



## Nitrogen and zinc scheduling for zinc biofortification in direct seeded rice (*Oryza sativa*)

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Rice (*Oryza sativa* L.) is the staple food of more than 65% world (FAO 2016) and 85% Indian population (Mohapatra 2014). It produced ~106 Mt from 44 M ha under different rice ecosystems (FAO 2016) in India of which, ~18.8 M ha is under rainfed (Mohapatra 2014). In which direct seeded rice (DSR) received much attention because that can be produced under limited resources (Bhushan *et al.* 2007). Rice is an important supplier of zinc in the diet of poor rural population (Babu 2013). It's deficiency is a major constraint for rice production and a global concern for human nutrition (Farooq *et al.* 2018). About one-third of the world's population is suffering from zinc deficiency (Hotz and Brown 2004) which contribute > 4% death of children under five years age group (Walker *et al.* 2009). Agronomic biofortification, enriching the micronutrient content in staple food is known to be potential strategy to combat Zn deficiencies. It provides temporary micronutrient increases through fertilizers and also rely on nutrient interactions (Prasad *et al.* 2014) particularly nitrogen status of crops (Shi *et al.* 2010). Nitrogen is the key nutrient, limiting yields of most crops (Shivay *et al.* 2016) and considered main input in augmenting cereal production (Ladha *et al.* 2005).

The proper nitrogen fertilization, combined with foliar Zn applications has a synergistic positive effect on grain Zn concentration (Kutman *et al.* 2010). Thus, in order to address the zinc deficiency issue in rice, various agronomic approaches of Zn biofortification were tested, among which foliar spray found more efficient (Wang *et al.* 2012, Singh and Prasad 2014, Farooq *et al.* 2018). However, in case of rice the appropriate stage of zinc biofortification in grain is not studied so far. Therefore, the present study was conducted

under field conditions to investigate the appropriate stage of 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, India during the *khari* (rainy) season of 2014. The soil of the experimental site was sandy clay loam, organic carbon 0.45%, pH 7.4, available NPK 197.6, 21.22, 220.21 kg/ha, respectively, and available zinc 0.51 ppm. The experiment was laid out in randomized block design (factorial) with 3 replications. The treatment consist of four nitrogen rates (0, 90, 120, 150 kg/ha) four zinc schedulings (as foliar spray) consist of 0.0% ZnSO<sub>4</sub> (control), 0.3% ZnSO<sub>4</sub> at anthesis, 0.3% ZnSO<sub>4</sub> at early milking and 0.3% ZnSO<sub>4</sub> at dough stage. Uniform rate of phosphorus and potassium at 60 and 60 kg/ha, respectively was applied. Nitrogen and zinc was applied in the form of urea and zinc sulphate (monohydrate), respectively. The certified seed of rice cultivar HUR-105 was purchased from Agricultural Research Farm, BHU. The plot size was 12 m<sup>2</sup> and in each plots 48 g seed @ 40 kg/ha was sown in unpuddled field on 10 August 2014. Light irrigation was given 4 days after sowing (DAS) for proper seed germination. Harvesting of rice crop was undertaken for harvest at maturity stage with the help of sickle. Grain yield thus obtained from each net plot were converted to kg/ha. Biological yield was obtained by addition of grain and straw yields and was expressed in kg/ha. The harvest index (HI) 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 0.1N HCl, solutions. Samples were then dried under shade followed by hot air oven at 60 ±1°C for 48 hours. Samples were then subjected to chemical analysis for nitrogen and zinc content. Nitrogen content in grain and straw was estimated by modified Kjeldahal method as described by Jackson (1973). The concentration of zinc was estimated by Atomic Absorption Spectrophotometer (Lindsay and Norvell 1978).

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Table 1 Yields, harvest index, nutrient content and uptake (nitrogen and zinc) of DSR under nitrogen rates and zinc scheduling

Treatment	Grain yield (kg/ha)	Biological yield (kg/ha)	Harvest index (%)	Grain content/uptake (N and Zn)			
				Nitrogen		Zinc	
				Content (%)	Uptake (kg/ha)	Content (mg/kg)	Uptake (kg/ha)
<i>N- rates</i>							
N0 (control)	3046.67	7629.67	40.21	0.80	25.05	22.18	0.070
N90	3626.52	8686.28	41.71	0.99	36.75	25.81	0.094
N120	3952.16	9354.58	42.48	1.17	47.43	27.80	0.112
N150	4340.93	10086.11	43.03	1.49	66.50	34.83	0.152
<i>Zinc scheduling (foliar spray)</i>							
0% ZnSO <sub>4</sub> (control )	2717.95	6639.08	41.04	0.79	21.76	21.98	0.060
0.3% ZnSO <sub>4</sub> at anthesis	4368.75	10273.45	42.63	1.11	50.47	23.03	0.103
0.3% ZnSO <sub>4</sub> at early milking	4026.76	9534.58	42.37	1.21	50.14	28.84	0.118
0.3% ZnSO <sub>4</sub> at dough stage	3852.83	9309.52	41.40	1.35	53.37	36.75	0.145
<i>CD (P= 0.05)</i>							
Nitrogen (N)	98.71	266.69	1.16	0.05	2.01	1.13	0.005
Zinc (Zn)	285.09	770.24	NS	0.15	5.79	3.28	0.013
N×Zn	SIG	NS	NS	SIG	SIG	SIG	SIG

Table 2 Interaction effect of N × Zn on grain yield, zinc content and uptake in grain of DSR

Treatment	Grain											
	Yield (kg/ha)				Zinc content (mg/kg)				Zinc uptake (kg/ha)			
	Nitrogen levels (kg/ha)											
	N0	N90	N120	N150	N0	N90	N120	N150	N0	N90	N120	N150
<i>Zinc scheduling (Foliar spray)</i>												
0% ZnSO <sub>4</sub> (control )	2270.83	2753.20	2866.66	2981.09	18.79	21.83	22.58	24.75	0.043	0.059	0.066	0.074
0.3% ZnSO <sub>4</sub> at anthesis	3525.63	4006.41	4360.90	5582.05	21.51	19.69	20.71	30.23	0.076	0.079	0.090	0.169
0.3% ZnSO <sub>4</sub> at early milking	3305.28	3879.80	4320.51	4601.44	22.20	29.45	30.96	32.77	0.072	0.115	0.134	0.151
0.3% ZnSO <sub>4</sub> at dough stage	3084.93	3866.66	4260.59	4199.13	26.23	32.29	36.95	51.57	0.081	0.125	0.156	0.216
CD (P=0.05) of N×Zn	570.18				0.30				6.57			

Scrutiny of data revealed that the grain and biological yield of DSR was significantly influenced by different nitrogen levels and zinc scheduling (Table 1). Significantly higher grain (~ 4340 kg/ha) and biological yield (~ 10086 kg/ha) were recorded with application of 150 kg N/ha, which were ~ 30% and 32%, higher than control, respectively. However, among the different zinc scheduling, the application of 0.3% ZnSO<sub>4</sub> spray at anthesis recorded the significantly highest grain (~ 4368 kg/ha) and biological yield (~ 10273 kg/ha), it might be due to zinc application at early growth stages, viz. anthesis, early milking, contributing more in growth and development than later stages. It was also observed that harvest index increased by an increase in nitrogen levels, not by zinc scheduling, but it was found non-significant. The higher yields of DSR might be due to involvement of N in many metabolic reactions, viz. proteins, nucleic acids (Hawkesford *et al.* 2012) and chlorophyll

formation (Zhao *et al.* 2015) and of zinc in N-metabolism of the plant. Our results also support the findings of Puteh and Mondal (2014).

Data showed that the content and uptake of nitrogen increased with the increasing levels of nitrogen from 0 kg/ha (control) to 150 N/ha (Table 1). Significantly highest grain N content (1.49%) and uptake (~ 66 kg/ha) were recorded with application of 150 kg N/ha and lowest (0.80% and ~ 25 kg/ha, respectively) with control. This might be due to nitrogen and protein content in grain directly related to increased level of N. Content and uptake of N was also influenced by zinc scheduling and highest N content (1.35%) and uptake (~ 53 kg/ha) were recorded with application of 0.3% ZnSO<sub>4</sub> at dough stage. Zinc content in grain was enhanced by nitrogen rate and highest zinc content (~ 34 mg/kg) was recorded with 150 kg N/ha, which is ~ 57% higher than control. While, the significantly higher zinc uptake (0.152

Table 3 Integrated effect of N × Zn on nitrogen content and uptake in grain of DSR

Treatment	Nitrogen content (%)				Nitrogen uptake (kg/ha)			
	N0	N90	N120	N150	N0	N90	N120	N150
<i>Zinc scheduling (Foliar spray)</i>								
0% ZnSO <sub>4</sub> (control )	0.70	0.72	0.85	0.88	15.90	19.35	25.20	26.57
0.3% ZnSO <sub>4</sub> at anthesis	0.75	1.01	1.16	1.51	26.88	40.64	50.05	84.30
0.3% ZnSO <sub>4</sub> at early milking	0.89	1.11	1.31	1.53	30.00	43.32	56.58	70.63
0.3% ZnSO <sub>4</sub> at dough stage	0.88	1.13	1.36	2.03	27.42	43.66	57.87	84.49
CD (P=0.05) of N × Zn			0.30				11.58	

kg/ha) also recorded with the application of 150 kg N/ha, however it was statistically at par with other N levels but each level was significantly superior over control. It was due to the involvement of N in growth and development and the increased availability of Zn, Mn and Fe (Lin *et al.* 2014). However, in case of zinc scheduling, the significantly highest zinc content (~ 36 mg/kg) and uptake (0.145 kg/ha) in grain were recorded with application of 0.3% ZnSO<sub>4</sub> at dough stage, followed by 0.3% ZnSO<sub>4</sub> at early milking stage (Table 1). It might be due to the maximum translocation of zinc from source to sink (grain), when; zinc was applied at later growth stage (Cakmak 2008). These findings also corroborate the finding of Zhang *et al.* (2012).

Interaction effect between the nitrogen rate and zinc scheduling significantly differed (Table 2 and 3). Results showed that the significantly highest grain yield (~ 5582 kg/ha) was recorded with N<sub>150</sub> × 0.3% ZnSO<sub>4</sub> at anthesis stage, which was ~ 87% higher than control. It might be due to the fulfillment of nutrient requirement and positive interaction of N and Zn. Our results corroborate the finding of Shivay and Prasad (2008). Zinc content and uptake in grain also interact significantly, resulted highest content (~ 51 mg/kg) and uptake (0.216 kg/ha) were recorded with N<sub>150</sub> × 0.3% ZnSO<sub>4</sub> at dough stage (Table 2).

This finding corroborates the zinc content and uptake could be enhanced by foliar zinc spray at later growth stage with high dose of N in rice (Singh *et al.* 2012). Similarly, uptake and content of nitrogen in grain also interact significantly (Table 3). The maximum N content (2.03%) and uptake (~ 84 kg/ha) in grain was recorded with N<sub>150</sub> × 0.3% ZnSO<sub>4</sub> at dough stage. Increasing dose of N along with Zn fertilization at later growth stage promotes protein synthesis, a major sink for Zn and enhances the expression Zn transporter proteins, resulted maximum nutrient translocate from source to sink (Cakmak *et al.* 2010).

#### SUMMARY

The study clearly showed the application of 150 kg N/ha and 0.3% ZnSO<sub>4</sub> spray at anthesis stage was found optimum for enhancement of grain and biological yield. However, 150 kg N/ha and 0.3% ZnSO<sub>4</sub> spray at dough stage found optimum for higher concentration of zinc and nitrogen in grain. Results showed that the combined fertilization of 150 kg N/ha and 0.3% ZnSO<sub>4</sub> at dough stage was proved highly effective for grain yield, content and uptake of nitrogen and

zinc in grain in the DSR condition.

#### REFERENCES

- Babu V R. 2013. Importance and advantages of rice biofortification with iron and zinc. *Journal of SAT Agricultural Research* **11**: 1–6.
- Bhushan L, Ladha J K, Gupta R K, Singh S, Tirol-Padre A, Saharwat Y S, Gathala M and Pathak H. 2007. Saving of water and labor in a rice–wheat system with no tillage and direct seeding technologies. *Agronomy Journal* **99**: 1288–96.
- Cakmak I. 2008. Enrichment of cereal grain with zinc: agronomic or genetic biofortification. *Plant and Soil* **302**: 1–17.
- Cakmak I, Pfeiffer W H and McClafferty B. 2010. Biofortification of durum wheat with zinc and iron. *Cereal Chemistry* **87**: 10–20.
- Donald C M and Hamblin J. 1976. The biological yield and harvest index of cereals as agronomic and plant breeding criteria. *Advances in Agronomy* **28**: 361–445.
- FAO. 2016. Food and Agriculture Organization of the United Nations Statistics Division. <http://faostat.fao.org/download/Q/QC/E>.
- Farooqa M, Ullaha A, Rehmana A, Nawaza A, Nadeema A, Wakeele A, Nadeema F, and Siddiquee K H M. 2018. Application of zinc improves the productivity and biofortification of fine grain aromatic rice grown in dry seeded and puddled transplanted production systems. *Field Crops Research* **216**: 53–62.
- Hawkesford M, Horst W, Kichey T, Lambers H, Schjoerring J, Møller I S and White P. 2012. Functions of macronutrients. (In) *Marschner's Mineral Nutrition of Higher Plants*, third ed. Marschner P (Ed), Academic Press.
- Hotz C and Brown K H. 2004. Assessment of the risk of zinc deficiency in populations and options for its control. *Food and Nutrition Bulletin* **25**: 94–204.
- Jackson M L. 1973. *Soil Chemical Analysis*. Perntice Hall of India Pvt Ltd, New Delhi.
- Kutman U B, Yildiz B, Ozturk L and Cakmak I. 2010. Biofortification of durum wheat with zinc through soil and foliar applications of nitrogen. *Cereal Chemistry* **87**: 1–9.
- Ladha J K, Pathak H, Kruprik T J, Six J and Kessel C V. 2005. Efficiency of fertilizer nitrogen in cereal production: retrospect and prospects. *Advances in Agronomy* **87**: 85–156.
- Lindsay W L and Norvell A. 1978. Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* **42**: 421–8.
- Lin Z M, Ning H F, Bi J G, Qiao J F, Liu Z H, Li G H, Wang Q S, Wang S H and Ding Y F. 2014. Effects of nitrogen fertilization and genotype on rice grain macronutrients and micronutrients. *Rice Science* **21**(4): 233–42.
- Mohapatra T. 2014. CRRRI Annual Report 2013-14. Central Rice Research Institute, Cuttack (Odisha), India.

- Prasad R, Shivay Y S and Kumar D. 2014. Agronomic biofortification of cereal grains with iron and zinc. *Advances in Agronomy* **125**: 55–91.
- Puteh A B and Mondal M A. 2014. Growth and yield performance of rice as affected by nitrogen rate. *Life Science Journal* **11**(8): 653–5.
- Shivay Y S and Prasad R. 2012. Zinc-coated urea improves productivity and quality of basmati rice (*Oryza sativa* L.) under zinc stress condition. *Journal of Plant Nutrition* **35**(6): 928–51.
- Shivay Y S, Prasad R and Pal M. 2016. Effect of nitrogen levels and coated urea on growth, yields and nitrogen use efficiency in aromatic rice. *Journal of Plant Nutrition* **39**(6): 875–82.
- Singh M K and Prasad S K. 2014. Agronomic aspects of zinc biofortification in rice (*Oryza sativa* L.). *Proceedings of the National Academy of Sciences, India Section B: Biological Science* **84**(3): 613–623.
- Walker C L F, Ezzati M and Black R E. 2009. Global and regional child mortality and burden of disease attributable to zinc deficiency. *Eur. J. Clin. Nutr.* **63**: 591–7.
- Wang W N, Lu J W, Ren T, Li X K, Su W and Lu M X. 2012. Evaluating regional mean optimal nitrogen rates in combination with indigenous nitrogen supply for rice production. *Field Crop Research* **137**: 37–48.
- Zhang Y, Sun Y, YouLiang Y M, Karim R, Xue Y, Yan P, Menga Q, Cui Z, Cakmak I, Zhang F. and Zou. 2012. Zinc biofortification of wheat through fertilizer applications in different locations of China. *Field Crops Research* **5**(125): 1–7.
- Zhao Y, Xi M, Zhang X, Lin Z, Ding C, Tang S, Liu Z, Wang S and Ding Y. 2015. Nitrogen effect on amino acid composition in leaf and grain of japonica rice during grain filling stage. *Journal of Cereal Science* **64**: 29–33.