Plant Archives Vol. 19 No. 1, 2019 pp. 1815-1820 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DISTRIBUTION OF FOLIAR APPLIED ^{65}ZN AND ^{59}FE IN TWO CONTRASTING RICE VARIETIES

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Abstract

Effect of foliar applied Zn (0, 0.25 and 0.50% $ZnSO_4$ solution tagged with ^{65}Zn) and Fe (0, 0.5 and 1.0% $FeSO_4$ solution tagged with ^{59}Fe) was examined on their distribution in plant parts of rice (Zn inefficient NDR359 and Zn efficient PD16). With no Fe or 0.5% $FeSO_4$, 0.5% $ZnSO_4$ increased ^{65}Zn accumulation in all plant parts of both varieties but decreased translocation to psuedostem in NDR359. At 1% $FeSO_4$, 0.5% $ZnSO_4$ decreased the accumulation of ^{65}Zn in all plant parts of both varieties. As regards Fe distribution, with 0.5% $FeSO_4$, 0.25 and 0.50% $ZnSO_4$ increased ^{59}Fe accumulation in most plant parts of both varieties but decreased translocation to psuedostem and dehulled grain of NDR359. With 1.0% $FeSO_4$, 0.25% $ZnSO_4$ increased ^{59}Fe accumulation in leaves and translocation to psuedostem but decreased translocation to dehulled grains of both varieties. With 1.0% $FeSO_4$, 0.50% $ZnSO_4$ increased ^{59}Fe accumulation in leaves of both varieties and translocation to psuedostem and grain in NDR359 but decreased translocation to psuedostem and dehulled grain of PD16.

Key words : Foliar fertilization, rice, translocation, zinc & iron interaction.

Introduction

Human populations consuming cereals as staple food exhibit nutritional deficiencies (Bonsmann and Hurrell, 2009). A large proportion of world population is severely affected by micronutrient malnutrition particularly in Asian countries where increasing population is one more serious issue (Subramanian *et al.,* 2008). Polished rice is a poor source of essential micronutrients (Zimmermann and Hurrell, 2002) and contains only 2 mg Fe and 12 mg Zn kg^{-1} (IRRI, 2006) while the recommended requirement of both the micronutrients for humans is 10-15 and 12-15 mg, respectively (Welch and Graham, 2004). Thus, increasing Zn and Fe content in grains is task of high priority for the health of human population dependent on cereals.

There are many factors responsible for the deficiency of Zn and Fe in agricultural soils. The main factors are deficiency of these nutrients in soil, alkaline soil pH, high content of carbonates, high salt level in irrigation water, high content of soil phosphorus and antagonistic interaction of nutrients with each other (Alloway, 2009). There are many reports indicating antagonistic interaction between Fe and Zn in soybean (Sliman, 1990) and flax (Lee *et al.,* 1969) due to competition for root absorption sites. High level of Fe in the soil had been reported to decrease the uptake of ^{65}Zn significantly in maize (Rathore *et al.,* 1974).

Breeding of cultivars is an important approach to develop efficient genotypes and to increase micronutrient content in grains. However, a very limited genotypic variation exists among cultivars for grain Zn and Fe concentration. Agronomic interventions like soil fertilization with Zn fertilizers are effective but limited due to poor translocation to grains (Cakmak, 2002). Foliar Zn and Fe supplementation can increase Zn and Fe concentration in grain (Yilmaz *et al.,* 1997; Sadana and Nayyar, 2000; Pahlavan-Rad and Pessarakli, 2009). However, not much information is available in the literature on the nature of Zn-Fe interaction if these nutrients are applied on the foliage.

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Therefore, an effort was done to investigate the interaction between foliar applied Zn and Fe and the accumulation of these nutrients in two rice varieties of varying tolerance to Zn deficiency.

Materials and Methods

Seeds of two contrasting rice genotypes viz., NDR359 (Zn inefficient) and Pant Dhan16 (Zn efficient) were collected from Genetics and Plant Breeding Department of the University. Seedlings were raised in Zn deficient soil filled in plastic trays and transplanted to plastic pots of 4 kg capacity after 21 days of sowing. Urea, KH_2PO_4 and KCl were used as a source fertilizer for basal dose of N (22.3 mg N kg^{-1} of soil), P (11.6 mg P kg^{-1} of soil) and K (18.5 mg K kg^{-1} of soil), respectively. Various levels of Zn (0, 0.25, 0.50% $ZnSO_4$ solution tagged with 925 KBq of ^{65}Zn pot^{-1}) and Fe (0, 0.5, 1.0% $FeSO_4$ solution tagged with 925 KBq of ^{59}Fe pot^{-1}) were foliarly applied 30, 60 and 90 days after sowing (DAS). Volume of solution used in each spray was 10 ml pot^{-1}. The experiment was carried out in two factorial completely randomized designs. At maturity, the crop was harvested and separated into leaves, psuedostem, grains with husk and dehusked grains. Accumulation of Zn and Fe was measured by measuring activities of ^{65}Zn and ^{59}Fe according to the method described by Mathpal *et al.,* 2018.

Results

Counts per minute of ^{65}Zn

The counts per minute of ^{65}Zn in leaves, psuedostem, grains and dehulled grains of rice varieties are presented in table 1. It is clearly apparent from the data that the main effects of varieties and levels of foliar applied Zn and Fe influenced the accumulation of ^{65}Zn in different plant parts of both rice varieties significantly. The accumulation of ^{65}Zn in leaves and grains of NDR359 was significantly higher than that of PD16. On the other hand, the accumulation of ^{65}Zn in psuedostem and dehulled grains of PD16 was significantly higher than that of NDR359. As regards the influence of Zn levels, an increase in Zn level in foliar spray increased the accumulation of ^{65}Zn in leaves but decreased in psuedostem, grains and dehulled grains significantly. Further, the main effect of Fe levels showed that an increase in Fe level in foliar spray reduced the accumulation of ^{65}Zn in leaves and psuedostem significantly but increased it in grains in comparison to no application of Fe (0% Fe), however, the effect was non significant in case of dehulled grains.

The interaction of variety × zinc × iron influenced the accumulation of ^{65}Zn in leaves, psuedostem, grains and dehulled grains of rice significantly. At 0% ferrous sulphate level, an increase in concentration of zinc sulphate from 0.25% to 0.5% significantly enhanced the accumulation of ^{65}Zn in leaves and dehulled grains of both rice varieties, in psuedostem of PD16 and in grain of NDR359 but decreased it in psuedostem of NDR359 and grains of PD16. At 0.5% ferrous sulphate level, an increase in concentration of zinc sulphate from 0.25% to 0.5% improved the accumulation of ^{65}Zn significantly in leaves and psuedostem of PD16 and in dehulled grains of NDR359 but decreased it in psuedostem of NDR359, grains of both varieties and dehulled grains of PD16. At 1.0% ferrous sulphate level in foliar spray, an increase in concentration of zinc sulphate from 0.25% to 0.5% reduced the accumulation of ^{65}Zn significantly in leaves, psuedostem, grains and dehulled grains of both rice varieties.

The results of the present investigation depicted that the phloem mediated translocation of Zn was likely to be promoted with no or at low Fe levels in the foliar sprays but interfered at higher level of Fe supply (1.0% ferrous sulphate). In yeast, ZmYS1 transporter has a broad specificity for nutrients and could transport mugineic acid bound micronutrients including Zn, Cu and Ni (Schaff *et al.*, 2004; Murata *et al.,* 2006). The phloem specific expression of OsYSL2 suggested its involvement in transport of Fe^{2+}-NA and Mn^{2+}-NA. The involvement of Zn-nicotinamine or Zn-mugineic acid transporters for translocation of Zn and also Zn-deoxymugineic acid for long distance transport of Zn has been indicated by Ishimaru *et al.*, (2011). Since the signaling and synthesis of these ligands is regulated by Fe supply, therefore, the observed response on Zn transport at low or high level of Fe in foliar spray was like to occur in rice.

Counts per minute of ^{59}Fe

The counts per minute of ^{59}Fe in leaves, psuedostem, grains and dehulled grains of rice varieties are presented in Table 2. It is clearly apparent from the data that the main effects of varieties and levels of foliar applied Zn and Fe influenced the accumulation of ^{59}Fe in different plant parts of both rice varieties significantly. The accumulation of ^{59}Fe in leaves and dehulled grains of PD16 was significantly higher than that of NDR359. On the other hand, the accumulation of ^{59}Fe in psuedostem and grains of NDR359 was significantly higher than that of PD16. As regards the main effect of Zn levels, the increase in Zn level from 0% to 0.25% increased the accumulation of ^{59}Fe in leaves, psuedostem and grain but decreased in dehulled grains significantly in comparison to no application of Zn (0% Zn). Further

Table 1: Effect of different levels of Zn and Fe in foliar spray on distribution of ^{65}Zn in different plant parts of two rice varieties.

A. Leaves

Fe levels	PD16 Zn levels			NDR359 Zn levels			Zn × Fe interaction Zn levels		
	0.25	0.50	Av.	0.25	0.50	Av.	0.25	0.50	Av.
0.0	30576	44653	37615	57708	67151	62430	44142	55902	50022
0.5	27913	35427	31670	60494	60389	60442	44203	47908	46056
1.0	29985	27734	28859	54705	29743	42224	42345	28739	35542
Av.	29491	35938	32715	57636	52428	55032	43563	44183	43873
Effect	V	Zn	Fe	V × Zn	Zn × Fe	V × Fe	V × Zn × Fe		
S.Em.±	77	77	95	109	134	134	189		
LSD at d≤0.05	225	225	27	319	390	390	552		

B. Stem

Fe levels	PD16 Zn levels			NDR359 Zn levels			Zn × Fe interaction Zn levels		
	0.25	0.50	Av.	0.25	0.50	Av.	0.25	0.50	Av.
0.0	3479	5992	4736	5667	2583	4125	4573	4288	4430
0.5	2433	3815	3124	4963	2531	3747	3698	3173	3435
1.0	3451	1621	2536	2667	1309	1988	3059	1465	2262
Av.	3121	3809	3465	4432	2141	3287	3777	2975	3376
Effect	V	Zn	Fe	V × Zn	Zn × Fe	V × Fe	V × Zn × Fe		
S.Em.±	10	10	12	14	17	17	24		
LSD at d≤0.05	29	29	36	41	51	51	72		

C. Grains with husk

Fe levels	PD16 Zn levels			NDR359 Zn levels			Zn × Fe interaction Zn levels		
	0.25	0.50	Av.	0.25	0.50	Av.	0.25	0.50	Av.
0.0	2083	1768	1925	2118	2419	2268	2100	2093	2097
0.5	2314	1922	2118	3385	2100	2742	2849	2011	2430
1.0	3521	2107	2814	4522	2286	3404	4022	2196	3109
Av.	2639	1932	2286	3341	2268	2805	2990	2100	2545
Effect	V	Zn	Fe	V × Zn	Zn × Fe	V × Fe	V × Zn × Fe		
S.Em.±	18	18	21	25	30	30	43		
LSD at d≤0.05	51	51	3	72	88	88	125		

D. Dehulled grains

Fe levels	PD16 Zn levels			NDR359 Zn levels			Zn × Fe interaction Zn levels		
	0.25	0.50	Av.	0.25	0.50	Av.	0.25	0.50	Av.
0.0	548	589	568	459	506	482	503	547	525
0.5	560	492	526	538	580	559	549	536	542
1.0	615	554	584	541	442	492	578	498	538
Av.	574	545	559	512	509	511	543	527	535
Effect	V	Zn	Fe	V × Zn	Zn × Fe	V × Fe	V × Zn × Fe		
S.Em.±	5	5	6	7	8	8	12		
LSD at d≤0.05	14	14	NS	19	24	24	33		

increase in Zn concentration from 0.25% to 0.5% increased the accumulation of ^{59}Fe in leaves and grains significantly in comparison to no application of Zn (0% Zn) but decreased it in psuedostem and dehulled grains. The main effect of Fe levels showed that an increase in Fe level from 0.5% to 1% decreased the accumulation

Table 2: Effect of different levels of Zn and Fe in foliar spray on distribution of ^{59}Fe in different plant parts of two rice varieties.

A. Leaves

Fe levels	PD16 Zn levels					NDR359 Zn levels				Zn × Fe interaction Zn levels		
	0.00	0.25	0.50	Av.	0.00	0.25	0.50	Av.	0.00	0.25	0.50	Av.
0.50	18575	24546	22124	21748	3920	21518	26985	17474	11247	23032	24554	19611
1.00	9594	22302	18508	16801	10878	18249	10941	13356	10236	20276	14725	15079
Av.	14084	23424	20316	19275	7399	19884	18963	15415	10742	21654	19639	17345
Effect	V	Zn	Fe	V × Zn		Zn × Fe		V × Fe		V × Zn × Fe		
S.Em. ±	39	47	39	7		55		67		95		
LSD at d≤0.05	113	138	113	195		160		195		276		

B. Stem

Fe levels	PD16 Zn levels					NDR359 Zn levels				Zn × Fe interaction Zn levels		
	0.00	0.25	0.50	Av.	0.00	0.25	0.50	Av.	0.00	0.25	0.50	Av.
0.50	1127	1971	1190	1429	2660	1614	1036	1770	1894	1792	1113	1600
1.00	1225	1666	784	1225	1064	2496	2370	1976	1145	2081	1577	1601
Av.	1176	1818	987	1327	1862	2055	1703	1873	1519	1936	1345	1600
Effect	V	Zn	Fe	V × Zn		Zn × Fe		V × Fe		V × Zn × Fe		
S.Em. ±	13	16	13	23		19		23		32		
LSD at d≤0.05	38	47	38	66		54		66		94		

C. Grain with husk

Fe levels	PD16 Zn levels					NDR359 Zn levels				Zn × Fe interaction Zn levels		
0.00	0.25	0.50	Av.	0.00	0.25	0.50	Av.	0.00	0.25	0.50	Av.	
0.50	872	1106	840	939	1327	1285	1001	1204	1099	1195	921	1072
1.00	865	812	910	862	1068	1257	1544	1289	966	1034	1227	1076
Av.	868	959	875	901	1197	1271	1272	1247	1033	1115	1074	1074
Effect	V	Zn	Fe	V × Zn		Zn × Fe		V × Fe		V × Zn × Fe		
S.Em. ±	10	12	10	17		14		17		24		
LSD at d≤0.05	28	35	NS	49		40		49		69		

D. Dehulled grains

Fe levels	PD16 Zn levels					NDR359 Zn levels				Zn × Fe interaction Zn levels		
0.00	0.25	0.50	Av.	0.00	0.25	0.50	Av.	0.00	0.25	0.50	Av.	
0.50	374	387	387	382	385	346	345	359	379	366	366	370
1.00	441	352	351	381	371	380	383	378	406	366	367	379
Av.	407	369	369	382	378	363	364	368	392	366	366	375
Effect	V	Zn	Fe	V × Zn		Zn × Fe		V × Fe		V × Zn × Fe		
S.Em. ±	5	7	5	9		8		9		13		
LSD at d≤0.05	15	19	NS	NS		NS		NS		38		

of ^{59}Fe in leaves and psuedostem significantly but increased it in grains. A non significant effect was noted in the case of dehulled grains.

The interaction of variety × zinc × iron affected the accumulation of ^{59}Fe in leaves, psuedostem, grains and dehulled grains of rice significantly. At 0.5% ferrous sulphate level, an increase in concentration of zinc sulphate from 0% to 0.25% increased the accumulation of ^{59}Fe significantly in leaves of both rice varieties, in psuedostem and grain of PD16 but decreased it in psuedostem and dehulled grains of NDR359 significantly. At 0.5% ferrous sulphate level, further increase in concentration of zinc sulphate in foliar application from 0.25% to 0.5% increased the accumulation of ^{59}Fe significantly in leaves of both rice varieties but decreased it in psuedostem, grain and dehulled grains of NDR359 in comparison to no application of Zn (0% Zn). At 1.0 % ferrous sulphate level, an increase in concentration of zinc sulphate from 0% to 0.25% enhanced the accumulation of ^{59}Fe significantly in leaves and psuedostem of both rice varieties, in grain of NDR359 but decreased it in dehulled grains of PD16 significantly. At 1.0 % ferrous sulphate, further increase in concentration of zinc sulphate in from 0.25% to 0.5% enhanced the accumulation of ^{59}Fe significantly in leaves of PD16 and in psuedostem and grain of NDR359 but decreased it in psuedostem and dehulled grains of PD16 in comparison to no application of Zn (0% Zn).

Rice being a Stategy II plant (Romheld and Marschner, 1986) possesses 18 YSL members (OsYSL1-18) and OsYSL2 has been reported to transport Fe^{2+}-NA and Mn^{2+}-NA into long distance sinks like leaves and grains (Ishimaru *et al.,* 2010; Koike *et al.,* 2004). OsYSL15, which transports Fe^{3+}-DMA is responsible for root absorption and internal translocation of Fe to long distances owing to its expression in vascular tissues (Inoue *et al.,* 2009; Lee *et al.,* 2009) while OsYSL18 gene is implicated in Fe transport to reproductive tissues (Tsukamoto *et al.,* 2009). In rice, and ferrous transporter OsIRT1 are also expressed in vascular tissue (Ishimaru *et al.,* 2006; Nozoye *et al.,* 2011) for Fe uptake and translocation. Nozoye *et al.,* (2011) reported expression of nicotianamine (NA) transporter (ENA1 and ENA2) in rice which is found to involve in NA transport. This enhanced level of NA inside plant facilitates efficient translocation of Fe and Zn towards grain (Kobayashi and Nishizawa 2012). The above mentioned results are also supported by the findings of Mathpal *et al.,* 2018, who also reported inhibited translocation of Fe with increasing levels of zinc sulphate.

Conclusion

Thus, the antagonistic interaction of foliar applied Zn and Fe levels on their distribution in rice plant was likely due to involvement of common transporters and varying expression of responsible genes in rice varieties.

Acknowledgments

We are thankful to ICAR, New Delhi, India for providing financial support during the period of this study.

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