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Folk rice: Genetic storehouse for Biofortification: A review

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Abstract
Micronutrient malnutrition, commonly known as 'hidden hunger' mainly due to iron, zinc and pro-vitamin A deficiencies, has emerged as one of the major health problems worldwide. According to recent finding by WHO and FAO, around 3 billion people are at risk for zinc deficiency, 2 billion people are anaemic due to iron deficiency and about 150 million are deficient in vitamin A. These micronutrient deficiencies impose a considerable disease burden to the society through creating adverse functional outcomes include stunting growth, increased susceptibility to infectious diseases, physical impairments, cognitive losses, blindness, and premature mortality. Rice is the major staple food and source of energy for more than half of the world's population. In comparison to other cereal crop rice contain meagre amount of micro nutrient like protein (6-7%), Zn (10-33 ppm), Fe (2-34 ppm) and deficit in pro-vitamin A. Unfortunately, due to gradual genetic erosion the genetic store house of land races and traditional varieties are gradually depleted by high yielding varieties which are poor source of essential micronutrients such as Zn, Fe and pro-vitamin A in their polished (white) form. Nowadays, utilization of molecular markers associated with target allele (MAS) helps in identifying desirable segregants thereby considerably shortening the breeding cycle. Once promising high-yielding, high-nutrient lines emerge then these lines are tested in multi-location trials for confirming their stable performance for growing in mega environment.

Introduction

Rice is the major staple food and source of energy for more than half of the world's population, and around 70% of Indians including large number of poor people rely on this crop. As information provided by FAO and WHO, the micro nutrients such as Fe, Zn, Cu, and Mn requirement on a daily basis for an adult on an average basis should be 15, 12, 1.5±3.0 mg and 2±5 mg respectively. Certain conditions as growth retardation, immune dysfunctions and cognitive impairment arises due to deficiency of Zn in diets. Deficit of Fe causes fatigue and decreases immunity because it restricts oxygen delivery to cells. Likewise Cu deficiency causes low immune competence, while shortage of Mn may cause hardening of veins. While Fe, Cu, Zn and Mn in cereals have nutritional value. Various traditional paddy varieties are considered in folk medicine as they possess high nutritive and therapeutic value. Out of which Some land races are used to cure anaemia in women during and after pregnancy, and many of them (e.g. Kalabhat, Navara, Norungan, etc.) are now known to have high levels of iron. Even lots of folk rice land-races are yet to be screened for their micronutrients content and therapeutic potential. Garibsal, an indigenous variety of rice has been used for treating gastro-enteric infection. The silver content available in this rice variety is likely to eradicate gut pathogens.

But unfortunately, due to gradual genetic erosion the genetic store house of land races and traditional varieties are gradually depleted by high yielding varieties which are poor source of essential micronutrients such as Zn, Fe and pro-vitamin A in their polished (white) form. Therefore, Genetic bio-fortification of rice with enhanced levels of pro-vitamins A, Zn and Fe in its polished form through genetic architecture and utilization of plant breeding and via biotechnological approaches could become a cost-effective and sustainable solution to assist in combating micronutrient malnutrition.

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Folk Rice Diversity Relating Biofortification

Folk rice or heirloom varieties are the genetic storehouse of valuable genes. Screening of folk rice varieties revealed genetic variation regarding micronutrient content and thereby indicating opportunity for selection of micronutrient dense rice genotypes and their further utilization in breeding programme. In folk rice the range of Fe and Zn content varies from 6.2-29.5 ppm and 11.0-37.0 ppm respectively suggesting vast genetic potential to increase the concentration of these micronutrients.

However, estimate of Zn in brown rice is in synergy with polished rice also; the story is different for Fe, as much of the Fe in the aleurone layer is lost during milling. The promising genotypes identified for high Fe and Zn content were Kalanamak, Bhutmuri, Champakhusi, Jayasilet, Kabirajsail, Seetabhog, Radhunipagal and Agniban, Badsabhog, Radhunipagal, Gitanjali, Lilabati respectively (Table 1), revealing the scope for introgression in the breeding program as a donor to develop high nutritional genotypes using mega varieties like Swarna, Nabin, IR-64, Samba Mahsuri as recipients. In general, short grain aromatic land races, Basmati genotypes, deepwater paddy and landraces were found to have high Fe and Zn contents in the grains.

Table 1: Rice varieties with high Zn and iron content in grain

Sl. No.	Folk rice genotypes	Grain type	Micronutrient in polished rice (ppm)	
			Fe	Zn
1	Kalanamak	Short Bold	30.0	34.0
2	Bhutmuri	Medium Bold	28.7	30.5
3	Champakhusi	Medium Bold	26.5	32.1
4	Jayasilet	Medium Bold	28.4	33.0
5	Kabirajsail	Medium Bold	27.0	33.4
6	Seetabhog	Short Bold	23.2	30.2
7	Radhunipagal	Short Bold	20.2	22.9
8	Badsabhog	Short Bold	20.0	22.3
9	Geetanjali	Long Slender	25.4	26.7
10	Leelabati	Medium Slender	22.2	22.3
11	Chittimutyalu	Short Bold	30.0	24.8
12	Kalinga III	Medium Bold	31.6	36.0
13	Bindli	Medium Bold	32.2	37.1

Breeding strategies for utilizing folk rice cultivars in bio-fortification programme

Before addressing the issue, certain breeding criteria should be formulated to mitigate the nutrient deficiency through developing nutrient enriched genotypes. The micronutrient rich genotypes should have high productivity in terms of farmers acceptance, considerable impact on human health, stable performance across various edaphic environments and climatic zones and of course in terms of bioavailability towards targeted people as well as in terms of organoleptic view point [5]. Bio-fortified rice can be developed by traditional breeding methods, exploiting the genetic variation present in folk rice cultivars for selection and hybridization programme. Breeders and Molecular biologist also mining the targeted genes through genotyping the folk rice. Using selective genotyping approach three loci associated with high content of iron and zinc in grain were mapped on chromosome 3, 4 and 8 in Chittimutyalu, a landrace and four loci on chromosome 3, 4, 6 and 12 were mapped in Ranbir Basmati at Directorate of Rice Research presently Indian Institute of Rice Research, Hyderabad [3]. However, the expression pattern of the genes involved in micronutrient

transport throughout plant development should be thoroughly understood before selecting a gene for use in a biofortification program. Nowadays, utilization of molecular markers associated with target allele (MAS) helps in identifying desirable segregants thereby considerably shortening the breeding cycle. Once promising high-yielding, high-nutrient lines emerge then these lines are tested in multi-location trials for confirming their stable performance for growing in mega environment. Not only that, plant breeder could breed for genotypes that contain lower concentration of anti-nutrient or elevated concentration of promoter or can modify plant genetic architecture to silent or over express the genes encoding anti-nutrient or promoter respectively [6]. The pleotropic nature of Fe and Zn association become an win-win opportunity to the plant breeder. Regarding bioavailability point of view it is important to determine the retention of micronutrients when screening and using genetic resources in breeding with food products in mind. Adoption of participatory selection can provides early feedback on farmer and consumer preferences and may speed delivery of biofortified varieties to targeted communities.

Rice Biofortification Programme

There is significant genetic diversity in rice genome to increase Fe and Zn concentration substantially in grain. In case of rice highest Zn and Fe concentration was observed mostly in case of aromatic lines, astonishingly the aromatic trait was not pleiotropic for grain Fe and Zn content. In rice generally Zn are concentrated in the endosperm whereas Fe are concentrated on embryo, pericarp, testa, and aleurone layers which are removed during milling, leaving only the endosperm causes losses of precious Fe in the polished rice [7]. There is a high correlation between Fe and Zn concentration in grains and under additive genetic control with high heritability. Additionally there is a very strong correlation between grain micronutrient and grain protein indicating that these might have the same genetic base to some extent, and could be simultaneously improved by breeding. Research efforts continue to identify quantitative trait loci (QTLs) associated with zinc and Fe content and to better understand their uptake, transport, and remobilization into the grain. Researchers are also trying to reduce the level of phytate and to add resorption enhancing factors and iron storage protein ferritin in rice grain. Genes encoding phytase (enzyme degrading phytate) and storage protein ferritin (from french bean and soybean) have been transferred into rice endosperm which results 3 fold increase of Fe and first successful bio-fortification effort was iron fortified rice [8]. It is noteworthy to mention that for achieving both iron and now higher zinc levels in the field requires both a ferritin and NAS (nicotianamine synthase) gene to be expressed correctly. Interestingly the losses of minerals during cooking will probably not be as important factor in achieving target levels in biofortified crops as losses due to milling of grains. Milestone is achieved in bio-fortification programme through development of pro vitamin A rich golden rice where through genetic engineering three beta-carotene biosynthesis genes were transferred into the rice endosperm. The biofortified rice was called Golden rice because of their golden grain colour. Replacing PSY (phytoene synthase) with genes from maize instead of from daffodil in case of Golden rice increased the level of β-carotene by 23 times in rice which was named as Golden Rice 2 [9]. The use of DNA-based techniques, such as association mapping studies, led to the identification of loci associated with provitamin-A, carotenoids and the

development of DNA markers that have led to accelerated genetic gain in breeding for increased provitamin-A content.

Summary

Now it is high time to utilize the folk rice cultivars in biofortification programme and also involvement of plant breeder for improving the health and livelihood of numerous resource poor, micronutrient deficit people of developing countries through developing bio-fortified staple food crops like rice.

Conclusion

The present investigation was conducted with seventy landraces. with the main objectives of studying the genetic variability among the landraces of rice for agro-morphological and grain quality parameters, estimating the genetic diversity among these genotypes using Mahalanobis D² analysis, identifying the promising genotypes possessing desirable traits and studying the extent of association among yield, its component traits and grain quality parameters and determining the direct and indirect effects of these characters on yield. Critical analysis of results obtained from character association and path analysis indicated that the number of productive tillers per plant exerted the highest positive direct effect on single plant yield but it had negative-non significant association with yield which might be due to the negative indirect effects manifested through other component traits. But stem length, panicle length, total number of grains per panicle, 100-grain weight, decorticated grain width and grain zinc concentration displayed significant positive correlation as well as positive direct effect on single plant yield. The positive direct effect of these traits on yield resulted in strong genetic correlation. Hence, due emphasis should be given to these traits in formulating selection criteria to bring yield as well as grain quality improvement.

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