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Anil Kumar
Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Bhudeo Rana Yashu
Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Uday Pratap Singh
Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Sanjay Kumar
Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence
Bhudeo Rana Yashu
Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Proximiation of zinc and iron of different indigenous and exotic lines of rice in Northern plain region

Anil Kumar, Bhudeo Rana Yashu, Uday Pratap Singh and Sanjay Kumar

Abstract

Rice (*Oryza sativa*) is a nutritive staple foods and one of the most important cereal crops, especially for people in Asia, but the consumption outside Asia has increased, recently. It provides the bulk of daily calories for many companion animals and humans. It has good digestibility, biological value and good quality protein due to the presence of higher amount of lysine. Rice has greater variability of the glycemic index depending on type, cooking method, etc. Rice is also one of food which is considered to be a potential food vehicle for the fortification of micronutrients because of its regularly consumption. Zn and Fe deficiency is a global nutritional problem and intensity of the issue is even severe in developing countries. Cereal grains are key to fulfill a person's daily energy requirements, but they have very low grain Zn-Fe concentrations, especially when grown in Zn-Fe deficient soils. Seeds of 52 lines of rice were used in this investigation indicated that line 383441 contains high amount of N, P, K, Na, Fe, Zn and nitrogen protein. Therefore, this line may be considered for utilization by breeders for development of high N, P, K, Na, Fe, Zn and nitrogen protein containing lines/varieties. With regards to Zn and Fe status in grains, line 282418 contained the maximum amounts of these elements. Therefore, this line may be utilized by breeder to develop high Zn and Fe containing lines/varieties.

Keywords: Zinc, iron, indigenous, exotic lines, rice, Northern plain region

Introduction

Rice (*Oryza sativa*) is a nutritive staple foods and one of the most important cereal crops, especially for people in Asia, but the consumption outside Asia has increased, recently (Orthoefer, 2005) [8]. It provides the bulk of daily calories for many companion animals and humans (Ryan, 2011) [9]. It has good digestibility, biological value and good quality protein due to the presence of higher amount of lysine. Milled rice has been shown to contain about 78% carbohydrate. Many reports on variability in protein content in rice are available. Usually, the average value of total crude protein content is taken as 7%, and rice having more than 10% total crude protein is considered to be of high protein type (Resurrection *et al*. 1979) [3]. Milled rice is referred to as "polished" or "whitened" and there are various degrees or fractions of polishing-white rice implies between 8 and 10% bran removal. In general, the more rice bran is removed from the grain during polishing, the more vitamins and minerals are lost. Protein loss due to milling is estimated at between 10 and 15 percent (Malik and Chaudhary 2002) [1]. The glycemic index is one of the popular issues in the world, and people are rethinking whether consume rice or not. Some study showed that rice consumption is related to the higher risk of diabetes mellitus (McKeown *et al*., 2002; Hu *et al*., 2002) [10]. The other studies showed the inverse one. In fact, rice has greater variability of the glycemic index depending on type, cooking method, etc. Rice is also one of food which is considered to be a potential food vehicle for the fortification of micronutrients because of its regularly consumption. Many studies tried to add iron and zinc to rice in order to reduce the nutritional problems, especially micronutrient deficiencies. A study in Bangladeshi children and their care givers showed that rice was the main source of zinc intake, providing 49% of dietary zinc to children and 69% to women (Arsenault *et al*., 2010) [12, 13]. Deficiencies of micronutrients (hidden hunger) are a major global health problem and more than 2 billion people in the world are estimated to be deficient in key vitamins and minerals, particularly vitamin A, iodine, iron and zinc (FAO, 2011). Mineral elements play numerous beneficial roles due to their direct and indirect effects in both plant and human metabolism and the deficiencies or insufficient intakes of these nutrients leads to several dysfunctions and diseases in humans (Welch and Graham, 1999) and also lead to entire failure of crops and lower content of trace elements in plant parts (Cakmak, 2008) [14].

Several strategies have been suggested as intervention programmers for the reduction of micronutrient malnutrition in human populations (Ng'uni *et al*., 2012). They include food fortification, dietary supplementation by use of complementary tablets. Other strategies are dietary diversification and micronutrient biofortification programs through plant breeding (Ng'uni *et al*., 2012). Comparatively, plant breeding has been identified as been potentially more sustainable and less expensive, since seeds could reach a larger number of people without necessarily changing consumer's behavior (Ortiz-Monasterio *et al*., 2007; Cakmak, 2008; Ng'uni *et al*., 2012) [16, 14]. This solution, however, requires a comprehensive exploration of potential genetic resources and indepth understanding of the physiological and genetic basis of nutrient- accumulation process in crops tissues (Chatzav *et al*., 2010) [17]. One major strategy to improve the level of mineral nutrients and vitamins in staple food crops is to exploit the natural genetic variation in food crops (Ortiz-Monasterio *et al*., 2007; Cakmak, 2008; Gomez-Becerra *et al*., 2010) [16, 14, 18].

Zn and Fe deficiency is a global nutritional problem and intensity of the issue is even severe in developing countries. Cereal grains are key to fulfill a person's daily energy requirements, but they have very low grain Zn-Fe concentrations, especially when grown in Zn-Fe deficient soils. Both nutrientdeficiency can be addressed in several ways viz., nutritional diversification, food enrichment and bio fortification. Several limitations regarding nutritional diversification and food enrichment favored Zn-Febio fortification as a perpetual solution of malnutrition. Among the potential bio fortification options to rectify Zn-Fe deficiency, plant breeding approaches and agronomic bio fortification offers major advantage. Current review appraised the possible role of Zn and Fe in plants, its uptake, translocation and partitioning efficiencies in cereal grains that is driven by various agronomic, breeding and biotechnological approaches. There is anhonest need to integrate Zn-Fe in rice production systems by using agronomic and conventional breeding tools. Likewise, agronomic biofortification is economically sustainable and practically adoptable solution to overcome the Zn-Fe deficiency issue in rice.

Material Methods

Seeds of 52 lines of rice were procured from National Bureau of Plant Genetic Resources, New Delhi (Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University).Seeds were dehusked by using palm dehusker. To obtain polished grains, dehusked grains were gently rubbed the between sand papers till aleurone layer was completely removed.

Polished rice grains were digested using di-acid digestion method. It consisted of concentrated H_2SO_4 and perchloric acid (60%). 100 mg of polished rice grains were taken in Kjehldahl flasks containing 2.0 mL of concentrated H_2SO_4. The samples were heated for 1-2 minutes and then 2.0 mL of perchloric acid (60%) was added to the samples. Then the samples were heated directly in the digestion block till it became colourless. Samples were cooled and volume was raised up to 100 mL with double glass distilled water and used for the estimation of mineral element Fe, Zn. A blank was prepared by taking same amount of H_2SO_4 and perchloric acid without samples and treating in the same manner.

Estimation of iron

Iron contents were estimated in the digested plant samples by Atomic Absorption Spectrophotometer (UNICAM-969). Atomic Absorption Spectro-photometer required standard solution of iron in three concentrations *viz.* 2 ppm, 5 ppm and 9 ppm were use as standards for estimation of iron content. The reading was obtained in ppm and they were expressed as µg g^{-1} dry weight of the sample.

Estimation of zinc

Zinc contents were estimated in the digested plant samples by Atomic Absorption Spectrophotometer (UNICAM-969). Atomic Absorption Spectro-photometer required standard solutions of zinc in three concentrations *viz.* 0.5 ppm, 1.0 ppm and 1.5 ppm. Values were obtained in ppm and then expressed as µg g^{-1} dry weight of the sample.

Statistical analysis

For determining the significance of difference among the genotypes means, and to draw the valid conclusions, the data obtained by various observations were subjected to statistical analysis by Duncan's multiple range test (DMRT) by using software version SAS 9.2. Correlation between analyzed parameters were also calculated and significance of correlation was determined at degree of freedom n-2 (n= number of observations).

Categorization of lines

Categorization of lines under high, medium and low mineral nutrient contents in grains was on the basis of relative amounts. It was calculated using formula as given below:

$$X = \frac{\text{Highest value of a nutrient in a line -lowest value of the nutrient in a line}}{3}$$

Lines under high mineral nutrient category (a) = Line with highest nutrient content - X

Lines under low mineral nutrient category (b) = Line with lowest nutrient content + X

Lines under medium mineral nutrient category = lines between a and b

Chemicals and glass wares and plastic wares

All chemicals used were of analytical reagent grade. Glass wares of ASGI make unless stated otherwise. Plastic wares were of TORSONS make.

Result and conclusion

Fe content (µg g^{-1} dry weight)

Data regarding Fe content (µg g^{-1} dry weight**)** are presented in Table 4.9. Line 282418 contained the highest Feand line 578485 the lowest. The collections were also classified in high, medium and low Fe content categories (Table 4.10). Lines 282418> 577568 contained high Feinpolishedgrains. Lines 298552> 337528> 383441 contained medium Fe and lines 426076>577587>450026>580440>468824>361732>98858>578030>282386>328439>282450>313140> 350107> 578485> 578465 contained low Fe in polished grains. However, Fe was in traces in lines 467349, 577324, 580272, 580270, 580439, 85722, 331166, 580185, 580344, 298479, 335396, 464906, 384176, 580254, 578478, 413609, 466532, 554782, 580290, 577282, 577109, 311862, 145594, 282212, 578118, 298559, 337569, 496926, 298572, 416700, 278774, 377560, as it was below the detection limit of the instrument in them.

Zn content (µg g^{-1} dry weight)

Data regarding Zn content (µg g^{-1} dry weight) are presented in Table 2. The Duncan’s Multiple Range Test (DMRT) indicated the difference among mean values of the 52 lines of rice.The collections were also classified in high, medium and low Zn content categories (Table1). Line 282418 contained the highest amount of Zn in polished grains and line 298559 the minimum Zn. Lines 282418> 578478> 383441 were categorized as lines with high Zn contents in polished grains. Lines 426076>450026>328439>298552>298479 were categorized as medium Zn containing lines, while lines 577587>337528>282450>580254>361732>85722>466532>580270>577568>467349>145594>278774>580290>98858>577282>466824>577324>580185>298572>337569>384176>580272>578465>580440>413609>578485>377560>311862>313140>282386>580439>577109>282212>578118>578030>350107>335396>331166>416700>496926>580344>464906>554782>298559 were categorized as low Zn containing lines.

It is the major food for people in third world countries where protein and mineral malnutrition, especially Fe and Zn, is of common prevalence (Batista *et al*. 2012) [6]. Though rice grains are good source of Na, K, P and Ca (Zubair 2012) [5], but are poor in Fe and Zn (Fang *et al.* 2008) [15].

In polished grains of rice concentration of different mineral element were found to follow a pattern as K> Mg >Ca> Si > Zn > Na > Al and Fe (Jung *et al*. 2005) [7].

Out of 52 lines, Fe in the detectable range was of the instrument was present only in the 20 lines. However, in remaining 32 lines it was below the detection limit of the instrument. Hence, it is inferred that in these lines Fe is in traces. In lines where Fe was detected, concentration ranged between 191.13-0.27 µg g^{-1} dry weights. Literature indicated variations in Fe concentration in rice grains; as between 186-317 µg g^{-1} dry weight (Zubair 2012) [5]; 0.01 mg g^{-1} dry weight (Babu *et al*. 2013) and 0.02 mg g^{-1} dry weight (Welch and Graham2004).

In contrast to Fe, presence of Zn was detected in all the 52 lines (Table 2). Zn concentration ranged between 11.13-0.11 µg g^{-1} dry weights. Zn content in polished rice grains is reported to be 0.027 mg g^{-1} dry weight (Mbatchou and Dawda 2013) [2], 0.19-0.32 mg g^{-1} dry weight (Zubair 2012) [5], 0.015-0.039 mg g^{-1} dry weight (Jung *et al*. 2005) [7], 0.044 mg g^{-1} dry weight (Babu *et al*. 2013) and 0.012-0.015 mg g^{-1} dry weight (Welch and Graham 2004).

It is recommended that daily intake of Fe and Zn is 10-15 mg and 12-15 mg, respectively normal Zn requirement in human beings (Welch and Graham 2004). However, in the present study content of Fe and Zn was too low to fulfill such requirement.

Correlation between different parameters indicated that Fe and Zn contents are positively correlated in rice grains (Table 2). Though other parameters also correlated with each other positively, but the significance of this investigation indicated that high Zn containing rice lines also contain higher amount of Fe and protein. However, this aspect needs further in depth investigation.

Present investigation indicated that line 383441 contains high amount of N, P, K, Na, Fe, Zn and nitrogen protein. Therefore, this line may be considered for utilization by breeders for development of high N, P, K, Na, Fe, Zn and nitrogen protein containing lines/varieties. With regards to Zn and Fe status in grains, line 282418 contained the maximum amounts of these elements. Therefore, this line may be utilized by breeder to develop high Zn and Fe containing lines/varieties.

In present investigation yield potential of studied lines were not observed. It will be worthwhile to study yield attributes, especially of lines rich in Fe and Zn in grains and to make breeding approaches to combine high yielding ability with high Fe and Zn contents.

Table 1: Zn content (µg g^{-1} dry weight) in polished grains of 52 lines of rice.

S. No.	Lines with high Zn	Zn content (µg g^{-1} dry weight)	S. No.	Lines with medium Zn	Zn content (µg g^{-1} dry weight)	S. No.	Lines with low Zn	Zn content (µg g^{-1} dry weight)
1.	282418	11.13 ^{a*}	1.	426076	7.13 ^{d}	1.	577587	3.67 ^{i}
2.	578478	9.90^{b}	2.	450026	7.02 ^{p}	2.	337528	3.43^{j}
3.	383441	8.15 ^{c}	3.	328439	6.02^{f}	3.	282450	3.32^{k}
			4.	298552	5.05^{g}	4.	580254	2.31^{l}
			5.	298479	4.66^{h}	5.	361732	2.30^{m}
						6.	85722	2.26 ^{n}
						7.	466532	1.74^{o}
						8.	580270	1.61 ^{p}
						9.	577568	1.52^{q}
						10.	467349	1.50^{r}
						11.	145594	1.46^{s}
						12.	278774	1.45^{t}
						13.	580290	1.35^{u}
						14.	98858	1.29^{v}
						15.	577282	1.29^{v}
						16.	466824	1.27^{w}
						17.	577324	1.24 ^{x}
						18.	580185	1.21^{y}
						19.	298572	1.21^{y}
						20.	337569	1.16^{z}
						21.	384176	1.113^{a}
						22.	580272	1.11^{b}
						23.	578465	1.05^{c}
						24.	580440	1.04^{d}

						25.	413609	1.01^{e}
						26	578485	1.01^{e}
						27.	377560	0.98^{f}
						28.	311862	0.97^{g}
						29.	313140	0.92^{h}
						30.	282386	0.90^{i}
						31.	580439	0.86^{z}
						32.	577109	0.85^{k}
						33.	282212	0.84^{l}
						34.	578118	0.76^{m}
						35.	578030	0.71^{n}
						36.	350107	0.69^{o}
						37.	335396	0.67^{p}
						38.	331166	0.67^{p}
						39.	416700	0.54^{q}
						40.	$496926^{\#}$	0.50^{r}
						41.	580344	0.45^{s}
						42.	464906	0.38^{t}
						43.	554782	0.36^{u}
						44.	298559	0.11^{v}

$^{\#}$ 496926 (other lines are indigenous lines).

*Indigenous lines with different letter differ significantly with each other.

Table 2: Fe content (µg g^{-1} dry weight) in polished grains of 52 lines of rice

S. No.	Lines with high Fe	Fe content (µg g^{-1} dry weight)	S. No.	Lines with medium Fe	Fe content (µg g^{-1} dry weight)	S. No.	Lines with low Fe	Fe content (µg g^{-1} dry weight)
1.	282418	191.13	1.	298552	128.39	1.	426076	43.25
2.	577568	159.29	2.	337528	123.78	2.	577587	32.67
			3.	383441	118.15	3.	450026	14.43
						4.	580440	11.70
						5.	468824	10.39
						6.	361732	8.27
						7.	98858	4.07
						8.	578030	2.72
						9.	282386	1.61
						10.	328439	1.16
						11.	282450	1.01
						12.	313140	1.00
						13.	350107	0.77
						14.	578485	0.74
						15.	578465	0.27
						16.	467349	Traces
						17.	577324	Traces
						18.	580272	Traces
						19.	580270	Traces
						20.	580439	Traces
						21.	85722	Traces
						22.	331166	Traces
						23.	580185	Traces
						24.	580344	Traces
						25.	298479	Traces
						26.	335396	Traces
						27.	464906	Traces
						28.	384176	Traces
						29.	580254	Traces
						30.	578478	Traces
						31.	413609	Traces
						32.	466532	Traces
						33.	554782	Traces
						34.	580290	Traces
						35.	577282	Traces
						36.	577109	Traces
						37.	311862	Traces
						38.	145594	Traces
						39.	282212	Traces
						40.	578118	Traces
						41.	298559	Traces
						42.	337569	Traces

						43.	496926	Traces
						44.	298572	Traces
						45.	416700	Traces
						46.	278774	Traces
						47.	377560	Traces

$^{\#}$ 496926 (other lines are indigenous lines).

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