Effect of soil micronutrients (Zinc and Boron) on yield and uptake of wheat in an acid soil of West Bengal, India

3 4

Abstract: Wheat The production of wheat (Triticumaestivum), an important staple food in the 5 world, production is often restricted due to micronutrients status in soil. Micronutrient deficiency 6 7 in soil including boron (B) and zinc (Zn) is quite widespread in Asian countries including India 8 due to prevalent soil and environmental conditions. A field experiment was conducted following randomized complete block design over a two-year period in an acid soil of Terai region of West 9 10 Bengal to study the effect of zinc and boron on the yield and uptake of nutrients by wheat. The highest grain yield (4.38 t/ha) was obtained after the combined application of Znand Bover that 11 of other treatment combinations (variable rates of B and Zn application with nutrients) or control 12 13 (no nutrients, B and Zn). Application of one micronutrient might have accelerated the uptake of other micro- and macro-nutrients resulting in higher yield. A positive correlation was observed 14 between the grain yield and the uptake of different nutrients with the weakest with Zn. A build-15 up of the nutrients in soils was also observed at the harvest. High response clearly demonstrated 16 the necessity of micronutrients for improving production in the studied regions with acid soils. 17 Key words: micronutrients, synergistic effect, Terai region, deficiency, growth stages 18

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20 Introduction

Wheat (Triticumaestivum L.) is the most important staple food for humans and is grown on 21 more land than any other commercial crops in the world. It was grown on 216.6 million ha land 22 in 2012[1] producing 674.9 million tonnes of wheat globally, the third most produced cereal 23 (perhaps any food crop) after maize (875.1 million tonnes) and rice (718.3 million tonnes) [1]. 24 Wheat has higher protein content than either maize (corn) or rice or any other cereals and is the 25 leading source of vegetable protein in human food globallyreference?. In 2012, India produced 26 94.9 million tonnes of wheat from a cultivated area of 29.9 million ha, the largest area devoted to 27 wheat production by any country in the world [2]. 28

With the demand of ever-increasing population, the present day agriculture became more and more intensive and mined available nutrients from soil over years. However, one of the major **Comment [MA1]:** Maybe wheat has more protein than other cereals, but I think there should be a reference. It seems as if the author says that wheat is a leading source of vegetable protein, but surely legumes will fit this role better?

triggering factors behind the dramatic improvement in the production and yield of wheat was the 31 32 supply of artificial nutrient source for plant growth and development especially the use of synthetic nitrogen fertilizer. Potash and phosphorus fertilizer in addition to the nitrogen fertilizer 33 34 supplied the major nutrients for the growth, development and production of wheat. In addition to 35 these major (macro) nutrients, there are some nutrients, which are essential for wheat growth but needed only in very small (micro) quantities. Among these, Boron (B), Zinc (Zn), Iron, (Fe), 36 Copper (Cu), Manganese (Mn), and Chlorine (Cl) are known to have effect on the grain- as well 37 as straw-yield of wheat. These micronutrients play a pivotal role in the yield improvement of 38 wheat crop [3]. They are needed in trace amount while the adequate supply improves nutrient 39 availability and positively affects the cell physiology that is reflected in yield as well [4, 5]. A 40 number of micronutrients are part of the photosynthesis and respiration processes, chlorophyll 41 formation, nucleic acid and protein synthesis, nitrogen-fixation and other biochemical pathways 42 [6-8]. However, the deficiency of micronutrients are wide spread in many Asian countries 43 including India due to calcareous nature of soils, high pH, low organic matter, salt stress, 44 45 prolonged draught, high bicarbonate content in irrigation water and imbalanced application of NPK fertilizers [9, 10]. The deficiency of micronutrients can induce the stress in plants including 46 low crop yield and quality, imperfect plant morphological structure (such as fewer xylem vessels 47 of small size), widespread infestation of various diseases and pests and low fertilizer use 48 efficiency. 49

50 Zinc is one of the important micronutrients, which is important in the production of various crops including wheat [11, 12]. It improves the number of grains per spike [13]. In addition to 51 52 the improvement of yield and yield components of wheat [14, 15], adequate supply of Zn can 53 improve the water use efficiency of wheat plants [16]. Zinc is also known to provide thermotolerance to the photosynthetic apparatus of wheat [17]. The presence of Zn is important in plant 54 metabolism and thus the growth and production [18]. The Zn deficiency is the third most 55 common deficient nutrient after nitrogen and phosphorus [19, 20]. Zinc deficiency in plants not 56 57 only reduces the grain yield, but also the nutritional quality [21].

Boron is another important micronutrient that is essential for plant growth and improves the production efficiency of wheat. However, the deficiency of B is the most frequently encountered one in field [22]. Boron is essential for cell division and elongation of meristematic tissues, floral organs and the flower male fertility, pollen tube germination and its elongation and the seed

andfruit formation. Lack of B can cause the 'wheat sterility' resulting in increased number of 62 open spikelets and decreased number of grains per spike [23]. The B deficiency in soil can affect 63 seedling emergence and cause an abnormal cellular development in young wheat plant [24]. 64 Deficiency of B also inhibits root elongation by limiting cell division in the growing zone of root 65 tips [25]. Deficiency of B is known to inhibit the leaf expansion and reduction in photosynthesis 66 though the exact role of boron in photosynthesis is still least understood of all the mineral 67 68 nutrients. In the field, sexual reproduction is often affected by low B reducing the grain yield significantly but without any visual symptoms expressed during vegetative growth. 69

The Terai region is located at the south of the outer foothill of the Himalaya and Siwalik hills 70 71 and the north of the Indo-Gangetic plain. It spreads over a number of states in India including Himachal Pradesh, Haryana, Uttaranchal, Uttar Pradesh, West Bengal, Sikkim and Assam. It also 72 covers a major part in Nepal, Bhutan and Bangladesh. The Terai region is the habitat of millions 73 of people. It is a very productive region and agriculture is the base of the economy of the 74 habitants. Rice and wheat are important crops of this region. The rice-wheat system is the most 75 76 important cropping pattern in this region and considered to be the major determinant factor of the agriculture-based economy. However, the intensive cultivation practices overexploited the 77 natural soil resource base, which was further enhanced by the imbalanced use of inputs [22, 26]. 78

The deficiency of B and Zn in soils of different agro-climatic zones isnot rare and *Terai* region is not an exception-of that in this regard. Deficiency of different micronutrients has been reported from this region. Among the most prevalent ones, the deficiency of Zn is estimated to be the highest [27, 28]. Incidence of B deficiency from the areas of West Bengal and Bihar has also been reported [26]. Comprehensive study on the effect as well as the interaction of these nutrients on the production of wheat at this part of the world would help understanding constraitns of cultivation and decreasing the yield gap to secure food for the future.

Based on the above perspectives the present study was undertaken in the *Terai* region of
West Bengal on the following objectives:

- To assess the effect of Zn and B on the yield of wheat.
- 90 To evaluate the residual status of Zn and B in soil at different stages of wheat crop.
- 91 Materials and Methods
- 92 Experimental site:

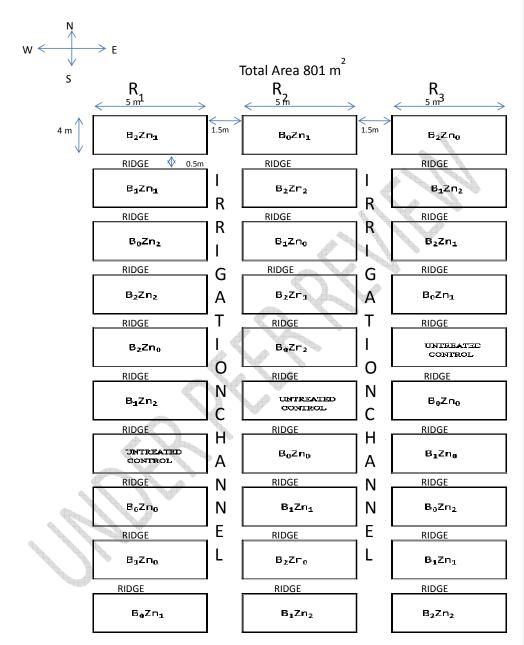
93 field experiment wascarried at the agricultural farm of Uttar А out BangaKrishiViswavidyalaya,Pundibari, Cooch Behar, West Bengal, India. The farm is located 94 within the Terai Agro-climatic zone and its geographic location is 26°19'86" N latitude and 95 89°23'53" E longitude. The elevation of the farm is 43 meters above the mean sea level. The field 96 experiment wascarried outin the same field during the winter season of 2010-11 and 2011-12. 97

98 Experimental plots:

The local topography of the study area is almost flat with good drainage facilities. The soil of the experimental site is sandy loam in texture. Before laying out the experimental plots, a set of surface soil samples were collected over the whole experimental area, composite together and tested in the laboratory following the methods described in the followingsub-section. The measured physical, chemical and physico-chemical properties were used as the baseline measurement for the experimental plots (Table 1).

105 Table 1: Initial characteristics of experimental soil for two years

Characteristics	Measur	ements
	2011	2012
рН	5	5
EC (dS/m)	0.05	0.05
Organic Carbon (%)	1.04	1.02
Nitrogen (kg/ha)	206.98	188.16
Phosphorus (kg/ha)	0.76	0.89
Potassium (kg/ha)	89.60	88.48
Boron (kg/ha)	0.68	0.62
Zinc (kg/ha)	0.73	0.84



109 Fig. 1: Layout of experimental plots for the field experiment. Same layout was used for

¹¹⁰ both years.

A set of 30 experimental plots (5 m × 4 m) were laid out following randomized complete 111 block design (RCBD) for this experiment (Fig. 1). Ten treatment combinations (Table 2) were 112 developed following three doses of B (0 kg/ha, 5 kg/ha and 10 kg/ha), three doses of Zn (0 kg/ha, 113 12.5 kg Zn sulphate/ ha and 25 kg Zn sulphate/ ha) and a treatment without application of any 114 115 nutrients (T10). Though the treatment T1 (B0Zn0) received recommended doses of N, P, and K, treatment T10 (control) did not receive any nutrient or micronutrients (B and Zn). The treatments 116 were replicated three times in this field experiment (Fig. 1). 117

Table 2: Details on the experimental plots and treatment combinations

Experimental details			
Crop	:	Wheat (Triticumaestivum	L.)
Variety	:	NW 1014	
Experimental design	:	Randomized Complete Ble	ock Design
Total Area	:	801m ²	
Plot size	:	$5 \text{ m} \times 4 \text{ m}$	
Number of replication	:	3	
Spacing	:	23 cm (Row to Row)	
Treatments	:	$T_1-B_0Zn_0, T_2-B_0Zn_1, T_3-B_0$	$Zn_2, T_4-B_1Zn_0, T_5-B_1Zn_1, T_6-B_1Zn_2,$
		$T_7-B_2Zn_0, T_8-B_2Zn_1, T_9-B_2$	$_2$ Zn ₂ , T ₁₀ - Control.
		B_0 = without Boron	Zn_0 = without Zinc Sulphate
		$B_1 = 5 \text{ kg/ha of Boron}$	$Zn_1 = 12.5 \text{ kg/ha of Zinc Sulphate}$
		$B_2 = 10$ kg/ha of Boron	$Zn_2 = 25$ kg/ha of Zinc Sulphate

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Field operations: 120

The land preparation for this experiment was started with a deep ploughing (21 and 22, 121 December 2010 and 12 and 13 December 2012) using a tractor. A laddering (similar to levelling 122 of soil surface) was performed after a day of soil drying following two secondary tillage using a 123 124 power tiller in order to prepare a good soil tilth. The weeds and stubbles were removed by hand picking and the final laddering was performed to prepare the seed bed. Bunds and channels were 125 prepared manually to prepare the experimental plots following the specifications mentioned in 126 Table 1. Nitrogen (N, 100 kg/ha), Phosphorus (P, 60 kg/ha) and Potassium (K, 30 kg/ha), in the 127 form of Urea, single super phosphate, muriate of potash; B as Borax(10 kg/ha, sodium borate), 128 129 and Zn as Zn Sulphate (25 kg/ha) were applied to the soil as per the treatments.Full dose of P andK and half of the recommended dose of N and full dose of B, Zn were surface applied as 130

Comment [MA2]: Is it necessary to write the elements' names in capital letters? If you decide to, then Borate and Potash should surely also be in capital letters?

basal dose and incorporated in the soil. The remaining half of the recommended dose of N was 131 applied as top dressing at 21 days after sowing (DAS), after completion of the first weeding. 132 The wheat variety of NW-104 was used for this experiment @100 kg ha⁻¹¹. Sowing was 133 completed in rows (spacing 23 cm) in North-South using a duck-foot type at a depth of 2.5 to 3 134 135 cm. Two weeding operations were performed manually on 21 DAS and 45 DAS. Two irrigations were applied on 21 DAS (after weeding and fertilizer application) and 65 DAS. The excess water 136 137 was drained out using drainage channels. The soil and plant samples were collected for laboratory analysis on 21 (CRI- crown root 138 initiation stage), 55 (tillering stage), 70 (booting stage) and 110 DAS (maturity). Leaving the 139 border rows, half of the area in each plot was marked for recording biometrical observation 140 including destructive plant sampling and other half for recording yield components and yield of 141 wheat. The height (from ground level) of five randomly selected plants were recorded and 142 averaged from each plot. The measured plants were tagged after first measurement for 143 subsequent measurements. Dry weight of both roots and shoots were also recorded. The number 144 of tillers per m² was recorded from 10 randomly selected plants. The crop was harvested from 145 net plot area discarding the border row. The number of panicles per plant was recorded from 10 146 randomly selected plants and converted to number of panicles per m². Length of panicles was 147 measured prior to harvest and average length was calculated. Number of grains per panicle as 148 well as - 1000 grain dry weight was- were also recorded for each treatment. The final yield of 149 wheat and straw was recorded after sun drying and thrashing. The yields were recorded and 150 calculated as tonne per ha following, 151 Grain yield (t ha⁻¹)= (Plot yield (kg) \times 10000 / Plot size (m²) \times 1000) 152 153 **Experimental methods:** Collected soil and plant samples were tested in laboratory following the methods described 154 below. 155

- a) Soil pH:pH of soil samples (soil:water 1:2.5) was determined in suspensions using a
 Systronics glass electrode-pH meter [29].
- b) Soil Electrical Conductivity (EC):Electrical Conductivity was measured in soil-water
 suspensions (soil:water 1:2.5)[29] using a digital conductivity meter of Systronics
 (Model No. 304).

Comment [MA3]: kg ha⁻¹ or kg/ha? You use both. Stick to one. See page 6 for example.

- c) Soil Organic Carbon (OC): Organic carbon content of samples was estimated by
 Walkely Walkley and Black's titration method [30].
- d) Mechanical Analysis of soils: Clay-content of soils was determined by the hydrometer
 method [31]. The texture of the soils was also ascertained from the particle-size
 distribution of sand, silt and clay.
- e) Available nitrogen (N): Available nitrogen in soil and plant was determined by alkaline
 KMnO₄ method developed by Subbiah and Asija[32].
- f) Available phosphorus (P): Available P in soil and plant content was determined by
 extracting the soil with a mixture of 0.03 M NH₄F and 0.025 M HCl[33] followed by
 colorimetric measurement using spectrophotometer (Systronics Model No. 167) [34].
- g) Available potassium (K): Available K concentration in soil and plantwas measured
 using a flame photometer (Systronics Model No128) [34]. The extraction was carried
 out with neutral normal ammonium acetate.
- h) Available Zinc (Zn): DTPA-(Diethylenetriaminepentaacetic acid) extractable Zn⁺² of s soil and plant samples weredetermined by extraction with the extractant containing 0.005M DTPA, 0.01M CaCl₂ and 0.1M Triethanol amine buffered at pH 7.3 [35] followed by the measurement using Atomic Absorption Spectrophotometer.
- i) Available Boron (B): Available Boron in soil and plant was extracted by boiling a known amount of soil with double distilled water (in 1:2.5 ratio) prepared by quartz glass distillation apparatus, for five minutes under a reflux condenser, followed by cooling and filtration [36]. The concentration was measured using Atomic Absorption Spectrophotometer.

The significant difference between the treatments was tested using ANOVA and LSD. The

between the yield components and nutrient uptakes were also calculated. The figures were

interaction between the effect of B and Zn was tested using two-way ANOVA. The correlation

Statistical analysis for the collected data was performed in SigmaPlot (Systat Software Inc.).

183 **Statistical** analyseis

prepared using the SigmaPlot. **Results and Discussion**

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190 191 **Comment [MA4]:** Surely there was more than one analysis? Then analyses.

was observed among the treatment combinations on yield components and grain yield of wheat.

The yield components and grain yield of wheat are shown in Table 3. A significant difference

The maximum mean grain yield (4.38 t/ha) was observed in the treatment T_6 (B₁Zn₂), while 192 minimum was observed in the control (1.68 t/ha) (Table 3). Relatively higher yield was obtained 193 from the treatments T_1 (B₀Zn₀) to T_9 (B₂Zn₂) over that of the control (T₁₀). The lowest harvest 194 195 index was observed in T₄ (B₁Zn₀) and the highest in controls (T₁₀) (Table 3). The application of 196 B and Z in combination significantly (p < 0.05) increased the grain yield of wheat. The grain yield increase with B and Zn addition was reported by Chaudryet al. [37]. Boron concentration 197 198 has been reported to increase grain yield of durum wheat by 16% [38]. This may be due to the requirement of B in wheat during the vegetative stage leading to high response to the grain yield 199 [39, 40]. Therefore, even a small amount of Zn and B directly affected the grain yield. Mandal 200 [41]reported a direct relationship between the number of grains and tillers and the wheat yield 201 202 under B deficient soils of Terairegion of West Bengal.

The effect of B on the grain and straw yield found was found to be significant at alpha = 0.05203 (95% significant level). However, the scenario was little different for Zn. For example, for no B 204 application, Zn had <u>not</u> any effect on crop yield.-w while with regular dose of B (5 kg B/ha), the 205 206 yield increased linearly. This indicated that with a regular application of B, the efficiency of Zn increased with the application rate considered in this study. However, with excess (more than 207 regular) application of B, the effect of Zn decreases indicating antagonistic effect between the 208 micronutrient at high dose, specifically the high dose of B.The two-way ANOVA following a 209 general linear model with alpha = $0.05 \frac{\text{found-showed}}{\text{showed}}$ a significant interaction between the effect 210 211 of B and Zn on the grain and straw yield of wheat. This means that the difference in the mean 212 values among the different levels of B and Zn is great enough to exclude the possibility that the 213 difference is just not due to random sampling variability after allowing for the effects of differences in Zn and B, respectively. Therefore, care should be taken in deciding the amount of 214 215 micronutrient application, which may have different effect. From this study, it could be suggested to choose a regular dose of B for better efficiency of Zn. Sometime a high dose of Zn 216 could be even beneficial with a controlled application of B. 217

In spite of the highest dry biomass production until the booting stage in T_6 (B₁Zn₂), the T_8 (B₂Zn₁) produced the highest dry straw at maturity(Fig. 2). Combination of B and Zn might have boosted the vegetative growth during the early stage, while the high amount of Zn along with a regular dose of B improved the yield and yield components of wheat at maturity [14, 15]. A combination of regular dose of Zn and B (T_5) could not produce high amount of straw compared

Comment [MA5]: Format: t/ha or t ha⁻¹

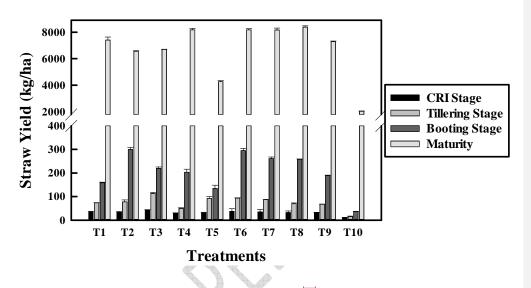
Comment [MA6]: Not sure: was found to be significant or was found to be not significant?

Comment [MA7]: format

- to other treatments with single or double dose of either Zn or B or in combination (Fig. 2). For
- example high straw yield with very little difference was observed among treatments T_4 (B₁Zn₀),
- 225 $T_6(B_1Zn_2)$, $T_7(B_2Zn_0)$ and $T_8(B_2Zn_1)$. The lowest biomass production was recorded in control

226 (T_{10}) at all stages of crop growth.

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Fig. 2:Effect of treatments on average straw yield (kg/ha) over two years at different stages of
wheat growth. The standard deviation of measurement is shown as error bars. The CRI stage
indicates crown root initiation.

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234 A significant difference in the nutrient uptake was recorded in different treatments and at 235 different growth stages. The highest uptake (kg/ha) of N over the entire growth period was recorded in treatment T₆(B₁Zn₂) and minimum in T₁₀ (control) (Fig. 3). The maximum amount of 236 237 N uptake at different growth stages was not consistent. For example, T_2 (B₀Zn₁) was recorded 238 with the highest amount of N uptake during booting stage (Fig. 3). While the highest amount of 239 P uptake was recorded in T7 (B2Zn0), the highest amount of K uptake was recorded in T8 (B_2Zn_1) (Fig. 3). Similar to N uptake, a variable amount of P and K uptake was also recorded at 240 241 different growth stages in different treatments. The highest amount of B and Zn uptake was Comment [MA8]: format

Comment [MA9]: format

242	recorded in treatment T ₉ (B	B_2Zn_2) (Fig. 3). High a	amount of B and Zn	application might show
242	recorded in deathent 19 (L	⁷ ² <i>L</i> ¹¹ ²) (1 ¹ g. 5). 111gii (amount of D and Zh	application might show

243	some	synergistic
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Comment [MA10]: Layout problem?

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Treatments		-			-				veight		A111. 101	1010h 010h	Harv	est Inde	ex (%)
	2011	2012	Mean	2011	2012	Mean	2011	2012	Mean	2011	2012	Mean	2011	2012	Mean
			(S.D.)			(S.D.)			(S.D.)			(S.D.)			(S.D.)
T1	171	168	169.5	37	36	36.5	45.72	45.75	45.74	2.89	2.77	2.83	27.7	27.6	27.6
			(2.12)			(0.70)			(0.02)	11		(0.08)			(0.07)
T2	156	156	156.0	41	42		42.74	42.80	ALC: 10000	2.73	2.80	2.77			29.7
			(0)			(0.70)			(0.04)		•	(0.05)			(0.21)
T3	165	162	163.5	39	39	39.0	45.92	45.72		2.95	2.89	2.92	30.6	30.1	
			(2.12)			(0)			(0.14)			(0.04)			(0.35)
T4	143	145	143.8	43	43		44.54	44.00			2.74		25.2	24.9	25.1
			(1.41)			(0)			(0.38)			(0.01)			(0.21)
T5	158	150	153.8	42	42		51.50	51.51		3.41	3.25	3.33	44.1	43.5	43.8
			(5.66)			(0)		\sim	(0.01)			(0.11)			(0.42)
T6	188	185	186.3	48	51		47.64	47.34	47.49	4.29	4.47	4.38			34.9
			(2.12)			(2.12)			(0.21)			(0.13)			(0.42)
T7	176	174	174.8	50	53		43.80			3.84	4.04		32.3	32.8	32.6
			(1.41)			(2.12)			(0.02)			(0.14)			(0.35)
T8	134	137	135.3	45	46	45.5	44.74	44.20		2.69	2.79	2.74	24.4	24.8	24.6
			(2.12)	\mathbb{R}		(0.70)			(0.38)			(0.07)			(0.28)
T9	143	142	142.3	52	50		41.38	39.38		3.07	2.80		29.7	27.6	28.7
			(0.71)			(1.41)			(1.41)			(0.19)			(1.48)
T10	122	124	122.8	36	39	37.5	37.00	36.00	36.50	1.62	1.74	1.68	44.1	46.4	45.2
			(1.41)			(2.12)			(0.71)			(0.08)			(1.63)
					w.										
	A														

Table 3: Effect of treatments on the yield components and grain yield (t/ha) of wheat. The data from 2011 and 2012 are shown along
with the average over two years. S.D. stands for standard deviation.

average 0	ver two y	cars. S.D	. stanus re	JI Stanua		ation.									
Nitrogen			Phosphorus H			Potassium Boron					Zinc				
Treatments			Mean			Mean			Mean	A.	141	Mean			Mea
	2011	2012	(S.D.)	2011	2012	(S.D.)	2011	2012	(S.D.)	2011	2012	(S.D.)	2011	2012	(S.D
T1	70.47	59.66	65.06	1.40	1.74	1.57	23.14	24.90	24.02	0.280	0.255	0.267	0.297	0.228	0.26
			(7.64)			(0.24)			(1.24)	L VP		(0.02)			(0.0
T2	58.17	58.89	58.53	1.58	1.50	1.54	19.14	22.43	20.78	0.249	0.253	0.251	0.314	0.168	0.24
			(0.51)			(0.06)		A	(2.33)		V	(0.01)			(0.1
T3	64.54	59.85	62.19	1.40	1.24	1.32	19.21	21.66	20.44	0.292	0.271	0.282	0.406	0.361	0.38
			(3.32)			(0.11)			(1.73)			(0.02)			(0.0
T4	69.54	66.06	67.80	2.29	1.90	2.09	25.93	27.43	26.68	0.211	0.239	0.225	0.300	0.645	0.4
			(2.46)			(0.28)			(1.06)			(0.02)			(0.2
T5	83.94	72.69	78.32	2.25	1.88	2.07	18.74	21.09	19.92	0.237	0.289	0.263	0.554	0.292	0.42
			(7.95)			(0.26)	\checkmark L	4	(1.66)			(0.04)			(0.1
T6	121.25	116.31	118.78	2.61	2.46	2.54	38.59	44.67	41.63	0.231	0.385	0.308	0.482	0.402	0.44
			(3.49)			(0.08)			(4.30)			(0.11)			(0.0)
T7	104.39	101.86	103.12	2.53	2.41	2.47(0.11)	28.83	36.38	32.60	0.215	0.246	0.230	0.413	0.333	0.3
			(1.79)						(5.34)			(0.02)			(0.0
T8	63.97	70.97	67.47	1.20	1.11		18.81	30.64	24.73	0.190	0.232	0.211	0.255	0.195	0.22
			(4.50)		P	(0.06)			(8/36)			(0.03)			(0.0)
T9	77.27	72.02	74.65	1.77	1.47	1.62	18.40	26.56	22.48	0.171	0.197	0.184	0.284	0.175	0.22
	10.1.1		(3.71)			(0.21)			(5.77)	0.0.61	0.0.60	(0.02)	0.000	0.047	(0.0
T10	13.14	9.75	11.45	0.76	0.79	0.77	7.28	9.58	8.43	0.061	0.060	0.060	0.020	0.065	0.04
			(2.40)			(0.02)			(1.62)			(0.00)			(0.0

Table 4:Effect of treatments on the uptake of nutrients (kg/ha) by seed. The data from 2011 and 2012 are shown along with the average over two years. S.D. stands for standard deviation.

255 effect to provide higher amount of uptake. The treatment T_{10} (control) always recorded with the 256 least amount of nutrient uptake.

257 A significant difference was observed in the uptake of different nutrients by seed (Table 4). The highest uptake of almost all nutrients (N, P, K, and B) were was recorded in treatment T6 258 (B_1Zn_2) except for Zn, the highest uptake of which was recorded in treatment T_4 (B_1Zn_0) (Table 259 4). The highest production as well as the interaction between the micro-nutrients (B and Zn) in 260 treatment T₆ facilitated higher amount of nutrients uptake in seed[42]. The lowest uptake of all 261 262 nutrients was recorded in treatment T₁₀ (control). A similar trend was observed for the uptake of nutrients by straw (Table 5). 263

> Comment [MA11]: If the brackets refer to the average? Unclear.

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able 5:Effect of treatments on the uptake of nutrients (kg/ha) by straw along with average(in brackets) grain yield and straw yield over two years.

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Treatments	Grain yield	Straw yield	Nitrogen	Phosphorus	Potassium	Boron	Zinc
	(S.D.)	(S.D.)	(S.D.)	(S.D.)	(S.D.)	(S.D.)	(S.D.)
T1	2829.83	7410.68	60.26	0.34(0.01)	109.61	0.118	1.130
	(89.10)	(220.6)	(7.66)		(2.83)	(0.02)	(0.01)
T2	2768.95	6558.93	19.26	0.38(0.00)	107.67	0.252	2.134
	(49.49)	(45.6)	(6.36)		(2.34)	(0.03)	(1.60)
T3	2921.77	6702.67	51.61	0.21(0.03)	111.44	0.172	0.821
	(46.67)	(13.2)	(1.43)		(12.46)	(0.02)	(0.21)
T4	2736.29	8177.83	27.29	0.36(0.05)	131.95	0.296	1.723
	(9.89)	(107.7)	(28.78)		(14.75)	(0.26)	(0.71)
T5	3325.93	4268.08	25.67	0.21(0.00)	73.80	0.230	0.581
	(114.55)	(68.7)	(2.12)		(1.44)	(0.01)	(0.06)
T6	4377.06	8171.53	84.69	0.23(0.01)	141.31	0.477	1.756
	(126.57)	(84.7)	(7.35)		(3.87)	(0.03)	(2.11)
Τ7	3942.73	8160.33	20.36	0.40(0.07)	149.46	0.413	1.947
4	(140.71)	(146.6)	(22.25)		(13.66)	(0.00)	(2.34)
Τ8	2736.62	8376.87	34.00	0.33(0.00)	155.96	0.227	5.001
8 - F	(68.59)	(96.9)	(1.26)		(8.56)	(0.03)	(6.15)
Т9	2931.12	7288.23	25.52	0.17(0.00)	120.57	0.567	3.448
	(190.92)	(44.6)	(4.48)		(5.46)	(0.01)	(4.61)
T10	1679.67	2033.42	2.28	0.06(0.04)	30.75	0.049	0.246
	(86.97)	(28.2)	(0.03)		(0.89)	(0.00)	(0.31)

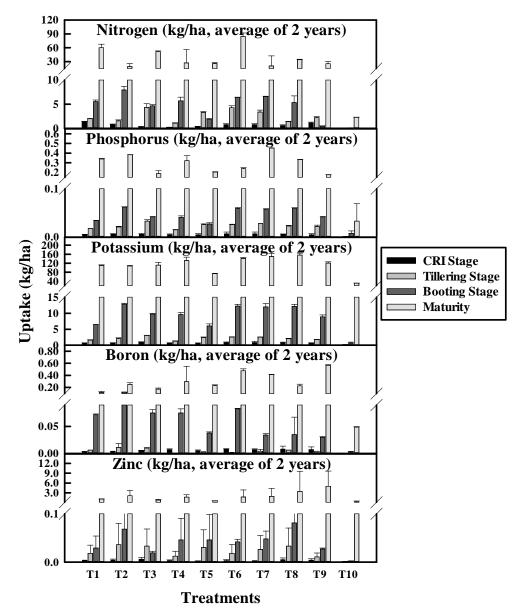


Fig. 3: Effect of treatments on the uptake of nutrients (N, P, K, B, and Zn) at different growth
stages (CRI, Tillering, Booting and Maturity) of wheat. The standard deviation of measurement is
shown as error bars. The CRI stage indicates crown root initiation.

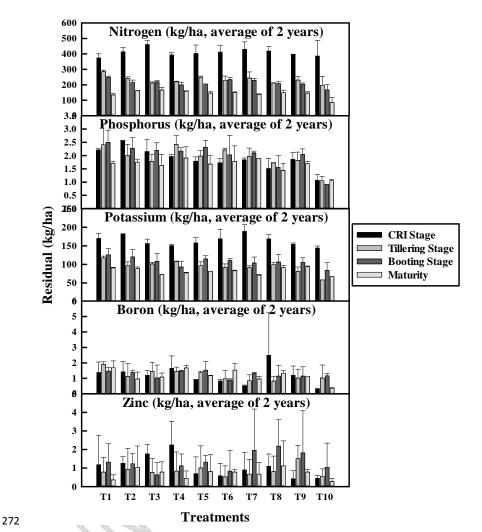


Fig. 4: Effect of treatments on the residual nutrient (N, P, K, B, and Zn) status at different
growth stages (CRI, Tillering, Booting and Maturity) of wheat. The standard deviation of
measurement is shown as error bars. The CRI stage indicates crown root initiation.

The B and Zn concentration in seeds (Table 4) and straw (Table 5) were calculated after dividing the total uptake of nutrients by the total grain and straw production. It clearly showed that with the increasing production, the concentration of nutrients, both B and Zn in seed and straw decreased.

There was significant difference in the residual N status of soil at different treatment plots 280 and at different growth stages. Initial application of N resulted a high amount of residual N at the 281 CRI stage and gradually decreased towards maturity, which had the least amount of residual N 282 (Fig. 4). Minimum demand of the applied N at the beginning of the growth stages resulted in a 283 284 high amount of residual N at the CRI stage, while the high demand towards maturity left the least amount of residual N. High demand during the peak growth stages such as tillering and booting 285 286 resulted in a very similar amount of residual N, which was lower than that at CRI stage. The highest amount of residual N was recorded in treatment T_3 (B₀Zn₂) and the least lowest amount 287 was recorded in treatment T₁₀ (control) (Fig. 4). The residual K status in soil at different growth 288 stages of wheat showed a very similar trend as that of N (Fig. 4). The CRI stage was recorded 289 with the highest amount of residual K, which in general decreased towards maturity. There was a 290 significant difference between the treatments at different growth stages. Treatment T_7 (B₂Zn₀) 291 was recorded with the highest of amount of residual K at the CRI stage, while Treatment T_1 292 293 (B_0Zn_0) was recorded with the highest amount of K at other growth stages (Fig. 4). The absence of micronutrients in treatment T₁ might have inhibited the uptake resulting in a high amount of 294 residual K. 295

The residual P in soil showed a little different trend than N and K (Fig. 4). There was no 296 specific trend of residual P at different growth stages. In general, a higher amount of residual P 297 298 was recorded at the CRI stage compared to tillering and booting stage. This might be due to the 299 presence of unavailable form of P at the beginning of the growth stage. While the difference 300 between the growth stages of wheat was not significant, the difference between the treatments 301 was significant. The highest amount of P was recorded in treatment T₂ (B₀Zn₁) for the CRI stage 302 while treatment T_4 (B₁Zn₀) at the maturity. The <u>least_lowest</u> amount of residual P was recorded in treatment T_{10} (control) (Fig. 4). 303

There was a significant difference in the residual B status in soil at different nutrient treatment combinations. However, the difference was not significant at different growth stages. There was no specific trend on the residual amount of B among the growth stages (Fig. 4). For example, while the treatment T_8 (B₂Zn₁) was recorded with the highest amount of residual B at the CRI stage, treatment T_1 (B₀Zn₀) was recorded with the highest amount of B at the tillering stage (Fig. 4). A similar trend in the residual Zn content was observed at different growth stages and at different treatments. For example, the highest amount of residual Zn was observed in Comment [MA12]: Least for something that cannot be measured/counted. Least for something measurable.

treatment T₄ (B₁Zn₀) at the CRI stage, while the highest amount of residual Zn was observed in 311

treatment T₈ (B₂Zn₁) at the booting stage (Fig. 4). A growth stage dependent Zn demand and the 312

residual Zn were also reported by Ozturk et al. [43]. The variation in the residual Zn might also 313

be due to the combined effect of pH, EC, organic carbon and P, which ultimately controls the Zn 314

availability [44]. A least The lowest amount of residual B and Zn was observed in treatment T10 315

(control). In general a lower amount of Zn was recorded at maturity, which indicates a demand 316

317 of Zn in the production of crop.

Table 6: Correlation (r) between soil available nutrient status at harvest and the nutrient content 318

319	in straw averaged over two years	
	Dlopt N	Dlo

		Plant N	Plant P	Plant K	Plant B	Plant Zn
	Soil N	0.41**	0.59**	0.66**	0.39**	0.33*
	Soil P	0.36**	0.63**	0.67**	0.60**	0.11
	Soil K	0.28*	0.39**	0.38**	0.31*	0.64**
	Soil B	0.68**	0.57**	0.63**	0.26*	0.25*
	Soil Zn	0.10	0.50**	0.48**	0.35	▲ 0.64**
*- p < 0.05, ** p	0 < 0.01				P V	

320 321

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Table 7: Correlation (r) between soil available nutrient status at harvest and the nutrient content 322

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323 in seed averaged over two years

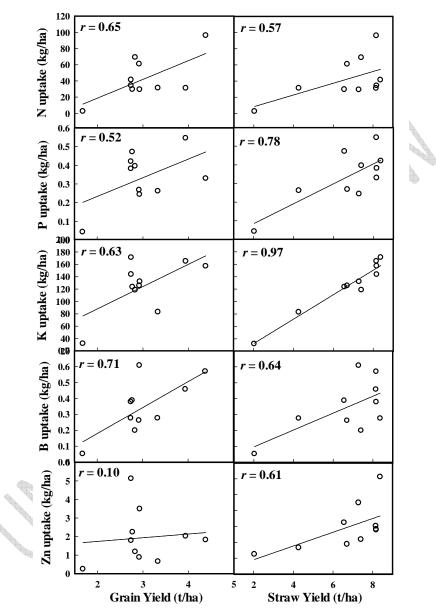
	Soil N	Soil P	Soil K	Soil B	Soil Zn
Seed N	0.54**	0.76**	0.23*	0.56**	0.37**
Seed P	0.40**	0.83**	-0.03	0.47**	0.07
Seed K	0.48**	0.71**	0.19	0.63**	0.25*
Seed B	0.80**	0.74**	0.32*	0.69**	0.41**
Seed Zn	0.70**	0.80**	-0.06	0.64**	0.15

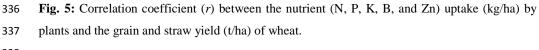
* p < 0.05, ** p < 0.01 A positive correlation was observed between the uptake of different nutrients and the grain 325 and straw yield irrespective of different treatments (Fig. 5). The correlation coefficient (r) was as 326 high as 0.97 between K uptake (kg/ha) and the straw yield (t/ha). There was a very weak 327 328 correlation between the Zn uptake and the grain yield (Fig. 5). The uptake of nutrients was governed by the soil, environmental and management practices. For example, the availability of 329 B was determined by the availability of Zn in soil [28]. Santra et al. [45] also reported an 330 increased amount of DTPA extractable Zn with the application of B. The relationship between B 331 332 and Zn was found to be synergistic making high amount of Zn available in soil. A high 333 correlation was also observed between the residual nutrient status in soil and the nutrient status is 334 straw(Table 6) or between residual status in soil and the nutrient status is seed (Table 7).

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339 Conclusions

This study examined the effect of Boron and Zinc on the yield and uptake of different 340 nutrients by wheat in the acid soil region of West Bengal, India. The yield components and grain 341 yield of wheat showed a significant difference among the treatment combinations. The maximum 342 343 average grain yield (4.38 t/ha) over two years was observed in the treatment T_6 with higher amount of Zn application along with recommended dose of Boron application. The minimum 344 grain yield was observed in treatment T_{10} (the control) (1.68 t/ha). A relatively higher yield was 345 obtained from the treatments with any nutrient combination over that of the control (T_{10}) . Along 346 with the difference in grain yield, a significant difference in straw yield was also observed 347 among the treatments. The application of Boron and Zinc might show some synergistic effects 348 leading to high grain and straw yield in the acid soil region. The presence of micro-nutrients and 349 their combination also affected the uptake of nutrients in different growth stages of wheat. The 350 interaction effect was also visible in the uptake nutrients by seeds. A positive correlation was 351 observed between the uptake of nutrients and the yield of grain and straw in this study region 352 353 with acid soils. The residual nutrient status showed a build-up of nutrients in soils.

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