

## Phenophase-based nitrogen and zinc scheduling for yield and zinc biofortification of direct-seeded rice (*Oryza sativa*)

SANDEEP KUMAR<sup>1</sup>, S.K. PRASAD<sup>2</sup>, POOJA<sup>3</sup>, M.K. SINGH<sup>4</sup> AND RAJENDRA PRASAD MEENA<sup>5</sup>

Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh 221 005

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### ABSTRACT

A field experiment was conducted during the rainy (*khari*) season of 2015 at Varanasi, Uttar Pradesh, to assess the effect of phenophase-based nitrogen and zinc scheduling on yield, and zinc content of direct-seeded rice (*Oryza sativa* L.). The experiment was laid out in a factorial randomized block design with 3 replications, having 3 nitrogen levels ( $N_0$ , 0 kg N/ha;  $N_1$ , 120 kg N/ha (50% at sowing + 25% at  $G_{21}$  + 25% at  $G_{32}$ );  $N_2$ , 160 kg N/ha (50% at sowing + 25% at  $G_{21}$  + 25% at  $G_{32}$ ) and 5 zinc scheduling ( $Zn_0$ , 0 kg Zn/ha;  $Zn_1$ , 5 kg Zn/ha basal + 0.3%  $ZnSO_4 \cdot H_2O$  spray at  $G_{45}$  and  $G_{61}$ ;  $Zn_2$ , 10 kg Zn/ha basal + 0.3%  $ZnSO_4 \cdot H_2O$  spray at  $G_{45}$  and  $G_{61}$ ;  $Zn_3$ , 5 kg Zn/ha basal + 0.3%  $ZnSO_4 \cdot H_2O$  spray at  $G_{51}$  +  $G_{73}$ ;  $Zn_4$ , 10 kg Zn/ha at basal + 0.3%  $ZnSO_4 \cdot 2H_2O$  spray at  $G_{51}$  +  $G_{73}$ ). Significantly highest effective tillers, panicle length, grains/panicle, panicle weight, 1,000-grain weight, grain and biological yields were recorded with independent application of  $N_2$  and  $Zn_2$  over the others, however the highest zinc content and uptake in grain recorded with  $N_2$  and  $Zn_4$  treatments. Nitrogen levels and Zn scheduling also positively interacted and recorded the highest grain yield with  $N_2 \times Zn_2$ , however the highest grain N and Zn content were recorded with  $N_2 \times Zn_4$ .

**Key words:** Direct-seeded rice, Zinc biofortification, Yield attributes, Grain yield, Phenophase (BBCH scale)

Rice (*Oryza sativa* L.) is the primary food of the world's population and is consumed by > 75% population in Asian countries (FAO, 2019). In India, ~ 118 Mt rice is produced from ~44 M ha area under different rice ecosystems (FAO, 2019). Cultivation of transplanted rice needs repeated puddling under stagnated water which adversely affects the soil physical properties (Sharma *et al.*, 2003). Direct-seeded rice (DSR) received much attention in upland water-scarce areas because that can be produced under limited water and labour without affecting soil physical properties (Bhushan *et al.*, 2007). It provides an opportunity to combat all problems of puddled transplanted rice (Meena *et al.*, 2017). Nitrogen plays a vital role in growth and develop-

ment by involving in many biosynthesis processes in plant system which finally contributes to yield (Shivay *et al.*, 2016) and is considered main input in augmenting cereal production. Zinc and nitrogen deficiency are a major constraint in rice production, especially in upland area, and global concern for human nutrition (Farooq *et al.*, 2018).

More than 33% of the world's population is suffering from zinc deficiency which causes > 4% death of children in 5 years age group (Walker *et al.*, 2009). Rice is an important supplier of bioavailable zinc and protein in the diet of poor rural population (Babu, 2013). Agronomic biofortification, enriching the micronutrient content in staple food through agronomic means, is known to be potential strategy to combat Zn deficiencies.

Phenophase referred as an observable stage or phase in the annual life-cycle of a crop plant that can be defined by duration of a few days or weeks (USA-NPN, 2020). Nitrogen fertilization, combined with soil and foliar Zn applications at various phenological growth stages of rice has a synergistic, positive effect on grain Zn concentration. Thus, in order to address the zinc deficiency issue in staple food, various agronomic approaches of Zn biofortification were tested in rice. However, for appropriate basal dose and phenological growth stage for foliar spray for zinc

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<sup>5</sup>Corresponding author's Email: rajagroicar@gmail.com

<sup>1</sup>M.Sc. Scholar, <sup>2,4</sup>Associate Professor Department of Agronomy, Banaras Hindu University, Varanasi, Uttar Pradesh 220 005; <sup>3</sup>Ph.D. Scholar, Department of Agronomy, Junagadh Agriculture University, Junagadh, Gujarat 362 001, <sup>4</sup>Scientist, Crop Production Division, ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand 263 636

biofortification in rice grain has not been studied in depth so far. Therefore, the present study was conducted under field conditions to investigate the appropriate stage of growth for soil and foliar zinc application in combination with nitrogen rate for enhanced Zn concentration in rice grain.

The experiment was conducted at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (25°18' N, 83°03' E, and 75.7 m above the mean sea-level), Uttar Pradesh, India, during the rainy (*kharif*) season of 2015. The climate of experimental site is typically subtropical with temperature range of 4.5 to 41.1°C and total precipitation received during crop-growing season was 570.7 mm. The soil of experimental field was Ustochrepts (Gangetic alluvium) of Inceptisols, with organic carbon 0.44%, pH 7.3, available N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O 195.4, 22.19, 223.13 kg/ha, respectively, and DTPA-extractable Zn was 0.53 ppm. The experiment was laid out in factorial randomized-block design with 3 replications. The treatment consisted of 3 nitrogen levels and 5 zinc scheduling (foliar spray and soil application). Uniform dose of phosphorus and potassium at 60 and 60 kg/ha, respectively, was applied. Nitrogen and zinc were applied in the form of urea and zinc sulphate (monohydrate) respectively. The sprouted seeds of rice cv. 'HUR 105' were broadcasted directly under condition on 10 August 2015 with seed rate of 50 kg/ha. Light irrigation was given 4 days after sowing (DAS) for proper seed germination. Rice crop was harvested at maturity stage with the help of

sickle. Grain yields obtained from each net plot was converted to t/ha. Biological yield was obtained by addition of grain and straw yields and was expressed in t/ha. The harvest index (H.I.) was calculated by dividing economical yield (grain yield) by the biological yield (grain and straw) and represented in percentage (Donald and Hamblin, 1976). Plant samples were cleaned properly by repeated washing followed by 0.1 N HCl, solutions. Samples were then dried under shade followed by hot air oven at 60 ± 1°C for 48 hours. Samples were subjected to chemical analysis for nitrogen, phosphorus, potassium and zinc content and uptake. Nitrogen content in grain and straw was estimated by modified Kjeldahl method as described by Jackson (1973). The concentration of zinc was estimated by Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978). The data were statistically analysed as per standard statistical procedure.

The results manifested that the phenophase-based nitrogen and zinc scheduling significantly improved the yield attributes, yields, content and uptake of N, P, K and Zn in direct-seeded rice (DSR). The yield attributes and yield of DSR increased with increasing level of nitrogen from the control to 160 kg N/ha. Among the treatments, the application of 160 kg N/ha (50% at sowing + 25% at G<sub>21</sub> + 25% at G<sub>32</sub>) recorded the highest effective tillers at harvesting time, panicle length, grains/panicle, panicle weight, test weight, grain yield, straw yield, biological yield and harvest index of rice, which were significantly higher than the control (Table 1). The adequate N at right stages (25% at

**Table 1.** Yield attributes and yields of direct-seeded rice under different phenophase-based nitrogen levels and zinc scheduling

Treatment	Yield attributes					Yields			
	Effective tillers/running meter	Panicle length (cm)	Grains/panicle	Panicle weight (g)	Test weight (g)	Grain yield (t/ha)	Straw yield (t/ha)	Biological yield (t/ha)	Harvest index (%)
<i>N (kg/ha)</i>									
N <sub>0</sub>	38	21.7	85	1.90	18.3	1.95	4.52	6.47	30.16
N <sub>1</sub>	57	23.5	101	2.46	20.7	2.80	5.13	7.85	34.06
N <sub>2</sub>	71	23.9	112	2.76	23.5	3.81	6.38	10.20	37.29
SEm±	2.10	0.6	2.37	0.07	0.5	0.07	0.13	0.20	0.68
CD (P=0.05)	6.07	1.7	6.85	0.19	1.4	0.21	0.40	0.59	1.96
<i>Zinc scheduling</i>									
Zn <sub>0</sub>	49	22.6	88	2.02	17.9	1.97	4.56	6.45	29.92
Zn <sub>1</sub>	52	22.2	96	2.34	20.3	2.85	5.45	8.24	33.56
Zn <sub>2</sub>	67	23.0	105	2.57	23.7	3.44	5.56	9.01	36.83
Zn <sub>3</sub>	54	24.2	102	2.50	21.3	2.98	5.65	8.63	34.13
Zn <sub>4</sub>	54	23.1	104	2.45	21.3	3.03	5.49	8.53	34.72
SEm±	2.71	0.8	3.05	0.09	0.6	0.09	0.17	0.26	0.87
CD (P=0.05)	7.84	NS	8.85	0.25	1.9	0.28	0.51	0.76	2.53
N×Zn	S	NS	S	S	S	S	S	S	NS

N<sub>0</sub>, 0 kg N/ha (control); N<sub>1</sub>, 120 kg N/ha (50% at sowing + 25% at G<sub>21</sub> + 25% at G<sub>32</sub>); N<sub>2</sub>, 160 kg N/ha (50% at sowing + 25% at G<sub>21</sub> + 25% at G<sub>32</sub>); Zn<sub>0</sub>, 0 kg Zn/ha (control); Zn<sub>1</sub>, 5 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>45</sub> and G<sub>61</sub>; Zn<sub>2</sub>, 10 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>45</sub> and G<sub>61</sub>; Zn<sub>3</sub>, 5 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>51</sub> and G<sub>73</sub>; Zn<sub>4</sub>, 10 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>51</sub> and G<sub>73</sub>

G<sub>21</sub> and 25% at G<sub>32</sub>) might have enhanced the rate of tillering and correspondingly increased the effective tillers, 1,000 grain weight and grain yield. This might be owing to active participation of N in chlorophyll formation and photosynthesis (Zhao *et al.* 2015). Our results also support the findings of Meena *et al.* (2017). Similarly, Baral *et al.* (2019) also reported that application of 100% NPK with 5 kg Zn/ha through ZnSO<sub>4</sub>·7H<sub>2</sub>O, recorded higher grain yield of aromatic rice (5.10 t/ha) than absolute control (2.68 t/ha).

Zinc application at early growth stages significantly contributed more in growth and development of plant than later growth stages and resulted in the highest effective tillers at harvesting, grains/panicle, panicle weight, test weight, grain yield and biological yield with Zn<sub>2</sub> (10 kg Zn/ha at basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>45</sub> + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>61</sub>). The Zn<sub>3</sub> and Zn<sub>4</sub> treatments were also found superior to the control. Our results support the finding of Meena *et al.* (2018). Yadav *et al.* (2018) also reported significantly highest panicle weight, panicle length and 1,000 grain weight of basmati rice with the application of 0.5% chelated Zn-EDTA solution at different growth stages (20, 40, 60 and 80 days after transplanting).

The content and uptake of N, P, K and Zn in direct-seeded rice (grain and straw) were significantly influenced by different nitrogen levels and Zn scheduling (Table 2). Among the nitrogen levels, significantly highest content

and uptake of nitrogen, phosphorus, potassium and potassium and zinc in grain were obtained with application of 160 kg N/ha (50% at sowing + 25% at G<sub>21</sub> + 25% at G<sub>32</sub>). It was owing to the involvement of N in growth and development and the increased availability of Zn (Lin *et al.*, 2014). However in zinc scheduling, significantly highest grain N content, straw N content, grain K content, grain zinc content, grain zinc uptake, straw zinc content and straw zinc uptake were recorded with Zn<sub>4</sub> (10 kg Zn/ha at basal + 0.3% ZnSO<sub>4</sub>·2H<sub>2</sub>O spray at G<sub>51</sub> + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>73</sub>), while the highest grain N uptake, straw N content and uptake were recorded with Zn<sub>2</sub> (10 kg Zn/ha at basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>45</sub> + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>61</sub>). Higher Zn content and uptake in grain might be owing to the maximum translocation of zinc from source to sink (grain), when higher dose of zinc was applied basal and zinc foliar spray at later growth stage (Cakmak, 2008).

Phenophase-based N and Zn scheduling interacts with each other for grain yield, N and zinc content in grain (Table 3). Nitrogen and zinc application at N<sub>2</sub> × Zn<sub>2</sub> recorded the the maximum grain yield followed by N<sub>2</sub> × Zn<sub>4</sub>, which were significantly higher than N<sub>0</sub> × Zn<sub>0</sub> treatment combination. It might be owing to the fulfilment of nutrient requirement and positive interaction of N and Zn. However, the highest N and zinc content in grain were recorded with N<sub>2</sub> × Zn<sub>4</sub>, which was significantly higher than N<sub>0</sub> × Zn<sub>0</sub>

**Table 2.** Nitrogen, phosphorus, potassium and zinc content and uptake of direct-seeded rice under different phenophase-based nitrogen levels and zinc scheduling

Treatment	Content/uptake (N, P, K and Zn)											
	Nitrogen				Phosphorus		Potassium		Zinc			
	Grain		Straw		Grain		Grain		Grain		Straw	
	Content (%)	Uptake (kg/ha)	Content (%)	Uptake (kg/ha)	Content (%)	Uptake (kg/ha)	Content (%)	Uptake (kg/ha)	Content (mg/kg)	Uptake (g/ha)	Content (mg/kg)	Uptake (g/ha)
<i>N (kg/ha)</i>												
N <sub>0</sub>	1	18.9	0.40	18	0.20	4.1	0.25	5.1	21.62	44.2	55.89	275.2
N <sub>1</sub>	1.1	31.8	0.51	27	0.24	6.8	0.27	8.2	30.47	99.4	60.18	339.4
N <sub>2</sub>	1.3	51.9	0.53	34.9	0.26	9.6	0.29	10.5	38.92	176.8	71.44	496.6
SEm±	0.04	1.9	0.018	1.2	0.009	0.4	0.009	0.3	1.218	4.35	2.30	16.30
CD (P=0.05)	0.11	5.5	0.053	3.5	0.026	1.1	0.025	0.8	3.53	12.6	6.68	47.24
<i>Zinc scheduling</i>												
Zn <sub>0</sub>	1	19.3	0.46	21	0.20	4.1	0.23	4.8	20.57	43.2	38.01	186.2
Zn <sub>1</sub>	1.1	32.0	0.46	26.2	0.25	7.2	0.29	8.2	27.09	88.1	56.06	341.4
Zn <sub>2</sub>	1.3	45.6	0.51	29.1	0.28	8.2	0.28	10.1	3 0.54	121.2	67.68	408.6
Zn <sub>3</sub>	1.1	35.0	0.46	26.9	0.26	7.6	0.26	8	34.18	119.5	72.11	449.7
Zn <sub>4</sub>	1.2	39.0	0.51	29	0.23	7	0.30	8.7	39.29	141.6	78.66	468.8
SEm±	0.053	2.5	0.24	1.7	0.012	0.5	0.011	0.4	1.57	5.6	2.97	21.05
CD (P=0.05)	0.154	7.2	0.06	4.6	0.034	1.4	0.03	1.1	4.55	16.2	8.62	60.99
N×Zn	S	S	NS	NS	S	S	S	S	S	S	NS	NS

N<sub>0</sub>, 0 kg N/ha (control); N<sub>1</sub>, 120 kg N/ha (50% at sowing + 25% at G<sub>21</sub> + 25% at G<sub>32</sub>); N<sub>2</sub>, 160 kg N/ha (50% at sowing + 25% at G<sub>21</sub> + 25% at G<sub>32</sub>); Zn<sub>0</sub>, 0 kg Zn/ha (control); Zn<sub>1</sub>, 5 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>45</sub> and G<sub>61</sub>; Zn<sub>2</sub>, 10 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>45</sub> and G<sub>61</sub>; Zn<sub>3</sub>, 5 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>51</sub> and G<sub>73</sub>; Zn<sub>4</sub>, 10 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>51</sub> and G<sub>73</sub>

**Table 3.** Interaction effect of phenophase-based nitrogen levels and zinc scheduling on grain yield, nitrogen and zinc content in grain in rice

Treatment	Grain yield (t/ha)					Grain									
						N content (%)					Zn content (mg/kg)				
	Zn <sub>0</sub>	Zn <sub>1</sub>	Zn <sub>2</sub>	Zn <sub>3</sub>	Zn <sub>4</sub>	Zn <sub>0</sub>	Zn <sub>1</sub>	Zn <sub>2</sub>	Zn <sub>3</sub>	Zn <sub>4</sub>	Zn <sub>0</sub>	Zn <sub>1</sub>	Zn <sub>2</sub>	Zn <sub>3</sub>	Zn <sub>4</sub>
N <sub>0</sub>	1.61	1.98	2.26	2.00	1.90	0.70	0.74	0.97	0.88	1.00	17.40	20.85	20.40	24.03	25.43
N <sub>1</sub>	2.03	2.62	3.44	3.06	2.82	0.97	1.13	1.31	0.94	1.16	23.04	24.09	31.59	32.77	40.89
N <sub>2</sub>	2.27	3.94	4.61	3.88	4.38	1.01	1.53	1.67	1.58	1.81	21.29	36.36	39.65	45.76	51.56
SEm±		0.16					0.09					2.72			
CD (P=0.05)		0.48					0.25					7.89			

N<sub>0</sub>, 0 kg N/ha (control); N<sub>1</sub>, 120 kg N/ha (50% at sowing + 25% at G<sub>21</sub> + 25% at G<sub>32</sub>); N<sub>2</sub>, 160 kg N/ha (50% at sowing + 25% at G<sub>21</sub> + 25% at G<sub>32</sub>); Zn<sub>0</sub>, 0 kg Zn/ha (control); Zn<sub>1</sub>, 5 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>45</sub> and G<sub>61</sub>; Zn<sub>2</sub>, 10 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>45</sub> and G<sub>61</sub>; Zn<sub>3</sub>, 5 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>51</sub> and G<sub>73</sub>; Zn<sub>4</sub>, 10 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>51</sub> and G<sub>73</sub>

treatment combination. Higher N rate along with Zn fertilization at later growth stage promotes protein synthesis, a major sink for Zn and enhances the expression Zn transporter proteins, resulted in the maximum nutrient translocations from source to sink (Cakmak *et al.*, 2010).

Thus, phenophase-based N and Zn scheduling, not only improved the grain and biological yields but also improved the content and uptake of N and Zn in grain. In case of zinc biofortification, 50% recommended dose of nitrogen RDN at sowing + 25% at G<sub>21</sub> + 25% at G<sub>32</sub> and 10 kg Zn/ha basal + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>51</sub> + 0.3% ZnSO<sub>4</sub>·H<sub>2</sub>O spray at G<sub>73</sub> significantly interacted and recorded the maximum zinc content in grain.

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