is influenced by the increased concentration of nitrogen in grains, which may have altered the proportion of grain constituents. Additionally, rice crops transplanted with one seedling/hill showed improvements in cooking qualities such as kernel elongation ratio upon cooking, bursting upon cooking, and alkaline spreading value. The enhancement in grain quality is credited to improved grain filling, facilitated by the planting density of rice (Jadhav et al., 2014). The highest volume expansion ratio, amylose content, and protein content in grains were recorded with iron and boron applied at both stages (MN₄), which was on par with MN₃ during 2019 and 2020, respectively. Boron supply improved cooking traits such as elongation ratio, bursting on cooking, and alkali spreading value in basmati rice. Adequate boron supply appears to be a prerequisite for obtaining optimum yields of good-quality basmati rice (Saleem et al., 2013; Rajput et al., 2019). It is noteworthy to observe that in this investigation, the application of iron and boron led to an increase in the content of iron and boron in rice grain. Although the accumulation of B and Fe in grains was enhanced, it did not impact the number of fertilized or filled grains during development (Songsriin et al., 2023). Therefore, the foliar application of B during the panicle initiation stage could be a factor in enhancing rice crop productivity. However, the optimal timing of application warrants thorough investigation in the future, along with a management strategy aimed at improving grain filling (Figure 3).



5 Conclusion

On the basis of the research study, it was concluded that the transplanting of three seedlings/hill produced the highest grain yield, but quality parameters such as volume expansion ratio and protein content (%) in grains were recorded at higher values with one seedling/hill as compared to two seedlings/hill. However, crop fortified with iron 0.1% and boron 0.04% at the maximum tillering (MT) and panicle initiation (PI) stages produce biofortified grains and increase the quality and quantity of grains.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

PR: Writing – review & editing, Conceptualization, Investigation, Methodology, Software, Writing – original draft. AS: Investigation, Writing – original draft. RR: Formal analysis, Writing – review & editing, Software. RB: Formal analysis, Validation, Visualization, Writing – review & editing. SA: Formal analysis, Writing – review & editing, Conceptualization, Funding acquisition. PK: Writing – review & editing. SG: Writing – review & editing, Methodology.

References

- Aslam, M. I., Mahmood, R. H., Qureshi, S., Nawaz, S., and Akhtar, J. (2002). Salinity tolerance of Rice as affected by boron nutrition. *Pak. J. Soil Sci.* 21, 110–118.
- Barua, R., Islam, M. N., Zahan, A., and Paul, Shamsunahar S. (2014). Effects of spacing and number of seedlings/hill on the yield and yield components of brri dhan47. *J. Eco-Friend. Agricul.* 7, 65–68.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgement

The authors sincerely acknowledge the researchers supporting project number (RSP2024R194), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

- Bommayasamy, N., and Durairaj, N. (2018). Influence of non-monetary inputs on growth, yield and economics of rice under system of rice intensification (SRI). *J. Pharmacog. Phytochem.* 7, 3046–3049.

- Devi, N., Narayan, K. G., Singh, K. K., Devi, M. A., Athokpam, H. S., and Singh, A. D. (2019). Effect of age and number of seedling/hill on growth and yield of black scented

- Rice (*Oryza sativa* L.) variety Chakhao Poiraiton under Manipur condition. *Int. J. Curr. Microbiol. Appl. Sci.* 8, 1738–1745. doi: 10.20546/ijcmas.2019.807.206
- Dhungana, R., Bhandari, R., Paude, R., Paudel, P., Bakabal, M., Bohora, S. L., et al. (2021). Effect of age and number of seedlings in productivity of Tilki Rice in dang, Nepal. *Nepalese J. Agricul. Sci.* 20, 05–18.
- Islam, M. S., Rashid, M. M., Mondal, M. K., Nath, S. C., and Karim, M. R. (2013). Effect of planting density on the performance of hybrid Rice (*Oryza sativa* L.) under waterlogged condition. *Scient. J. Krishi Found.* 11, 109–113.
- Jadhav, K. T., Lokhande, D. C., and Asewar, B. V. (2014). Effect of ferrous and zinc nutrient management practices on Rice under aerobic condition. *Adv. Res. J. Crop Improve.* 5, 131–135. doi: 10.15740/HAS/ARJCI/5.2/131-135
- Kannan, K., Kundu, D. K., Singh, R., Thakur, A. K., and Chaudhari, S. K. (2015). Productivity and water use efficiency of aerobic Rice under different moisture regimes in eastern India. *Indian J. Soil Conserv.* 43, 170–174.
- Khampuang, K., Lordkaew, S., Dell, B., and Prom-u-thai, C. (2021). Foliar zinc application improved grain zinc accumulation and bioavailable zinc in unpolished and polished rice. *Plant Prod. Sci.* 24, 94–102. doi: 10.1080/1343943X.2020.1797512
- Kumar, V., Kumar, D., Singh, Y. V., and Raj, R. (2015). Effect of iron fertilization on dry-matter production, yield and economics of aerobic rice (*Oryza sativa*). *Ind. J. Agrono.* 60, 547–553. doi: 10.59797/ija.v60i4.4491
- Kumar, M. B., Subbarayappa, C. T., and Ramamurthy, V. (2018). Effect of graded levels of zinc and boron on growth, yield and chemical properties of soils under Paddy. *Int. J. Curr. Microbiol. App. Sci.* 6, 1185–1196.
- Murthy, P. S. N., and Govindaswamy, S. (1967). Inheritance of grain size and its correlation with the hulling and cooking qualities. *Oryza* 4, 12–21.
- Negalur, R. B., Halepyati, A. S., and Rao, K. N. (2016). Influence of age and number of seedlings on yield and nutrient uptake by machine transplanted Rice (*Oryza sativa* L.). *Int. J. Bio-resource Stress Manag.* 7, 393–397. doi: 10.23910/IJBMS/2016.7.3.1548
- Patel, S. K., Singh, R. P., Srivastava, S., Pandey, A. K., and Chandel, S. K. (2019). Effect of foliar application of boron at different stages of crop growth on nutrient utilization and yield of Rice (*Oryza Sativa* L.). *Ind. J. Scient. Res.* 9, 1–6. doi: 10.32606/IJSR.V9.I2.00001
- Pradhan, A., and Dixit, A. (2021). Effect of age and number of seedlings on rice (*Oryza sativa*) under SRI in rainfed agro-ecosystem. *Indian J. Agric. Sci.* 91, 857–860. doi: 10.56093/ijas.v91i6.114286
- Prasad, R., Shivay, Y.S., Kumar, D., and Sharma, S.N. (2006). Learning by doing exercises in soil fertility (a practical manual for soil fertility), Division of Agronomy, IARI, and New Delhi: 68.
- Promsomboon, P., Sennoi, R., Puthmee, T., Marubodee, R., Ruanpan, W., and Promsomboon, S. (2019). Effect of seedlings numbers/hill on the growth and yield of Kum Bangpra Rice Variety (*Oryza sativa* L.). *Int. J. Agricul. Technol.* 15, 103–112.
- Rajput, P., Singh, A. K., Rajput, R. K., and Singh, A. (2020). Effect of nitrogen levels on yield and yield attributes of Rice (*Oryza sativa*) grown under different planting geometry. *Ind. J. Agronom.* 65, 235–237.
- Rajput, P., Singh, A., Rajput, R. K., and Verma, J. (2019). Agronomic bio-fortification in wheat through zinc and iron nutrition: a review. *Int. J. Chem. Stud.* 7, 2900–2906.
- Reddy, M., Padmaja, B., Veeranna, G., and Vishnu Vardhan Reddy, D. (2012). Evaluation of popular *kharif* rice (*Oryza sativa* L.) varieties under aerobic condition and their response to nitrogen dose. *J. Res. ANGRAU* 40, 14–19.
- Saleem, M., Khanif, Y. M., Fauziah, C. I., Samsuri, A. W., and Hafeez, B. (2013). Efficacy of crushed ore colemanite as boron fertilizer for rice grown under calcareous soil conditions. *Pak. J. Agric. Sci.* 50, 37–42.
- Singh, K., Singh, S. R., Singh, J. K., Rathore, R. S., Pal, S., Singh, S. P., et al. (2013). Effect of age of seedling and spacing on yield, economics, soil health and digestibility of Rice (*Oryza sativa*) genotypes under system of Rice intensification. *Indian J. Agric. Sci.* 83, 479–483.
- Songsriin, J., Yamuangmorn, S., Lordkaew, S., Jumrus, S., Veeradittakit, J., Jamjod, S., et al. (2023). Efficacy of soil and foliar boron fertilizer on boron uptake and productivity in Rice. *Agronomy* 13:692. doi: 10.3390/agronomy13030692
- Stein, A. J. (2010). Global impacts of human mineral malnutrition. *Plant Soil* 335, 133–154. doi: 10.1007/s11104-009-0228-2
- Thimmaiah, S. K. (1999). “Estimation of starch by anthrone reagent” in *Text book of biochemistry*. 1st ed. Eds. Lakshmappa Ratha, Seema Mishra, V. Ramachandran, Manmohan Singh Bhatia. (Mumbai: Himalaya Publ. House), 11–12.
- Verghese, E. J. (1950). A standard procedure for cooking Rice for experimental purposes. *Madras Agric. J.* 37, 217–221. doi: 10.29321/MAJ.10.A04487
- Yadav, G. S., Shivay, Y. S., Kumar, D., and Babu, S. (2013). Enhancing iron density and uptake in grain and straw of aerobic rice through mulching and rhizo-foliar fertilization of iron. *J. Plant Nutr.* 8, 5447–5454.
- Zhu, C., Naqvi, S., Gomez, G. S., Pelacho, A. M., Capell, T., and Christou, P. (2007). Transgenic strategies for the nutritional enhancement of plants. *Trends Plant Sci.* 12, 548–555. doi: 10.1016/j.tplants.2007.09.007