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Effect of nitrogen fertilizer levels on Fe and protein content, grain breakage and grain yield of rice (*Oryza sativa* L. cv. Khazar)

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Abstract. In order to evaluate the effect of nitrogen fertilizer levels on Fe and protein content and breakage of rice grain (cv. Khazar), a field experiment was conducted at Rice Research Institute of Iran, in 2011. Nitrogen fertilizer levels (10, 20 and 30 kg.ha^{-1}) were applied according to three chlorophyll meter (SPAD-502 reading threshold) (36, 38 and 40) on fully developed leaf of rice plant. Result showed that the nitrogen fertilizer levels had significant effect on grain Fe and protein content and grain breakage. Maximum protein and Fe content was observed in $S_{40}N_{30}$ ($SPAD_{40}$+ 30 kgN) treatment (9.46% and 12.43 mg.kg^{-1}, respectively). Grain breakage reduced in accordance to nitrogen fertilizer increment and there was a negative correlation between nitrogen fertilizer application and grain breakage. Minimum rate of grain breakage was observed in higher nitrogen treatment without any fissure. Maximum grain yield and milling recovery was also observed in $S_{40}N_{30}$ treatment (5960 kg.ha^{-1} and 69.93%, respectively) which that 2.5% higher than recommended fertilizer treatment and 14.6% higher than control. It seems that split application of nitrogen fertilizer may improve rice grain protein content, reduce rice grain breakage and enhance rice grain quality and milling recovery.

Key words: Chlorophyll meter, Fe, Grain breakage, Nitrogen fertilizer, Protein and Rice.

Introduction

Iron (Fe) deficiency anemia has become a worldwide problem in recent year. For most people, more than half of their daily Fe intake comes from cereals (Juliano 1993). Unfortunately among cereals, rice has the lowest Fe concentration in grain (Senadhiraet al. 1998). In Asia, some 60-70% of preschool children and pregnant women are reported to be affected by Fe deficiency (IFPRI 1999).The usual range of Fe in rice grain is about 7.5 to 24.4 mg.kg^{-1}with a mean value 12.1 mg.kg^{-1} (Anonymous 2010). It has been suggested that nitrogen fertilizer, may help to increase the Fe content of rice grain. With split application of nitrogen fertilizer in proper time corresponding to plant requirement, may increase Fe content of rice grain and enhance rice grain nutritional value (Feiet al.2008). Hao et al. (2007) reported that Fe content in different parts of plant may be affected by nitrogen fertilizer, thus with applied 160 kgN.ha^{-1} the Fe amount in the flag leaves and the leaf beneath, brown rice and hull reached 85.5, 30.93 and 28.96% over the control, respectively. It has been reported that rice cultivars with higher protein content are less vulnerable to breakage (Nangju et al. 1970). High protein stored in the seed endosperm cells in the region of breakage, causes the resilience of grain and reduces the rice grain cracking during milling processes. As a result, increased grain protein content increases its hardness and thus increases the milling recovery (Leesawatwong et al. 2003). It has been reported that grain protein content in rice in Asian countries is from 6.3 to 9.2 percent (Abilgos-Ramos et al. 2004). Hao et al. (2007) reported that grain protein content increases with nitrogen input fertilizer application. They reported that grain protein content in three levels of nitrogen (80, 160 and 320 kg.ha^{-1}) increased 0.23, 0.63 and 1.17% over the control, respectively.

The objectives of present experiments was the assessment of split application of nitrogen fertilizer on grain protein content and grain fissuring during milling along with milling recovery in rice cv. Khazar.

Materials and Methods

Field experiment was conducted in Rice Research Institute of Iran, Guilan (37°, 16´ N, 51°, 3´ E and 7 m elevation from sea level)in 2011. The experiment was carried out using a Randomized Complete Block Design with 11 treatments and 3 replications in rice (*Oryza sativa* cv. Khazar). Nitrogen fertilizer rates (10, 20 and 30 kg.ha^{-1}) were applied according to three chlorophyll content (SPAD reading threshold) (36, 38 and 40) using the chlorophyll meter [SPAD-502, Soil and Plant Analysis Development (SPAD), Minolta Camera Co. Osaka, Japan]. Then the mean SPAD value reached below the threshold, nitrogen fertilizer in the form of urea with predeterminedamount was applied. Two control (without N fertilizer) and the recommended nitrogen fertilizer were also considered (Table 1).

Fe content was measured by using Atomic absorption (Varian Spectra 220, USA). Grain N content was determined by Kjeldahl digestion and distillation and then protein content calculated using the factor 6.25 (Feiet al. 2008). In order to determine the breakage of grain from each treatment, grains were observed with laboratory fissure detector. Milling recovery is defined as the ratio of head rice to the total amount of paddy. Analysis of variance and Fisher LSD mean comparisons test were carried out using SAS program (SAS Institute 1996). Correlation coefficients and regression analysis performed using SPSS 11.5 software and for drawing diagrams EXCEL 2007 software were used.

Results

Fe concentration of grains increased in accordance to nitrogen fertilizer increment (Table 3). Also result showed that nitrogen fertilizer application at correct timing and rate, increased nitrogen content in grain and also increased grain protein content (Table 3). Minimum and maximum grain protein content (5.44 and 9.46% respectively) observed in $S_{40}N_{30}$ ($SPAD_{40}$ +30 kgN) that received maximum rate of nitrogen (103 kg.ha^{-1}) and control, respectively. Grain protein content in $S_{40}N_{30}$ was 33% higher than control and 10.5 percent higher than recommended treatment (Table 2). In present experiment the split application of N fertilizer reduced grain breakage of rice (Table 2), the maximum rate of grain

Table 1. Nitrogen split application ratesat different days after transplanting.

Treatments	Time of N fertilizer application					
	Basal	Days after transplanting				Total N application (kg.ha^{-1})
		23	42	55	69	
Control (without N)	0	0	0	0	0	0
Recommended N	45	-	-	22.5	22.5	90
$S_{36}N_{10}$	33	10	-	10	-	53
$S_{36}N_{20}$	33	-	20	-	-	53
$S_{36}N_{30}$	33	30	-	10	-	73
$S_{38}N_{10}$	33	10	10	10	-	63
$S_{38}N_{20}$	33	20	10	10	10	83
$S_{38}N_{30}$	33	30	10	-	30	103
$S_{40}N_{10}$	33	10	-	10	10	63
$S_{40}N_{20}$	33	20	20	20	-	93
$S_{40}N_{30}$	33	30	10	30	-	103

S_{36}, S_{38} and S_{40} = SPAD value 36, 38 and 40; N_{10}, N_{20} and N_{30} = Nitrogen fertilizer levels 10, 20 and 30 kg.ha^{-1}, respectively.

Table 2. Analysis of variances for Fe and protein content, grain breakage, grain yield and milling recovery in rice grain (*cv*. Khazar) under N fertilizer treatments.

S.O.V	d.f	Mean square					
		Grain N content	Grain protein content	Grain Fe content	Grain breakage	Grain yield	Milling recovery
Block	2	0.01	0.02	0.09	12.89	68230.11	0.17
N fertilizer	10	0.88**	5.06**	3.20**	28.79*	1360846.67**	25.58**
Error	20	0.02	0.03	0.46	8.99	208640.20	0.80
C.V (%)		1.95	1.01	6.59	5.47	9.36	1.71

"*" and "**": Significant at 0.05 and 0.01 probability levels, respectively.

Table 3. Nitrogen, Fe and protein content of rice grain and grain breakage under N fertilizer treatments.

Treatments	Grain N content (%)	Grain protein content (%)	Grain Fe content (mg.kg^{-1})	Grain breakage (%)	Grain yield (kg.ha^{-1})	Milling recovery (%)
Control (without N)	0.96f	5.44f	9.06c	2.15a	3790.0d	55.34e
Recommended N	1.58ab	9.19ab	10.67b	0.54b	5210.6b	67.30ab
$S_{36}F_{10}$	1.13e	6.58e	9.32c	1.27ab	4606.1c	57.37d
$S_{36}F_{20}$	1.34cd	7.76cd	9.47c	0.03b	4565.0c	58.34cd
$S_{36}F_{30}$	1.34cd	7.77cd	9.60c	0.73ab	4935.6c	62.74b
$S_{38}F_{10}$	1.36c	7.91c	9.93c	1.67ab	4522.8c	59.55c
$S_{38}F_{20}$	1.48b	8.57b	10.52b	0.00b	5043.3c	64.29b
$S_{38}F_{30}$	1.59a	9.32a	11.42ab	0.00b	5262.9ab	68.87a
$S_{40}F_{10}$	1.28d	7.40d	9.91c	1.68a	4473.3c	59.34c
$S_{40}F_{20}$	1.52b	8.80b	11.17b	0.00b	5651.7ab	66.19b
$S_{40}F_{30}$	1.63a	9.46a	12.43a	0.00b	5960.0a	69.93a

Means in each column followed by similar letter(s) are not significantly different at 5% probabilityusing to LSD test (0.05)

breakage was observed in control (without N fertilizer). With increasing N fertilizer to 103 kg.ha^{-1}, the protein content was elevated and the grain breakage was reduced.

Maximum grain yield (5960 kg.ha^{-1}) was observed in $S_{40}N_{30}$ ($SPAD_{40}$ +30 kgN), which was significantly higher than other treatments, especially than recommended fertilizer (Table 3). Results showed that there was a positive significant correlation ($P \leq 0.01$) between grain yield and nitrogen content of rice grain (r= 0.907**) (Table 4). Maximum milling recovery (69.93%) was observed in $S_{40}N_{30}$ ($SPAD_{40}$ +30 kgN) with no significant difference with $S_{38}N_{30}$ ($SPAD_{38}$ +30 kgN) and minimum milling recovery (55.34%) was observed in control (Table 3).

Regression analysis indicated that there was a positive linear correlation between Fe and nitrogen content of grain and showed that grain nitrogen content predicted 75% of grain Fe content variations (Fig. 1). Correlation coefficients also showed that there was a negative correlation between grain nitrogen content and grain breakage (Table 4) (Fig. 2). Regression relation between grain protein content and grain breakage showed a negative significant relationship, thus 71% of grain breakage may be predicted by grain protein content (Fig. 3).

Discussion

Protein content of rice grain increased in parallel with nitro-

Table 4. Correlation coefficients *(r)* between grain nitrogen, Fe and protein content, grain breakage, yield and milling recovery in rice (*cv*. Khazar).

	Grain N content	Grain yield	Grain Fe content	Grain protein content	Milling recovery	Grain breakage
Grain N content	1					
Grain yield	0.907**	1				
Grain Fe content	0.868**	0.875**	1			
Grain protein content	0.999**	0.907**	0.863**	1		
Milling recovery	0.936**	0.905**	0.927**	0.934**	1	
Grain breakage	-0.801**	-0.810**	-0.667*	-0.801**	-0.751**	1

** Significant at 1% probability level
n = 33

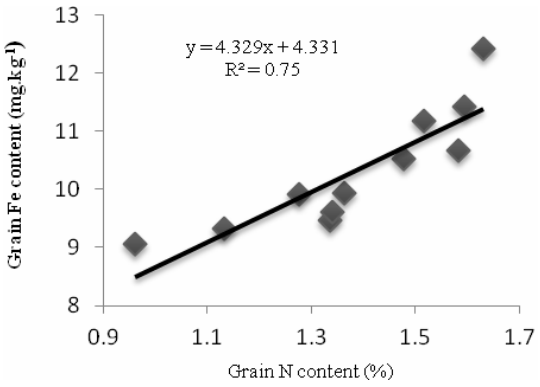

Figure 1. Regression relation between grain Fe content and grain N content in rice (*cv*. Khazar).

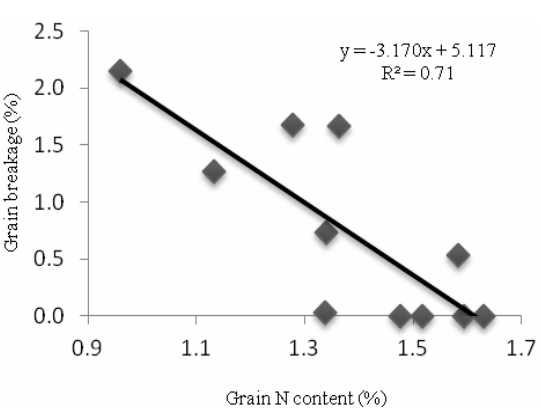

Figure 2. Regression relation between grain breakage and grain N content in rice (*cv*. Khazar).

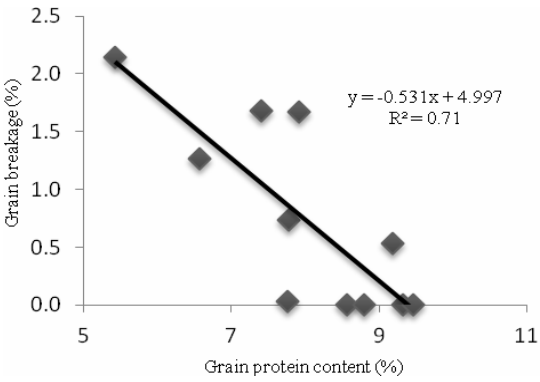

Figure 3. Regression relation between grain breakage and grain protein content in rice (*cv*. Khazar).

gen fertilizer incrementand nitrogen fertilizer application at boot stage reduced fissured grain and had significant effect on head rice yield (Hao et al. 2007, Wopereis-Pura et al. 2002). Hasalsobeen reported that the head rice rate affected by time and rate of nitrogen fertilizer and nitrogen application at boot stage increased head rice yield to 55.7% (Fei et al. 2008). In present experiment the difference between nitrogen treatments and control showed that there was a positive effect of nitrogen fertilizer on rice grain rigidity. The difference between nitrogen treatments and recommended treatment also showed that nitrogen fertilizer in proper time and proper rate, according to plant requirement, increased grain integrity and reduced breakage.

Higher nitrogen mobility from growing organs to grains and higher leaf N concentration during grain filling lead to higher grain yield in rice (Shi et al. 1994 and Kamiji&Horie 1988). Nitrogen application during maximum tillering and panicle initiation stages in rice, enhances some nutritional attributes of rice grain. Application of N at these stages may lead to improvement of nitrogen contents and antioxidant enzymes activity (e.g. catalase and superoxidase activities), the delay of senescence, enhancement of photosynthetic activities and accelerated translocation of photoassimilates toward grains and as a result improvement of rice grain quality (Mo et al. 2004). Results showed that nitrogen split application according to plant requirement and its application during maximum tillering and panicle initiation stages, resulted in higher rate of accumulation of nitrogen in grain, increased grain hardness and reduced grain breakage in rice. This may have led to increased head rice rate and reduced broken grain and increased milling recovery.

In conclusion, based on the results of the present study, nitrogen split application in proper time and rate according to the plant demands, leads to higher nitrogen accumulation in rice grain. The positive correlation between grain nitrogen content, grain Fe and protein content and the significant negative correlation between grain nitrogen content and grain breakage demonstrated that the nitrogen fertilizer split application increased the Fe and protein content of grain and additionally raised the nutritional value of rice grain. This may lead to increase grain resistance to milling process and improve milling recovery and quality of product.

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