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Research Article

THE ROLE OF VARIABILITY IN BIOFORTIFICATION AND IN VITRO CONSERVATION OF DIVERSED RICE LANDRACES OF WEST BENGAL

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ABSTRACT

In developing countries like India and south east Asia, biofortification is an emerging cost-effective strategy to address global malnutrition. This strategy involves supplying of micronutrients such as iron and zinc in the staple foods by using conventional plant breeding and biotechnology. *In vitro* techniques that can be applied to plant breeding programs in order to accelerate the process of obtaining a pure line, its conservation, transgenic and developing the somaclonal varients. An efficient *in vitro* plant regeneration system is required for successful crop improvement through genetic engineering and hence present investigation aims at estimation of iron and zinc from fifteen traditional rice cultivars of gangetic alluvial zone and to define its role in biofortification programme, conservation of these landraces with *in vitro* approach. Significant variability was found for the traits taken for the study. Iron concentration ranged from 35.2 μg/g (Ladhula) to 95.3 μg/g (Gandhibiroin) and zinc concentration ranged from 18.7 μg/g(Nakrasal) to 28.5 μg/g (Daharnagra) in traditional rice. Also *in vitro* study showed good response to development of somaclonal variations in a promising aromatic line Gandhibiroin (GB). Number of embryos per explants and percent culture with somatic embryo was found to be 9.7 embryos per explants and 65 percent of somatic embryo development at the concentration of 10.5 μM. This study will help the researchers in the area of biotechnology and bioinformatics to produce the quality food with full of micronutrients keeping pace with time using *in vitro* techniques.

Keywords: Conservation strategy, iron content, traditional rice, variability, TDZ, BAP

1. INTRODUCTION

In the Asian diet rice contributes almost 28-54% proteins. The ability of a species to adapt to changing environment and cope with stress and its maximum utilization in breeding depends upon the genetic diversity it possesses. Rice is one of the most important cereals and is the main food crop to the lives of billions of people around the world and genetic improvement of any crop mainly depends upon the amount of genetic variability present in the population. Malnutrition is the most common cause of zinc deficiency and 25% of the world's population is at risk of zinc deficiency [1]. Iron deficiency (ID) is the most prevalent micronutrient deficiency worldwide, affecting mainly children under 5 and women of childbearing age living in the poorer communities of the developing world. The leading nutrition-related causes of disability in Latin America and the Caribbean (LAC) are childhood and maternal underweight, iron-deficiency anemia, zinc deficiency, and vitamin. Iron deficiency during childhood and adolescence impairs physical growth, mental development, and learning ability. In adults, it reduces the ability to do physical labor. Severe anemia increases the risk of death for women in childbirth. Deficiencies of zinc, iron and vitamin A in human population of developing countries were noticed and particularly, zinc deficiency is the fifth major cause of diseases and deaths in these countries. Health problems caused by zinc deficiency include anorexia, dwarfism, weak immune system, skin lesions, hypogonadism and diarrhoea [2]. Males aged 15 to 74 years need about 12 to 15 mg of zinc daily while females aged 12 to 74 years need about 68 mg of zinc daily [3]. Iron dependent anemia in turn leads to maternal mortality, preterm births [4] decreases immunity [5] and increases placental weight [6] during pregnancy. Biofortification of staple foods could be a more cost-effective and sustainable strategy. Biofortification is the process of increasing the level and/or bioavailability of essential nutrients in crops by traditional plant breeding or genetic engineering. Rice is a major source of dietary carbohydrate for more than half of the world's population [7]. Rice protein ranks high in nutritional quality among cereals, though protein content is unassuming. Unmilled rice (brown rice) provides 4.3% to 18.2% protein, averaging 9.5% based on 17,587 cultivars in the IRRI germplasm [8]. The nutritional value of rice and estimated that 100 gm of rice consist of following components: calories (kcal)-345, Moisture-13.7 gm, Carbohydrate-13.7 gm, Protein-6.8 gm, Fat-0.5 gm, Fibre-0.2 gm, Phosphorus-160.0 mg, Minerals-0.6 gm, Essential amino acid-1.09 mg, Calcium-10.0 mg, Iron-0.7 mg, Magnesium-90.0 mg, Riboflavin-0.06 mg, Thiamine-0.06 mg, Niacin-1.9 mg, Folic acid-8.0 mg and Copper-0.14 mg [9]. All staple food crops only rice accounts for the dietary energy requirement of almost half of the World population and over 90 percent of it is produced and consumed in Asia. Due to high dependency on rice production to meet the growing challenges for food security many country have evolved specific strategy for sustainable rice production [10].

Modern agriculture had reasonable success in meeting the energy needs of developing countries. In the past 40 years, agricultural research in developing countries has met Malthus' challenge by placing increased cereal production at its centre. However, agriculture must focus on a new paradigm that will not only produce more food but also better quality food. Biofortification of staple food crops for enhanced micronutrient content through genetic manipulation is the best option available to alleviate hidden hunger with little recurring costs [11, 12]. Rice production needs to be increased to keep pace with the growing population; however, its productivity may be affected by several biotic and abiotic stresses. The genetic diversity for these stresses is limited in the current rice cultivars. There is urgent need to broaden the genetic base of this important crop by introgressing genes from diverse sources. Land races genotypes, wild and weedy relatives are an important source of useful genes [13, 14]. Iron and zinc contents in brown and milled rice of national and international germplasm need to be estimated for identification of donors for future deployment in the nutritional breeding program and also to get mapping information on association of iron and zinc contents in grains [15]. The objective of this work was to study the variability present among the landraces of rice with remarkable iron and zinc content to combat malnutrition, their conservation and *in vitro* studies for their conservation for future breeding to improve human nutrition.

2. MATERIAL AND METHODS

2.1. Estimation of Fe and Zn

A total of 15 rice cultivars, collected from Zonal Adaptive Research Station, Krishnagar including one collected from Assam were used for mineral content analysis. These cultivars were developed in a randomized complete block design with three replications at the research farm of Zonal Adaptive Research Station, (23°24'N latitude and 88°31'E longitude with an altitude of 9.75 meters above mean sea level) Krishnagar, Nadia, West Bengal, India during kharif season for three consecutive years of 2010, 2011 and 2012. The soil was slightly acidic with pH of 6.0, low soluble salts (EC of 0.15 dS m^{-1}), medium organic carbon content (0.57%), Total N (0.056%), medium in available P (25.28 kg ha^{-1}) and K (148.77 kg ha^{-1}). The experimental site belongs to tropical humid climate having the average rainfall of 1464 mm, most of the amount falls in between June to September. The minimum temperature reaches 7.6°C in the month of January and the maximum 41.1°C in the month of May. It has been observed that 74.7% of the annual rainfall is obtained during June to September and more than 83.6% during June to October. The materials were transplanted in 3.0×2.85m^2 plot with plant to plant spacing 15 cm within a row and row to row spacing of 20 cm. plot to plot distance was 60 cm. A random sample of five competitive plants was used for observations on different traits under study. Crop was raised following recommended package of practices. Fertilizers ($N:P_2O_5:K_2O$) @ 50:25:25 kg ha^{-1} were applied. Iron and zinc Content of the soil samples varied between 20.1-39.0mg/g. The observation of various characteristics was recorded at different stages of growth with appropriate procedures as per the DUS test guidelines of PPV & FR Act, 2001.

One gram oven dried ground dehusked seed samples were collected in a 150ml conical flask. To this, 25-30ml diacid mixture (HNO_3 :$HClO_4$; 5:4 v/v) was added and kept overnight. Next day, it was digested by heating till clear white precipitates at the bottom. The crystals were dissolved by diluting in doubled distilled water. The contents were filtered through Wattmann no. 42 filter paper. The filtrate was made to 50ml with doubled distilled water. The acid digested samples were used for the determination of Fe and Zn contents by Atomic Absorption Spectrophotometer 2380, Perkin Elmer (USA) according to the method of Lindsey and Norwell. Mean values were taken from the measurements of three replicates and standard error of the means was calculated. Difference between means was determined by one way ANOVA. Analyses were done using statistical package for social sciences (SPSS) for window, version 13.0.

2.2. *In vitro* response of the promising cultivar Gandhibiroin

Investigation on *in vitro* response was done initially at the plant tissue culture research laboratory of Sripat Singh College for observation of variability pattern in rice. The seeds were surface-sterilized with 70% ethanol for 2 min followed by 0.2% (w/v) aqueous $HgCl_2$ solution for 5 min and finally rinsed 5-6 times with sterilized distilled water. The sterilized seeds were germinated by soaking in sterile distilled water for 16 hrs in the dark on a orbital shaker at 200 rpm as well as on semisolid medium either on MS9 basal medium or MS containing TDZ (10μM) up to 6 days at 27±2°C under light conditions. For the initiation of callus and induction of somatic embryos, different types of explants were taken and cultured on MSB medium (MS salts and B5 vitamins) containing % (w/v) sucrose and supplemented with various type of growth regulators such as TDZ and BAP in different concentrations and combinations.

3. RESULTS AND DISCUSSION

3.1. Breeding strategies

The first pre-requisite for initiating a breeding program to develop micronutrient-rich genotypes is to screen the available germplasm and identify the source of genetic variation for the target trait, which can be used in crosses, genetic studies, molecular marker development and to understand the basis of micronutrient uptake process. Iron and Zinc contents in grains also depend on the micronutrient uptake and translocation efficiency from root to grains. With the help of molecular markers, the loci associated with nutrient content in grains can be identified and used for marker-assisted selection in regular breeding programs. Rice breeders are expected to concentrate on increasing the total nutrient content in the endosperm of the grain, the part that remains after milling. The range of iron and zinc concentrations in brown rice is 6.3-24.4 g g-1 and 13.5-28.4 μg g^{-1} respectively. There was approximately fourfold difference in iron and zinc concentrations, suggesting vast genetic potential to increase the concentration of these micronutrients in rice grains [16].

Variation in respect to iron and zinc contents in rice cultivars was studied. There was a significant difference in iron contents of dehusked grains (p=0.001) among the various rice cultivars. It ranged between 95.3 μg/g (in Gandhibiroin) to 35.2 μg/g (in laldhula) and zinc concentration from 28.5 μg/g (in Daharnagra) to 18.7 μg/g (in Nakrasal) (Table 1). It was reported wide range of variations of iron contents in brown rice with in the eight sets of genotypes [17]. Biofortification is defined as the enhancement of micronutrient levels of staple crops through biological processes, such as plant breeding and genetic engineering [18]. It could be effective in reducing the problem of malnutrition as part of a strategy that includes dietary diversification, supplementation, commercial fortification and other aspects. HarvestPlus is a CGIAR initiative which started "biofortification" umbrella through which international agricultural and research centres have made efforts to develop new breeds of staple foods that are rich in vitamins and minerals. Biofortification has multiple advantages, including the fact that it capitalizes on the regular daily intake of a consistent amount of staple food by all family members. Rice is one of the global staple foods being cultivated since 10,000 years and provides 70–80% or more daily calorie intake for 3 billion people, which is almost half of the world's population. The grain has large genetic variability in micronutrient concentration. Hence, rice was included in biofortification program [19], which implicitly targets low income households who have limited access to commercially marketed fortified foods that are more readily available in urban areas.

3.2. Findings from *In vitro* study

A promising aromatic ice rice landrace was taken for *in vitro* study and the results (Table 2) showed that the continuous supply of TDZ was required for the induction of somatic embryo. Combined treatment of TDZ and BAP showed a different picture on its embryo development and its reflection in counting the percentage culture with somatic embryo. Number of embryos per explants and percent culture with somatic embryo was found to be 9.7 and 65 at the concentration of 10.5 μM of TDZ and 4.9 and 32 in combination of BAP and TDZ respectively. The proliferation of embryogenic cells, maturation and conversion of somatic embryos is apparently influenced by a variety of factors, some of which can be controlled during the culture process, and some of which are as yet undefined. Therefore, further knowledge regarding better production, maturation and germination of embryos may make this system more suitable so that could be use as routine experiment for improvement of this variety. Still it is a challenge for the researchers to know about the molecular events associated with the callus differentiation in indica rice. A thiaduazuron dependent regeneration protocol was developed for efficient embryogenesis in indica rice. The regenerating embryogenic calli induced by TDZ for 10 days showed transcriptional modulation of a number of genes associated with photosynthesis, hormone metabolism, plant development, signal transduction, light response and plant defense. 18 candidate mi RNA was predicted to target genes expressed differentially in the embryogenic calli grown in TDZ containing medium. The majority of the photosynthesis related genes unregulated in differentially calli were not expressed. Most of the genes down regulated in the developing seeds. The transcriptome of differentially callus were mostly closely resembled with that germinating whole seeds [20].

Table 1: Rice *landraces* with iron and Zinc content in grain

Sl. No.	Name of the Cultivars	Iron Content (μg/g)	Zinc Content (μg/g)
1	Ranisal	40.2	23.1
2	Badhabna	50.1	24.4
3	Machkata	86.5	19.2
4	Laldhula	35.2	25.6
5	Dhuladhan	55.1	21.5
6	Dhuri	70.1	23.9
7	GandhiBiroin (GB)	95.3	20.1
8	Suakalama	77.6	22.4
9	Nakrasal	92.2	18.7
10	Asanlaya(white)	45.8	27.8
11	Asanlaya(red)	69.3	23.6
12	Pubalgara	94.2	24.4
13	Daharnagra	57.9	28.5
14	Kalonunia	47.8	23.3
15	Tulsibhog	95.2	20.8
		Highest value-99.2 (12) Lowest value-35.3 (4)	Highest value-28.5 (13) Lowest value-18.7 (9)

Table 2: Treatment of different plant growth regulators, concentration and combinations on the callus obtained from seed explants on TDZ for induction and proliferation of somatic embryos of rice landrace Gandhibiroin

Treatments (μM)	No. Of embryos per explants	% culture with somatic embryo
5.0 TDZ	5.13	25
10.5 TDZ	9.17	65
15.0 TDZ	5.5	33
10.5 TDZ+15.5 BAP	4.9	32

4. CONCLUSION

Biofortification in addition to molecular marker technology and *in vitro* studies expedites the development of rice varieties with improved iron and zinc contents through identified genomic regions. Landraces of rice have beneficial genes in them and have immense potentiality to fight under different environmental hazards. Lethal effects during pregnancy in women and iron deficiency in mass people and children can be controlled by feeding, cultivating and hence conserving these invaluable rice landraces. Different coloured rice present nationally or at global level needs characterization and estimation of iron and zinc content present in the rice followed by observation of *in vitro* response. Rice breeding can be improved by varietal development through somaclonal variation and genetic engineering techniques. Rice lines in the genetic background of elite rice varieties possessing optimum concentration of zinc in the endosperm will be developed and released for cultivation.

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Conflict of Interest

The author declares no conflict of interest

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