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Zinc-Coated Urea for Enhanced Zinc Biofortification, Nitrogen Use Efficiency and Yield of *Basmati* Rice under *Typic Fluvents*

Ramesh Chand Bana [1,2], Ashok K. Gupta [1], Ram Swaroop Bana [2,*], Yashbir Singh Shivay [2], Shanti D. Bamboriya [2], Narendra P. Thakur [1], Ramphool Puniya [1], Meenakshi Gupta [1], Shish Ram Jakhar [3], Kailash [4], Raj Singh Choudhary [1], Ranjeet Singh Bochalya [1], Tejpal Bajaya [5], Vipin Kumar [2,6], Parshotam Kumar [1] and Anil K. Choudhary [2,7]

1 Department of Agronomy, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu 180 009, India; banajaitpura11@gmail.com (R.C.B.); ashokgupta1970@rediffmail.com (A.K.G.); npthakur08@gmail.com (N.P.T.); ramagro@gmail.com (R.P.); meenakg13@gmail.com (M.G.); rajsinghchoudhary88880@gmail.com (R.S.C.); rbacholya651@gmail.com (R.S.B.); rcbana@outlook.com (P.K.)
2 Division of Agronomy, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India; ysshivay@hotmail.com (Y.S.S.); sbamboriya93@gmail.com (S.D.B.); vipindudi56@gmail.com (V.K.); anilhpau2010@gmail.com (A.K.C.)
3 Department of Soil Science & Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur 482 004, India; 444sjakhar@gmail.com
4 Krishi Vigyan Kendra, Delhi 110 073, India; jakharkailash3112@gmail.com
5 Department of Agronomy, Sri Karan Narendra Agricultural University, Jobner 303 329, India; tejpalbajya93@gmail.com
6 Eastern Shore Agricultural Research and Extension Center, Virginia Tech, Blacksburg, VA 23420, USA
7 ICAR-Central Potato Research Institute, Shimla 171 001, India
* Correspondence: rsbana@gmail.com

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Citation: Bana, R.C.; Gupta, A.K.; Bana, R.S.; Shivay, Y.S.; Bamboriya, S.D.; Thakur, N.P.; Puniya, R.; Gupta, M.; Jakhar, S.R.; Kailash; et al. Zinc-Coated Urea for Enhanced Zinc Biofortification, Nitrogen Use Efficiency and Yield of *Basmati* Rice under *Typic Fluvents*. *Sustainability* **2022**, *14*, 104. https://doi.org/10.3390/su14010104

Academic Editors: Ram Swaroop Meena, Marc A. Rosen, Sandeep Kumar and Manoj Kumar Jhariya

Received: 1 November 2021
Accepted: 15 December 2021
Published: 23 December 2021

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Abstract: Deficiency of Zn in human diet is an emerging health issue in many developing countries across the globe. Agronomic Zn biofortification using diverse Zn fertilization options is being advised for enhancing Zn concentration in the edible portion of rice.A field study was carried out to find out the Zn fertilization effects on biofortification of *basmati* rice and nutrient use efficiencies in the Himalayan foothills region. Amongst the Zn nutrition treatments, 4.0% Zn-coated urea (ZnCU) + 0.2% Zn foliar spray (FS) using $ZnSO_4{\cdot}7H_2O$ recorded the highest grain (3.46 t/ha) and straw (7.93 t/ha) yield of *basmati* rice. On average, the rice productivity increase due to ZnCU application was ~25.4% over Commercial Urea. Likewise, the same Zn fertilization treatment also resulted in the maximum Zn (35.93 and 81.64 mg/kg) and N (1.19 and 0.45%) concentration in grain and straw of rice, respectively. Moreover, N use efficiency (NUE) was also highest when ZnCU was applied at 4.0% ($ZnSO_4{\cdot}7H_2O$) in comparison to soil application. From the grain quality viewpoint, Zn ferti-fortification had significant effect on elongation ratio and protein concentration of grain only and respective Zn fertilization treatment recorded highest quality parameters 1.90 and 7.44%, respectively. Therefore, ZnCU would be an important low-cost and useful strategy for enhancing yield, NUE and biofortification, and also in minimizing the Zn malnutrition related challenges in human diet in many developing economies.

Keywords: biofortification; protein content; rice grain quality; rice yield; zinc nutrition

1. Introduction

Globally, almost half of the humanity subsists, partially or wholly, on rice (*Oryza sativa* L.) crop and 90% of it is being cultivated and consumed in Asia [1–3]. Similarly, the crop also contributes to ~21% of the world's energy and protein requirements [4]. However, unfortunately, rice grains are inherently poor in both protein and Zn content and their bioavailability is low as well [5,6]. Rice products contribute ~70% to the per day calorie intake of the rural world, leading to Zn malnutrition mainly owing to low Zn content in rice grains. Nearly one-third of global humans (2.7 billion people) are facing the Zn malnutrition challenge severely [7], ranging from 4 to 73% of the population in different nations [8] and ~90% of these are inhabitants of Africa and Asia [9]. Like other developing countries, the prevalence of Zn deficiency in India is more common in rural populations. Today, Zn deficiency has emerged a serious challenge for nutritional security in both humans and plants [10]. A WHO report highlights that Zn deficiency is the 11th major risk factor responsible for the development of illnesses, diseases and death on the entire planet and 5th among the 10 major factors of risk in the emerging economies [11,12]. An inadequate supply of Zn has caused severe human-health-related complexities such aschildhood dwarfism, abnormal brain development, increased susceptibility to various infectious diseases, low learning ability, DNA damage, reduced physical work productivity and pregnancy related ailments [13–17]. For the entire life cycle, beginning with gametogenesis and up to old age, Zn is required continuously for normal human growth, development and metabolic activities [18,19]. Around 10% of all the human body proteins are Zndependent. Therefore, the research thrust on enhancing grain Zn concentrations in rice is obligatory to overcome Zn-related malnutrition, particularly in the developing world [20,21].

Nearly 50% soils in the world, mostly in majority of rice growing belts, are deficient in available Zn [22,23] and this leads to poor crop productivity levels [21,24,25], lower nutritional quality of rice grain [6,25,26] and consequently results in reduced nutritional value of human diet [22]. Based on the above facts, it has been hypothesized that Zn malnutrition in humans is linked to the soil Zn content and the relative intake of cereals in diet [27]. In India, among deficient micronutrients, Zn is now considered as 4th nutrient which limits the yield, after N, P and K [28]. Presently, 48% of the Indian soils are Zn deficient and it is expected to grow up to 63% by the year 2025 [29]. Low Zn availability is a serious problem in soils of developing Asian, African and several other countries [12,30] and good response of rice crop to Zn application has been reported by many researchers in India [30,31], Philippines [32] and China [33]. On the other hand, poor N use efficiency and loss of fertilizer N through leaching, denitrification and volatilization is another severe problem in rice production [34,35], specially under puddled transplanted rice systems. To reduce N losses in diverse rice ecologies, coated urea materials were found effective in various studies [21] across diverse agroclimatic scenarios.

To tackle the twin challenges of poor N use efficiency and low Zn concentration in rice grains, innovative zinc coated urea (ZnCU) could be a great N and Zn management option. Coating of Zn on commercial urea (CU) alters its release pattern [35,36] and minimizes the N losses due to leaching. The Zn applied as coating material, supplements the Zn requirements of the rice crop simultaneously [21,36] and consequently leads to Zn biofortification. There are several reports that to overcome the problem of Zn malnutrition, biofortification via agronomic practices is a promising strategy [6,37,38], as post-harvest biofortification of grains with micronutrients is quite expensive and time consuming [39]. The ZnCU is considered to be a potential alternative for combined application of N and Zn fertilizer [36]. In the past, few field studies have reported positive effects of ZnCU on *basmati* rice productivity and Zn biofortification at lower Trans Gangetic Plains [21,23,40]. However, not much information is available on ZnCU effects on N use efficiency of *basmati* rice, specifically under soil application of ZnCU, and correlation of N and Zn concentration in grain with *basmati* rice yields. Likewise, information is lacking on ZnCU on enhancing the productivity, grain biofortification, and nutrient use efficiencies of *basmati* rice under hills ecologies. Our efforts were thus focused towards assessing the effect of ZnCU under soil application as well as foliar fertilization on Zn biofortification, N use efficiency and yield enhancement of *basmati* rice under western Himalayas.

2. Materials and Methods

2.1. Experimental Site

The experiment was carried out the Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-J), Jammu and Kashmir, India, during *kharif* season 2015-2016 and 2016-2017. The studied site's geographic coordinates are latitude 32°40′ N and longitude 74°58′ E (Supplementary Figure S1), and is situated in the north-western foothills of the Himalayan Mountain arc at an altitude of 332 m. The region's climate is classified as sub-tropical cold desert zone [41] with severe cold winters, hot and humid summers [42], with average (mean of last 30 years) annual rainfall of 1050–1115 mm. The weather data for the entire cropping season obtained from the SKUAST-J Chatha meteorological observatory (Supplementary Figure S2).

The experimental field soils belong to the soil order *Inceptisol*, typic fluvents [43] and the textural class was sandy loam. Soil samples from 0–50 and 50–150 mm deep layers were collected from the experimental field prior to experimentation using core sampler and later, the analysis for various physico-chemical properties was carried out. The details of the initial soil properties of the experimental site are presented in Table 1.

Table 1. Initial physico-chemical properties of experimental site.

S. No.	Parameters	Value	Methods Employed
A	**Mechanical Properties**		
(i)	Sand (%)	61.8	Bouyoucous Hydrometer method [44]
(ii)	Silt (%)	14.0	
(iii)	Clay (%)	24.3	
	Textural Class	Sandy loam	
B	**Chemical Properties**		
(i)	pH	7.85	1:2.5 Soil water suspension measured with Glass electrode pH [45]
(ii)	EC (dS/m)	0.18	1:2.5 Soil water suspension measured with Systronics conductivity meter [45]
(iii)	Organic Carbon (g/kg soil)	4.3	Walkley and Black method [46]
(iv)	Available N (kg/ha)	240.3	Alkaline Potassium Permagnate method [47]
(v)	Available P (kg/ha)	12.8	Sodium Bicarbonate method [48]
(vi)	Available K (kg/ha)	143.6	Ammonium acetate method [45]
(vii)	Available Zn (ppm)	0.56	DTPA-extractable [49]

2.2. *Experimentation and Crop Husbandry*

This present study was designed in randomized block design with eleven treatment combinations of two coating materials and foliar formulation with three replications (Table 2). Different Zn coated urea materials were prepared just before the transplanting of rice as per the protocols described by Pooniya et al. [50].

Transplanting of 4-week old rice seedlings of variety "*Basmati*-370" was done in the puddled field with a crop geometry of 20×10 cm in first fortnight of July during both the experimentation seasons. Disk-ploughing was done twice followed by three-times puddling in partially ponded conditions. For nutrient management, 8.75 kg P/ha and 8.3 kg K/ha were applied using single super phosphate and muriate of potash, respectively, after last puddling. N doses of 30 kg/ha as ZnCU or CU, as per treatment, were applied in two equal splits at transplanting and panicle-initiation stage, whereas 0.2% Zn foliar sprays (ZnFS) (ZnO as well as $ZnSO_4 \cdot 7H_2O$) were done at the panicle-emergence stage. Rice was cultivated as per standard agronomic package of practices.

Table 2. Details of experimental treatments.

Treatment Abbreviations	Treatment Details	Amount of Zn Applied (kg/ha)
CU (control)	Commercial urea (only N)	0
2.0% ZnCU (ZnO)	2% Zn coated urea through ZnO	1.3
2.0% ZnCU ($ZnSO_4{\cdot}7H_2O$)	2% Zn coated urea through $ZnSO_4{\cdot}7H_2O$	1.3
4.0% ZnCU (ZnO)	4% Zn coated urea through ZnO	2.6
4.0% ZnCU ($ZnSO_4{\cdot}7H_2O$)	4% Zn coated urea through $ZnSO_4{\cdot}7H_2O$	2.6
0.2% ZnFS (ZnO)	0.2% Zn foliar spray (ZnO)	1.2
0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$)	0.2% Zn foliar spray ($ZnSO_4{\cdot}7H_2O$)	1.2
2.0% ZnCU + 0.2% ZnFS (ZnO)	2% Zn coated urea + 0.2% Zn foliar spray through ZnO	2.5
2.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$)	2% Zn coated urea + 0.2% Zn foliar spray through $ZnSO_4{\cdot}7H_2O$	2.5
4.0% ZnCU + 0.2% ZnFS (ZnO)	4% Zn coated urea + 0.2% Zn foliar spray through ZnO	3.8
4.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$)	4% Zn coated urea + 0.2% Zn foliar spray through $ZnSO_4{\cdot}7H_2O$	3.8

2.3. Measurement and Sampling

2.3.1. Yield Performance

The treatment plots were harvested manually. Sun-dried samples were manually threshed and, the grain yield was recorded after removing border rows. Straw yield was obtained by deducting the grain weight from the biomass yield.

2.3.2. Chemical Analysis of Plant Samples

Basmati rice grain and straw samples collected after harvest from all the field plots were oven-dried at 60 ± 2°C for 72 h and then ground in a Willey Mill and sieved through a 1 mm sieve. Zn concentration (mg/kg) in grain and straw samples was determined by a di–acid digestion method ($HClO_4$ + HNO_3 in 3:10 ratio) using atomic absorption spectrophotometry [30]. The N concentrations (%) in these plant parts were estimated by the Micro-Kjeldahl method [51]. Thereafter, the Zn (expressed as g/ha) or N (expressed as kg/ha) uptake were calculated by multiplying their respective concentrations by the weights of grain and straw. Total Zn or N uptake was computed by summing the uptake by grain as well as straw.

2.3.3. Nutrient Use Efficiencies

Various nutrient-use efficiencies/indices [partial factor productivity (PFP), agronomic efficiency (AE), recovery efficiency (RE), physiological efficiency (PE), harvest index (HI) and mobilization efficiency index (MEI)] of applied Zn and N were calculated using the equations given below, refs [21,52,53] for Equations (1)–(3); refs [21,52,54] for Equations (4) and (5); refs [54,55] for Equation (6):

$$PFP = Y_t / Zn_a \text{ or } N_a \tag{1}$$

$$AE = (Y_t - Y_c) / Zn_a \text{ or } N_a \tag{2}$$

$$RE = \{(U_{Zn \text{ or } N} - U_c) / Zn_a \text{ or } N_a\} \times 100 \tag{3}$$

$$PE = (Y_t - Y_c) / (U_{Zn \text{ or } N} - U_c) \tag{4}$$

$$HI = GU_{Zn \text{ or } N} / U_{Zn \text{ or } N} \tag{5}$$

$$MEI = GC_{Zn \text{ or } N} / SC_{Zn \text{ or } N} \tag{6}$$

where in Y_t and $U_{Zn \text{ or } N}$ refer to the yield of rice (kg/ha) and total uptake of Zn/N (kg/ha), respectively, in the treated plots; Y_c and U_c refer to yield (kg/ha) and total uptake of Zn/N (kg/ha), respectively, of *basmati* rice in control plots; Zn_a or N_a refers to the Zn/N applied (kg/ha); GU_{Zn} or GU_N represents Zn/N uptake (kg/ha) in grain, respectively; GC_{Zn} or GC_N refers to Zn (mg/kg) or N (%) concentration in grain, respectively, and SC_{Zn} or SC_N refers to Zn (mg/kg) or N (%) concentration in straw.

2.4. Qualitative Studies

2.4.1. Morphological Properties of Milled Rice

Length and breadth of milled rice grain were measured using a vernier caliper. The measurements were repeated 10times in each sample and the mean length and breadth of 10 grains were expressed in millimetres [56]. Length/breadth ratio was calculated by dividing the grain length by grain breadth [57]. Head rice recovery was computed by using the following equation, ref [26] for Equation (7):

$$Head\ rice\ recovery\ (\%) = \left(\frac{Weight\ of\ whole\ milled\ grains\ (g)}{Weight\ of\ paddy\ (g)}\right) \times 100 \quad (7)$$

2.4.2. Chemical Properties of Milled Rice

Protein content of rice grain was estimated by micro Kjeldahl digestion to determine N concentration, which is then converted to protein content by multiplying respective N concentration in rice grain with a factor 5.75 [58]. Amylose content was determined by the procedure described by Williams et al. [59]. Ten whole milled rice-grains were placed evenly in a 5 cm-wide Petri plate containing a 10 ml solution of 1.7% potassium hydroxide. The Petri-plates were then covered and were incubated by maintaining 30 $\pm$ 1 °C temperature for 16 h. After removal of the samples from the incubator, the gelatinization temperature was scored on a 7-point numerical scale as suggested by Jennings et al. [60].

2.4.3. Cooking Properties of Milled Rice

The grain length expansion ratio was calculated using the following expression, refs [21,24] for Equation (8):

$$Elongation\ ratio\ (ER) = L_1/L_2 \quad (8)$$

where L_1 and L_2 are grain length after and before cooking, respectively.

2.4.4. Statistical Analysis

The data obtained from the field experiment and laboratory analyses were statistically analyzed through the F-test as per the procedure described by Gomez and Gomez [61]. The least significant difference (LSD) values (at $p = 0.05$) were used to determine the significance of difference between treatment means. Correlation analyses and treatment means were compared at 5% level of significance.

3. Result

3.1. Yield Performance

Application of ZnCU along with ZnFS significantly increased ($p < 0.05$) the *basmati* rice grain yield over CU, and the highest grain yield (3.46 t/ha) was recorded with 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$), which was significantly superior over ZnFS, 2.0% ZnCU (ZnO), 4.0% ZnCU (ZnO) and CU, and at par with remaining Zn fertilization treatments (Figure 1). The Zn application via foliar spray or ZnCU was found to be equally effective.Further, yield was low in case of ZnO application either in ZnCU or ZnFS compared to $ZnSO_4 \cdot 7H_2O$ application. Among the Zn fertilization treatments, the least grain yield (2.76 t/ha) of *basmati* rice was recorded under CU. Straw yield also followed almost the same pattern. Strong correlations of 0.85 and 0.86 wereobserved between rice productivity and Zn and N concentrations in grain, respectively (Figure 2).

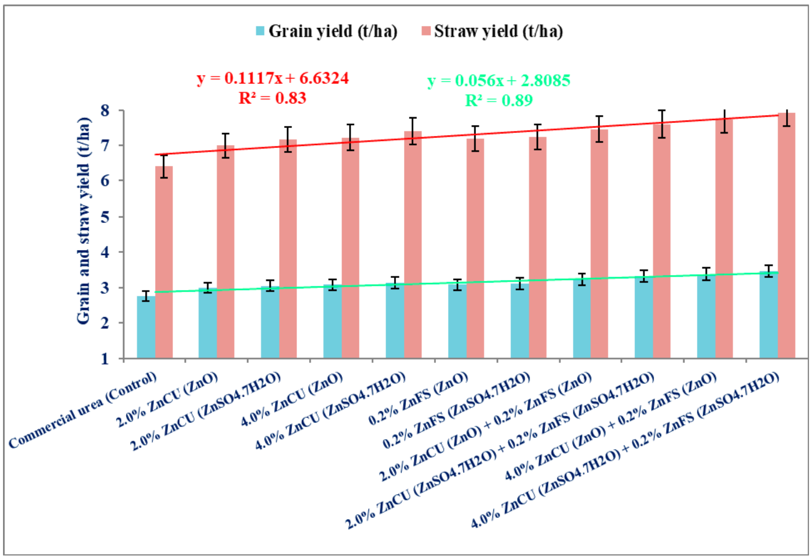
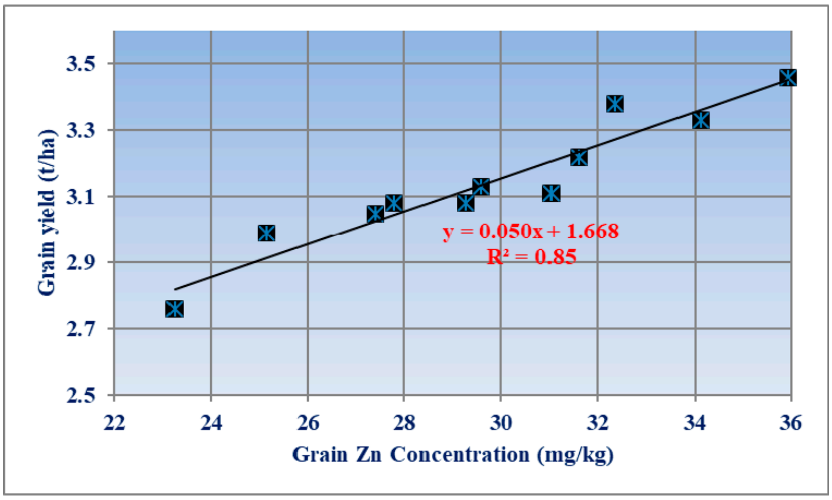
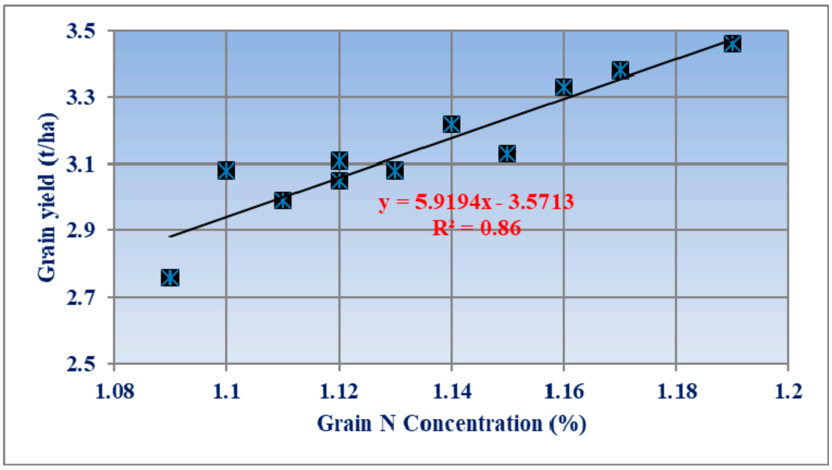


Figure 1. Effect of Zn ferti-fortification on grain and straw yield of *basmati* rice.



(A)



(B)

Figure 2. Regression and correlation of *basmati* grain yield (y) and nutrient concentration (x), (**A**) grain Zn concentration; (**B**) grain N concentration.

3.2. Zn Concentration and Uptake in Plant Sample

With respect to control, grain Zn was numerically greater in treatments with Zn addition at all rates and with either source (Table 3). The highest value of Zn concentration in rice grain and straw was recorded with 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$) treatment and it remained at par with 2.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$) and 4.0% ZnCU + 0.2% ZnFS (ZnO) w.r.t. rice grain and straw Zn concentrations. Application of 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$) enhanced the grain Zn concentration by 54.5% over CU and 2.3–2.5 times higher Zn concentration was observed in grain as compared to straw. A nearly identical trend was also observed with Zn uptake in rice grain, straw and total (grain + straw).

Table 3. Effect of Zn ferti-fortification on Zn concentration and uptake of *basmati* rice.

Treatments	Zn Concentration (mg/kg)		Zn Uptake (g/ha)		
	Grain	Straw	Grain	Straw	Total
CU (control)	23.25 ± 2.48	59.16 ± 6.03	64.2 ± 5.83	378.7 ± 20.50	443.0 ± 21.53
2.0% ZnCU (ZnO)	25.15 ± 1.98	59.97 ± 3.10	75.4 ± 8.40	419.4 ± 13.26	494.8 ± 18.03
2.0% ZnCU ($ZnSO_4{\cdot}7H_2O$)	27.41 ± 2.06	64.87 ± 5.77	83.7 ± 5.32	464.5 ± 15.36	548.2 ± 20.84
4.0% ZnCU (ZnO)	27.78 ± 3.18	65.05 ± 4.97	85.7 ± 5.18	469.8 ± 15.11	555.5 ± 30.08
4.0% ZnCU ($ZnSO_4{\cdot}7H_2O$)	29.59 ± 2.64	69.3 ± 5.58	92.8 ± 6.05	513.1 ± 25.10	605.8 ± 18.97
0.2% ZnFS (ZnO)	29.26 ± 1.46	68.29 ± 3.09	90.2 ± 4.24	491.2 ± 16.79	581.4 ± 20.89
0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$)	31.03 ± 0.95	71.92 ± 6.09	96.5 ± 6.77	520.8 ± 17.61	617.3 ± 18.92
2.0% ZnCU + 0.2% ZnFS (ZnO)	31.6 ± 2.38	73.26 ± 5.94	101.9 ± 5.02	546.9 ± 20.10	648.7 ± 19.55
2.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$)	34.12 ± 2.11	77.79 ± 3.87	113.7 ± 6.75	591.5 ± 24.27	705.3 ± 17.00
4.0% ZnCU + 0.2% ZnFS (ZnO)	32.35 ± 2.04	75.43 ± 4.90	109.4 ± 8.25	584.1 ± 20.44	693.5 ± 22.15
4.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$)	35.93 ± 1.98	81.64 ± 4.75	124.4 ± 9.61	647.8 ± 18.48	772.1 ± 29.30
LSD (p = 0.05)	3.95	7.72	18.3	83.8	92.7

3.3. N Concentration and Uptake in Plant Sample

Application of 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$) resulted in enhanced N concentration rice in grain over rest of the Zn application treatments except 4.0% ZnCU + 0.2% ZnFS (ZnO), 2.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$), 2.0% ZnCU + 0.2% ZnFS (ZnO) and 4.0% ZnCU ($ZnSO_4{\cdot}7H_2O$). Rice grain N concentration remained 1.71-9.17% higher under different Zn fertilization treatments as compared to CU (Table 4). When ZnCU was applied at 4.0% ($ZnSO_4{\cdot}7H_2O$), concentration of N in the grain of basmati rice was highest as compared to other soil application treatments. The N concentration in straw of *basmati* rice also followed an identical trend.

Application of 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$) recorded the highest N uptake in rice grain, straw and total (grain + straw). In the grain, application of 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$) remained on par with 4.0% ZnCU + 0.2% ZnFS (ZnO), 2.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$) and 2.0% ZnCU + 0.2% ZnFS (ZnO), while in case of *basmati* rice straw, 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$) remained at par to 4.0% ZnCU + 0.2% ZnFS (ZnO) and 2.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$). In a similar manner, total N uptake was also found to be maximum in 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4{\cdot}7H_2O$) treatment. Overall, a 38% increase in total N uptake was recorded due to ZnCU as the mean total N uptake in control plot was ~55 kg/ha, which was increased to 76.9 kg/ha with Zn.

Table 4. Effect of Zn ferti-fortification on N concentration and uptake of *basmati* rice.

Treatments	N Concentration (%)		N Uptake (kg/ha)		
	Grain	Straw	Grain	Straw	Total
CU (control)	1.09 ± 0.13	0.4 ± 0.09	30.1 ± 2.62	25.6 ± 1.57	55.7 ± 3.87
2.0% ZnCU (ZnO)	1.11 ± 0.09	0.41 ± 0.11	33.3 ± 1.72	28.7 ± 2.10	62.0 ± 4.05
2.0% ZnCU ($ZnSO_4·7H_2O$)	1.12 ± 0.20	0.42 ± 0.10	34.2 ± 2.75	30.1 ± 1.89	64.3 ± 5.90
4.0% ZnCU (ZnO)	1.13 ± 0.16	0.42 ± 0.10	34.8 ± 2.09	30.3 ± 2.01	65.2 ± 3.99
4.0% ZnCU ($ZnSO_4·7H_2O$)	1.15 ± 0.21	0.43 ± 0.09	36.1 ± 1.62	31.8 ± 2.10	67.9 ± 4.35
0.2% ZnFS (ZnO)	1.1 ± 0.11	0.41 ± 0.10	33.9 ± 3.45	29.5 ± 1.76	63.4 ± 2.74
0.2% ZnFS ($ZnSO_4·7H_2O$)	1.12 ± 0.18	0.42 ± 0.07	34.8 ± 2.21	30.4 ± 2.23	65.3 ± 3.80
2.0% ZnCU + 0.2% ZnFS (ZnO)	1.14 ± 0.27	0.43 ± 0.09	36.7 ± 1.74	32.1 ± 1.18	68.8 ± 6.07
2.0% ZnCU + 0.2% ZnFS ($ZnSO_4·7H_2O$)	1.16 ± 0.20	0.44 ± 0.10	38.7 ± 0.68	33.5 ± 1.53	72.1 ± 5.93
4.0% ZnCU + 0.2% ZnFS (ZnO)	1.17 ± 0.11	0.44 ± 0.09	39.6 ± 1.58	34.1 ± 3.06	73.6 ± 3.04
4.0% ZnCU + 0.2% ZnFS ($ZnSO_4·7H_2O$)	1.19 ± 0.21	0.45 ± 0.10	41.2 ± 2.92	35.7 ± 2.19	76.9 ± 5.22
LSD (p = 0.05)	0.05	0.02	5.1	2.9	7.3

3.4. Zn Use Efficiency

The PFP, RE and AE indices of Zn were almost found inversely related to applied Zn (Table 5). ZnFS was found more efficient than soil application in terms of PFP, RE and AE of applied Zn. The maxima of PFP, RE and AE values of Zn were obtained under application of 0.2% ZnFS ($ZnSO_4·7H_2O$). The 0.2% ZnFS ($ZnSO_4·7H_2O$) treatment remained at par to 0.2% ZnFS (ZnO) w.r.t. PFP and AE of applied Zn in both years of study, while significantly highest value of PE was recorded with 2.0% ZnCU (ZnO) treatment. Among the soil Zn fertilization treatments, these indices were greater with 2.0% ZnCU ($ZnSO_4·7H_2O$) application. The Zn fertilization and CU treatment showed significant difference from each other with respect to HI and MEI of applied Zn. Application of 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4·7H_2O$) recorded the greatest HI and MEI of Zn. ZnHI of 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4·7H_2O$) was significantly higher than 2.0 and 4.0% ZnCU ($ZnSO_4·7H_2O$) and 2.0% ZnCU (ZnO), meanwhile ZnMEI was found statistically higher than 2.0% ZnCU and CU, whereas the higher HI and MEI of applied Zn were obtained when rice was grown with 4.0% ZnCU ($ZnSO_4·7H_2O$) treatment.

Table 5. Effect of Zn ferti-fortification on Zn use efficiency indices of *basmati* rice.

Treatments	PFP (kg Grain/kg Zn)	AE (kg Grain Increased/kg Zn Applied)	RE (%)	PE (kg Grain/kg Zn Uptake)	ZnHI (%)	ZnMEI
CU (control)	-	-	-	-	14.5 ± 1.92	0.39 ± 0.05
2.0% ZnCU (ZnO)	2306 ± 79.78	180 ± 10.59	4 ± 0.52	4504 ± 153.69	15.2 ± 1.83	0.42 ± 0.03
2.0% ZnCU ($ZnSO_4·7H_2O$)	2348 ± 71.69	222 ± 16.75	8 ± 0.31	2740 ± 122.44	15.3 ± 2.23	0.42 ± 0.05
4.0% ZnCU (ZnO)	1186 ± 52.44	123 ± 10.75	4 ± 0.36	2839 ± 58.71	15.4 ± 1.40	0.43 ± 0.05
4.0% ZnCU ($ZnSO_4·7H_2O$)	1206 ± 38.83	143 ± 14.54	6 ± 0.63	2275 ± 102.33	15.4 ± 1.70	0.43 ± 0.04
0.2% ZnFS (ZnO)	2569 ± 85.97	266 ± 18.65	12 ± 0.67	2301 ± 62.02	15.5 ± 1.91	0.43 ± 0.05
0.2% ZnFS ($ZnSO_4·7H_2O$)	2593 ± 111.01	289 ± 20.87	15 ± 0.48	1990 ± 105.87	15.6 ± 1.69	0.43 ± 0.03
2.0% ZnCU + 0.2% ZnFS (ZnO)	1289 ± 65.60	184 ± 15.54	8 ± 0.38	2232 ± 97.54	15.7 ± 2.21	0.43 ± 0.06
2.0% ZnCU + 0.2% ZnFS ($ZnSO_4·7H_2O$)	1333 ± 41.38	227 ± 10.11	10 ± 0.57	2168 ± 73.30	16.1 ± 1.94	0.44 ± 0.04
4.0% ZnCU + 0.2% ZnFS (ZnO)	890 ± 44.58	163 ± 17.98	7 ± 0.70	2465 ± 54.22	15.8 ± 1.79	0.43 ± 0.05
4.0% ZnCU + 0.2% ZnFS ($ZnSO_4·7H_2O$)	911 ± 63.47	184 ± 15.71	9 ± 0.64	2119 ± 104.63	16.1 ± 2.14	0.44 ± 0.06
LSD (p = 0.05)	209	31	2	429	0.7	0.0204

3.5. N Use Efficiency

Various Zn nutrition treatments had significantly affected various N use efficiencies of the rice crop except MEI (Table 6). Application of 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4·7H_2O$) resulted in significantly greater PFP, RE and AE of applied N over rest of the Zn nutrition treatments, however PEP and AE were found at par with 4.0% ZnCU + 0.2% ZnFS (ZnO) and 2.0% ZnCU + 0.2% ZnFS ($ZnSO_4·7H_2O$) and 4.0% ZnCU + 0.2% ZnFS (ZnO) alone, respectively. The highest PE was registered with 0.2% ZnFS (ZnO) and it was statistically better to the remaining treatments, while the highest HI of applied N was observed in 4.0% ZnCU + 0.2% ZnFS (ZnO) treated plot and it was on par to all the Zn fertilization treatments except control plot Mere soil N and Zn fertilization treatments, the greater PFP, RE and PE of applied N were recorded when *basmati* rice was grown with 4.0% ZnCU ($ZnSO_4 \cdot 7H_2O$) treated plot, while PE and HI was found higher in 2.0% ZnCU (ZnO) treatment.

Table 6. Effect of Zn ferti-fortification on N use efficiency indices of *basmati* rice.

Treatments	PFP (kg Grain/kg N)	AE (kg Grain Increased/kg N Applied)	RE (%)	PE (kg Grain/kg N Uptake)	NHI (%)	NMEI
CU (control)	92 ± 7.89	-	-	-	53.1 ± 3.01	2.70 ± 0.26
2.0% ZnCU (ZnO)	100 ± 7.23	8 ± 1.01	21 ± 3.24	38 ± 2.01	53.7 ± 5.28	2.71 ± 0.30
2.0% ZnCU ($ZnSO_4 \cdot 7H_2O$)	102 ± 3.14	10 ± 0.99	28 ± 2.20	34 ±.81	53.2 ± 3.47	2.67 ± 0.34
4.0% ZnCU (ZnO)	103 ± 10.20	11 ± 0.88	32 ± 2.86	34 ± 2.93	53.5 ± 3.26	2.69 ± 0.30
4.0% ZnCU ($ZnSO_4 \cdot 7H_2O$)	104 ± 3.68	12 ± 0.92	41 ± 3.18	30 ± 1.93	53.2 ± 2.48	2.67 ± 0.41
0.2% ZnFS (ZnO)	103 ± 8.48	11 ± 1.30	26 ± 3.57	42 ± 1.45	53.5 ± 1.03	2.68 ± 0.28
0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$)	104 ± 7.84	12 ± 0.72	32 ± 2.17	36 ± 3.50	53.4 ± 3.21	2.67 ± 0.17
2.0% ZnCU + 0.2% ZnFS (ZnO)	107 ± 9.28	15 ± 0.75	44 ± 1.30	35 ± 2.73	53.4 ± 2.20	2.65 ± 0.37
2.0% ZnCU + 0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$)	111 ± 4.32	19 ± 0.78	55 ± 2.18	35 ± 3.21	53.6 ± 2.55	2.64 ± 0.26
4.0% ZnCU + 0.2% ZnFS (ZnO)	113 ± 7.26	21 ± 1.59	60 ± 5.45	34 ± 2.75	53.7 ± 1.80	2.66 ± 0.24
4.0% ZnCU + 0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$)	115 ± 5.76	23 ± 1.72	71 ± 3.06	33 ± 1.79	53.6 ± 2.26	2.64 ± 0.39
LSD (p = 0.05)	4	3	7	2	0.5	NS

3.6. Qualitative Studies

Grain quality parameters such as protein concentration and elongation ratio of *basmati* rice were affected significantly due to various Zn treatments, while non-significant variation was recorded with respect to kernel length and breadth, L:B ratio, head rice recovery and amylose content (Table 7). The higher mean quality traits (L:B ratio, kernel length and breadth, head rice recovery, protein content, elongation ratio and amylose content) were obtained when rice was grown in 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$) treated plots, compared to other treatment. Zn application, by any source and method, increased grain quality over control. Protein content and elongation ratio ranged from 6.81% and 1.69 without Zn fertilization to 7.44% and 1.90 grown in the same soil with Zn fertilization, respectively. There was 9.25% and 12.4% increase in protein content and elongation ratio with 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$) over control, respectively. Zn fertilization did not influence gelatinization temperature (GT) significantly. The quality parameters of grain except amylose content were less with ZnO than $ZnSO_4 \cdot 7H_2O$ at similar dose of fertilization.

Table 7. Effect of Zn ferti-fortification on quality parameters of *basmati* rice.

Treatments	Kernel Length (mm)	Kernel Breadth (mm)	L:B Ratio	Head Rice Recovery (%)	Elongation Ratio	Protein Content (%)	Amylose Content (%)	Geletinization
CU (control)	6.33 ± 0.57	1.96 ± 0.28	3.30 ± 0.22	53.3 ± 1.92	1.69 ± 0.14	6.81 ± 0.87	20.8 ± 3.55	Low
2.0% ZnCU (ZnO)	6.34 ± 0.41	1.98 ± 0.23	3.20 ± 0.21	53.5 ± 3.24	1.75 ± 0.10	6.94 ± 0.58	20.5 ± 1.82	Low
2.0% ZnCU ($ZnSO_4 \cdot 7H_2O$)	6.36 ± 0.35	2.03 ± 0.25	3.13 ± 0.23	53.6 ± 4.61	1.76 ± 0.09	7.00 ± 1.03	20.4 ± 1.93	Low
4.0% ZnCU (ZnO)	6.37 ± 0.49	2.06 ± 0.22	3.09 ± 0.20	53.9 ± 3.11	1.77 ± 0.12	7.06 ± 0.85	20.2 ± 1.97	Low
4.0% ZnCU ($ZnSO_4 \cdot 7H_2O$)	6.39 ± 0.56	2.10 ± 0.23	3.04 ± 0.13	54.2 ± 3.47	1.79 ± 0.14	7.19 ± 0.96	20.1 ± 2.85	Low
0.2% ZnFS (ZnO)	6.34 ± 0.72	1.93 ± 0.16	3.29 ± 0.11	54.3 ± 2.84	1.75 ± 0.10	6.88 ± 0.64	20.4 ± 1.58	Low
0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$)	6.35 ± 0.55	1.96 ± 0.26	3.24 ± 0.09	54.3 ± 2.88	1.77 ± 0.05	7.00 ± 0.80	20.2 ± 1.15	Low
2.0% ZnCU + 0.2% ZnFS (ZnO)	6.38 ± 0.47	2.04 ± 0.18	3.13 ± 0.26	54.7 ± 3.46	1.84 ± 0.08	7.13 ± 0.86	20.1 ± 2.62	Low
2.0% ZnCU + 0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$)	6.40 ± 0.59	2.10 ± 0.19	3.05 ± 0.19	55.3 ± 1.82	1.87 ± 0.15	7.25 ± 0.67	20.0 ± 2.29	Low
4.0% ZnCU + 0.2% ZnFS (ZnO)	6.40 ± 0.66	2.12 ± 0.14	3.02 ± 0.23	55.2 ± 2.97	1.88 ± 0.09	7.31 ± 0.73	20.0 ± 3.57	Low
4.0% ZnCU + 0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$)	6.42 ± 0.50	2.15 ± 0.21	2.98 ± 0.19	55.9 ± 2.89	1.90 ± 0.07	7.44 ± 0.71	19.9 ± 1.93	Low
LSD (p = 0.05)	NS	NS	NS	NS	0.12	0.35	NS	-

4. Discussion

Earlier studies have shown that the soil Zn deficiency is a well-documented problem which is one of the leading factor of crop productivity reduction as it results in a significant penalty in plant performance, reported in several crops in countries such as India, China, Pakistan and Australia [21,62] and Zn applications had significant positive effects on growth and development of plants leading to increased yield attributes and culminating in improved biomass of agronomical crops such as rice [21,23,26,63], wheat [64–67] maize [68], chickpea [69,70], green gram [71,72], black gram [73], cowpea [74], lentil [75], clusterbean [76] and soybean [77]. However, the role of diverse source and grade of ZnCU, in conjunction with foliar fertilization in the Himalayan foothills region has not been undertaken. The present experiment strongly supported the hypothesis that application of Zn would improve the *basmati* rice yield performance, quality, nutrient use efficiencies and grain Zn biofortification for Zn nutrition. Nevertheless, excessive or inappropriate nutrient applications does not guarantee persistently and proportionately increasing biomass production, whereas, it might lead to reduced nutrient use efficiency, and may also invite new-generation environmental threats [78]. The comparatively better performance of rice under $ZnSO_4 \cdot 7H_2O$ as compared to ZnO was probably owing to its relatively higher water solubility [23,79]. Pooniya and Shivay [36] in rice and Imran and Rehim [80] in maize also revealed the productivity improvement and other yield traits with the application of soil applied Zn in combination with foliar spray compared to separate application. However, foliar Zn fertilization alone may not perform best owing to its application at later growth stages and Zn deficiency in plant tissues during early stage of ontogeny [81]. Additionally, N application in soil through CU faces problem such as leaching and denitrification losses, resulting in poor supply of N to the plants during later stage of development [37,82]. This is why split application of ZnCU with ZnFS is a better option to improve crop performance [23]. Compared with CU fertilization, grain and straw yields were significantly increased by all the Zn nutrition treatments except 2.0% ZnCU (ZnO) (Figure 1), which indicates the importance of Zn fertilization for enhancing rice productivity under these areas. Among all treatments, 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$) treatment produced the highest rice, which might be due to enhancement in N and Zn availability up to the harvesting stage through synchronized release from ZnCU might be responsible for increased leaf surface area and number, resulting in enhanced metabolism, photosynthesis and cell division, which consequently led to increased growth and yield attributes [83]. A strongly positive correlation between respective nutrient and rice grain yield has also been reported by Paramesh et al. [84].

In our study, modern agronomic techniques of Zn application caused a significant variation in fortification of respective nutrients, and these techniques with synergistic relationship of N and Zn are major players in enhanced availability of respective elements in the plough layer of soil due to acid formation effect of N [54]. In the study, 4.0% ZnCU + 0.2% ZnFS ($ZnSO_4 \cdot 7H_2O$) resulted in maximum Zn and N uptake in rice grain and straw, which were owing to higher grain and straw yields, and more concentration of these nutrients in the plant tissue [21,36,79,85]. The ZnFS was extremely effective in the rice plant because of high mobility of Zn in phloem, therefore, quicker re-translocated from the vegetative tissues via the phloem and finally accumulated into grain [79]. On the other hand, highest N concentration in rice grain and straw were due to an increased N availability through sustained and synchronized N released from the ZnCU, which finally improved the grain and straw N concentrations proportionately and ultimately resulted in higher N uptake [21].

The PFP, AE and RE efficiencies of applied Zn was almost found inversely related to applied Zn and was higher with foliar fertilization because its faster adsorption and translocation over soil application and subsequent slow desorption [36,67]. Greater response of *basmati* rice to applied Zn was another reason under Zn deficient condition [23]. Significantly highest value of PE recorded with 2.0% ZnCU (ZnO) treatment. Although HI and MEI of applied Zn were much influenced by Zn application, both efficiencies of

4.0% ZnCU ($ZnSO_4{\cdot}7H_2O$) was much higher with ZnFS. This was due to the fact that the greater partitioning of absorbed amount of Zn in grains [36]. Significant changes in N use efficiency might be due to response of *basmati* rice to Zn and N fertilization, leading to differential growth and biomass production and N uptake. CU is extremely soluble when applied to a puddled soil which could cause various losses, crops will only take up small amount N and which will not be sufficient in meeting the crop demands. In this way, the application of Zn-coated urea fertilizers would not only result in enhanced crop productivity but also minimize environmental footprints [86].

Application of Zn significantly influenced the elongation ratio and protein content of *basmati* rice. Rice protein content can be severely affected if N is insufficient during flowering [87]. To attain high *basmati* rice yields with high protein contents, sufficient N supply is necessary [88]. Adequate N fertilization increased N influx in rice, which induced nitrate reductase activity, intern leading to higher redistribution of proteins in rice grains [89]. Additionally, Protein biosynthesis serves as sinks for Zn owing to the important role of Zn in biosynthesis of proteins and protein composition [90,91]. That is why ZnCu in combination with foliar fertilization increases the availability of respective nutrients in plants in a way that results in higher protein content in basmati rice [26,36].

5. Conclusions

Based on the present findings, it may be concluded that Zn biofortification via ZnCU along with foliar fertilization not only enhances the Zn concentration in edible portion but also improves nutrient use efficiencies in *basmati* rice cultivation, which is extremely beneficial under puddled transplanted rice. In addition, ZnCU also leads to higher productivity andbetter grain quality (biochemical, and morphological parameters) of *basmati* rice. Further, considering the agronomic Zn biofortification viewpoint, ZnFS is better option and reduces the requirements of Zn fertilizers than its soil application. Among soil application treatments, 4.0% ZnCU ($ZnSO_4{\cdot}7H_2O$) results in highest N use efficiency. Hence, ZnCU and agronomic biofortification would be an attractive alternative and a useful strategy in tackling the twin challenge of Zn malnutrition related health issues and low N use efficiency problem in flooded *basmati* rice growing regions. In future, understanding process or mechanism by which ZnCU improves these parameters in rice and other crops can be an innovative line of research work. Further, threshold of ZnCU or foliar fertilization for increasing the yield, NUE and the content of Zn needs to be studied thoroughly.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su14010104/s1, Figure S1: Map of location of study site, Figure S2: Standard meteorological weekly data observed during crop growing season; (**A**) *Kharif* 2015-16; (**B**) *Kharif* 2016-17.

Author Contributions: Conceptualization, R.S.B. (Ram Swaroop Bana), Y.S.S. and A.K.C.; methodology, R.S.B. (Ranjeet Singh Bochalya); software, S.D.B. and V.K.; validation, R.C.B. and A.K.G.; formal analysis, R.P. and P.K.; investigation, R.C.B., A.K.G. and R.P.; resources, R.C.B., N.P.T., M.G., S.R.J., R.S.C. and R.S.B. (Ranjeet Singh Bochalya); data curation, R.P. and S.D.B.; writing—original draft preparation, R.C.B. and T.B.; writing—review and editing, R.S.B. (Ram Swaroop Bana), S.D.B., Kailash, V.K. and A.K.C.; visualization, Y.S.S. and R.S.B. (Ram Swaroop Bana); supervision, A.K.G.; project administration, A.K.G. and N.P.T.; funding acquisition, A.K.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors gratefully acknowledge the Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu for providing necessary facilities for carrying out the experiment. The senior author is also thankful to Division of Agronomy, ICAR-IARI, New Delhi for guiding for preparations of research material and assisting during conceptualization stage.

Conflicts of Interest: The authors declare no conflict of interest.

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