Chapter 2

Genetic Analysis of Biofortification of Micronutrient Breeding in Rice (*Oryza sativa* L.)

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Additional information is available at the end of the chapter

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Abstract

Rice is a staple food for millions of people and has great importance in food and nutritional security. Rice is the second most widely consumed in the world next to wheat. The poorest to the richest person in the world consumes rice in one or other form. New research on the importance of micronutrients, vitamins and proteins aims at biological and genetic enrichment. Vital nutrients that the farmer can grow indefinitely without any additional input to produce nutrient-packed rice grains in a sustainable way is the only feasible way of reaching the malnourished population in India. In the present study, an attempt has been made to improve the nutritional quality of rice.

Keywords: rice, biofortification, iron, zinc, malnutrition

1. Introduction

Rice is the very life and the main staple food for more than 50% of the world's population. It provides more carbohydrate and protein in the average daily diet to supplement with essential micronutrients. The per capita consumption of rice is very high, ranging from 62 to 190 kg/year. Therefore, it is one of the most important crop plants on Earth. It provides 35–75% of the calories consumed by more than three billion Asians. Major advances have occurred in rice production during the last four decades due to the adoption of green revolution technologies. Rice production increased 136%, from 257 million tonnes in 1966 to 600 million tonnes in 2000. Rice production grew at the rate of 2% during 1970–1980 and 1.1% during the 1990s. There has been no substantial increase in rice production during the last four years. Worldwide, rice is cultivated in an area of about 152.99 million hectares with a production and productivity of 418 million metric tonnes and 4.07 tonnes per hectare, respectively. In India, rice is cultivated in 43.7 million hectares with production of 93.35 million metric tonnes and productivity of 3.18

metric tonnes per hectare (USDA, World Agriculture production, 2008). In terms of area under cultivation, India ranks first in the world, and with respect to production, China ranks first. To be healthy, human beings require more than 20 mineral elements and more than 40 nutrients, particularly vitamins and essential amino acids, all of which can be supplied by an appropriate diet. Malnutrition deficiencies common in rice consuming countries are iron (Fe) and zinc (Zn) deficiencies that occur mostly in developing countries.

2. Micronutrient deficiencies

Iron deficiency anemia is by far the most common micronutrient deficiency in the world, affecting more than two billion people. It is estimated that about 79% of the children aged between 6 and 35 months and 56% of women between the age of 15 and 49 years are anemic in India. Iron deficiency during childhood and adolescence impairs physical growth, mental development and learning capacity. In adults, iron deficiency anemia reduces the capacity to do physical labor and increases the risk of women dying during childbirth or in the postpartum period.

Zinc is required as a co-factor in over 300 enzymes and plays critical structural roles in many protein and transcriptional factors. Zinc deficiency is more extensive in developing countries, where more than 60% of the population is at risk. Zn deficiency in older children and adolescent males causes retarded growth and dwarfism, retarded sexual development, impaired sense of taste, poor appetite and mental lethargy.

To address micronutrient deficiencies, nutritionists focus on supplementation, fortification and dietary diversification. Fortified food and food supplements do not reach all those affected in the developing countries, because of weak market infrastructure and high recurring cost. Sustainable solutions to the micronutrient problem in these countries can be developed through agricultural approaches. One such approach is crop diversification and the other is to enhance the level of micronutrients in major staple food crops through plant breeding strategies, that is, biofortification of staple in rice production for both rural and urban people.

"Harvest Plus" is one of the programs to motivate the breed crop varieties to enriching in iron and zinc. The ultimate goal of the biofortification strategy is to reduce mortality and morbidity rates related to micronutrient malnutrition and to increase food security, productivity and the quality of life for poor populations of developing countries.

Exploiting genetic variation in crop plants for micronutrient content is one of the most powerful tools to change the nutrient level of a given diet on a large scale. Genetic studies are required to obtain information on the mode of inheritance of the targeted trait, variability and heritability, which are the true key phenomena determining the efficiency of the breeding programs. Based on the information generated through genetic analysis studies, desirable plants with improved levels of Fe and Zn could be fixed from advanced generation breeding materials. A human being needs 49 essential nutrients for normal metabolic activities. Inadequate consumption of even one of these nutrients will result in adverse metabolic disturbances leading to malnutrition. To consider the micronutrient malnutrition to arising world health consequences, to concentrate poor people to balance the daily diet with micronutrient enriched diet. The major micronutrient deficiencies common in rice-consuming countries are iron and zinc deficiencies.

According to the FAO (2010), lack of micronutrients in food, leading to hidden hunger, is seriously damaging the health of billions of people all over the world. Over 9 billion people are affected by undernourishment and the numbers are on the rise. Nearly 5 billion people from Asia and Pacific region and billions of people in developing and underdeveloped countries suffer due to lack of micronutrients, of which 40% of people are affected by lack of iron and zinc.

The nutritional disorder anemia due to iron deficiency is widespread in rice-consuming countries. South East Asia shows the highest prevalence of anemia in women with over 50% of pregnant women being affected. Anemia lowers work performance. It has been linked to reduced resistance to infection. Severe anemia is a significant cause of maternal deaths, while mild anemia may also affect cognitive functions. The magnitude of micronutrient (Fe and Zn) deficiency is alarming, particularly among children, women of reproductive age and pregnant and lactating women. Zinc is a critical micronutrient needed for structural and functional integrity of biological membrane and for diversification of highly aggressive radicals.

Decrease in zinc concentration in the human body results in a number of cellular disturbances and impairments such as immune dysfunctions and high susceptibility to infection of diseases, retardation of mental development and stunted growth in children.

The main strategies addressing micronutrient disorders are dietary diversification, food fortification, supplementation and biofortification. A sustainable solution for micronutrient deficiency is to enhance the level of micronutrients in major staple food crop through plant breeding strategies, that is, biofortification. According to high yielding cultivars combine effort of enriched micro nutrient rice possible through rice improvement strategies.

Successful biofortification strategies must be initiated with screening of diverse germplasm for desired micronutrient content, followed by suitable breeding methods. Genetic engineering through modification of functional pathways to improve the functional novel genes is another major approach. Genetic studies generally reveal the information on the mode of inheritance of the targeted trait. Variability and heritability are the true key phenomena determining the efficiency of the breeding program. Estimates of heritability would be helped in predicting the parents to advance generation for further selection improvement. It facilitates the breeder in finding out the heritable portion of phenotypic variance for effective selection. Genetic advance is yet another parameter for knowing the quantum of desired genes transferred to the progenies. Information on inter-relationships existing among these traits and their association with yield is also important for suggesting suitable guide for selection. Crop improvement for specific trait has been achieved through effective use of F_2 and F_3 segregating population and fixing desirable combinations [1].

Major micronutrient deficiency affected people to lose the valuable life. Since rice is the principal cereal crop in most rice-growing countries and is the staple food of the world's population, it bears significant impact on human health. Biofortification has emerged as a new way to eradicate micronutrient deficiencies. Biofortification is likely to reach rural households, especially subsistence farmers who grow and consume the harvested cereal grains, which in turn is expected to have impact in an affordable and sustainable manner.

Rice (*Oryza sativa* L.) is basically a starchy crop and it has low nutritional elements compared to millets. Though rice provides 50–80% of the energy intake of the poor, it does not provide enough essential micronutrients to eliminate hidden hunger, iron deficiency anemia (IDA) and zinc deficiency. Sufficient micronutrient in the daily diet is one of the prerequisites for human health. The main strategies addressing micronutrient disorders are dietary diversification, food fortification, supplementation and biofortification. Most of the micronutrient deficiencies can be addressed, to some extent, through biofortification. One of the ways to enhance the micronutrient level in the staple crops is biofortification breeding.

To develop new varieties with high amount of micronutrients in the rice kernel, a population with high variability serves as prime source for effective selection. Particularly, the role played by F_2 segregants, contributing much variability, is highly recognized. The F_2 and F_3 generation is the correct stage for selection in any hybridization program and fixing desirable traits in the early segregants of rice by selecting and evaluating them for desirable characters.

A scrutiny of available literature is invaluable in gaining an insight into the research problem under study. This review helps to acquire broad and general background in the given field or discipline. Comparative views of past approaches and findings can also be had through this compiled information. This could orient researchers in the desired lines of thinking, which is supposed to be a prerequisite for a scientific study.

A sincere attempt has been made to review the available literature relevant to the study and is presented under the following sub-heads:

1. Studies on variability
2. Studies on heritability and genetic advance
3. Studies on association of characters
4. Studies on path coefficient analysis
5. Studies on parent-progeny regression

2.1. Variability

The development of an effective plant breeding program is dependent upon the existence of genetic variability and it is a prerequisite for a plant breeder to work with any crop species. Genetic improvement for quantitative traits can be achieved through a clear understanding of the nature and extent of variability present in the material. The efficiency of selection in any crop largely depends on the magnitude of genetic variability available in the population.

The simple measures of variability partitions the variation into phenotypic, genotypic and environmental components. Phenotypic coefficient of variation (PCV) is the measure of total variability resulting from the genotype, environment and interaction of both. Phenotypic and genotypic coefficients of variation give the real picture of variability concealed in a population.

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2.2. Heritability and genetic advance

Knowledge of heritability serves as an effective tool to the plant breeder to estimate the relative importance of the inheritance and environment on the variation observed for a character. The concept of heritability helps to discern whether phenotypic differences among individuals are due to genetic differences or due to environmental causes. Heritability in a narrow sense is defined as that fraction of the observed variance which is caused by additive genetic effect.

Similarly, the estimate of genetic advance or genetic gain for a particular character is an important parameter to evaluate the effectiveness of selection [2].

i. The amount of genetic variability

ii. The intensity of selection

Knowledge of variability, heritability and genetic advance is of great value in both stages. Estimation of heritability along with genetic gain is more useful in predicting the resultant effect through selection of the best individuals.

Estimates of heritability for different traits of economic importance are available on a variety of materials.

2.3. Association of characters

Inter-relationship of yield with other traits is considered as the most valuable while formulating selection program for yield improvement in any crop. Correlation studies pave the way to know the association between highly heritable characters with the most economic, namely the grain yield. Several authors have worked in this aspect to bring about the relationship of different characters with yield and also within the yield contributing character.

A number of independent components will influence yield, since it is a complex quantitative trait. Simultaneous selection for more characters can easily be done with the knowledge of association between yield and yield components. This association between highly heritable characters with the most economic character, the yield, can be obtained by correlation studies. Various authors have brought out the relationship between yield and yield attributing economic traits by computing genotypic and phenotypic correlation coefficients.

2.4. Path coefficient analysis

Path coefficient analysis provides an efficient means of partitioning of correlation coefficients into direct and indirect effects of the component character. Selection on the basis of direct and indirect effect is much more useful than selection for yield *per se* alone. Several authors have reported the extent of direct and indirect influence of characters on yield in rice.

2.5. Studies on parent-progeny regression analysis

Parent-offspring regression analysis in advancing generations makes it possible to study how far the genetic potentials from one generation is transferred to the next; the higher the values of regression, the higher will be the genetic effect with less environmental influence. The analysis will be helpful to select the early fixing characters in a segregating population. Parent-progeny regression analysis method helps us to ascertain the influence of environment on different characters in progenies obtained from individual selection from early generation and to study the real genetic potentiality of the progenies, which was inherited from their parents [3]. Parent-progeny regression analysis assumes no environment association between generations, so genotype × environment interaction and co-variances between parents and offspring will be zero [4].

Multiple regression analysis to fix yield attributing characters found that percentage of filled grains per panicle, biological yield and harvest index were major selection criteria for yield improvement [5]. A comparative analysis to estimate heritability for grain yield and plant height using parent-offspring regression and variance components in maize revealed broad sense heritability estimated from variance components of the progeny greater than those based on the parent–offspring regression. It was found that selection is not effective for other traits like plant height.

High narrow sense and realized heritability for the characters, 1000 grain weight, and number of grains per panicle in early generation showed the prospects of selecting for these traits in early generation itself. Multiple regression analysis in pearl millet to fix yield-attributing characters found that number of grains per spike, 1000 grain weight, totally contributed a 60% variation in grain yield, suggesting that selection should be based on the above characters for improvement in grain yield.

With regard to heritability and environmental effects of yield and yield-related traits using parent-offspring regression in F_1 progenies, environmental effects and heritability estimates were high for culm length, tillers per plant, panicles per plant and 1000 grain weight. Based on the variance estimates of the parent-offspring regression model, it was suggested that these traits with high heritability, considerable phenotypic correlation and low seasonal variability could be used for further improvement of the F_1 progenies [6].

The most widely used breeding method in rice is the hybridization of homozygous diverse genotypes followed by pedigree method of handling the segregating population in order to isolate genotypes possessing desirable characteristics of parents. The usefulness of these methods is limited because of limited parental participation, low genetic diversity, reduced recombination and rapid fixation of genes. The selection and choice of breeding method for the improvement of quantitative or qualitative character largely depends on the nature and magnitude of additive and dominance variance. The success in the improvement of cultivated variety for yield, grain quality and resistance to biotic and abiotic resistance largely depends upon the natural variability present in the population. Through hybridization among the selected genotypes, it is possible to reshuffle desired characteristics, provided the segregating generations contain large variability. Selection for quantitative characters is generally taken up in the early segregating generations. For characters like grain yield, the selection is continued till the material becomes homozygous, because such characters are controlled by large number of genes and huge number of population has to be raised for making the selection effective. This is not always true, because the effective selection was known to be restricted by close linkages between desirable and undesirable component characters and these undesirable linkages delay the utilization of full recombination potential.

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Among the methods of increasing the frequency of desirable recombinants, the biparental mating or disruptive mating gets importance in the improvement of self-pollinated crops, because it increases the possibility of obtaining desirable and valuable recombinants [7]. The system of biparental mating is reported to alter the phase of linkage through forced recombination. As a result, greater amount of concealed genetic variation is released, particularly of the additive type. Since the additive genetic variance is the only variance which responds to selection, a perceptible genetic gain can be expected after biparental mating.

Quality breeding in rice has assumed greater significance due to varied consumer preference in recent years. Developing a better variety with respect to higher yield with good grain quality and multiple resistances to biotic and abiotic tolerance than the already existing ones is the prime goal of plant breeders. Thus, high yield is the foremost goal of any crop improvement program but consumer preference varies from region to region. Next to grain yield, grain quality is the important criterion considered by the plant breeders. If the newly developed variety is not accepted by the consumers due to its poor taste, texture, aroma or appearance, its usefulness is greatly impaired. In developed countries, as well as in the rice-exporting countries, physical appearance of the grain or kernel is often more important than the yield [8]. In developing countries, like India, grain quality has become greatly important as the country has become more prosperous and self-sufficient in food production.

Quality of rice is determined by a combination of many physio-chemical properties. Rice kernel size and shape are important quality components that directly influence the market value. Gelatinization temperature and amylose content are the principal determinants for cooking and eating quality of milled rice. In addition to this trait, kernel length after cooking and linear elongation ratio is desirable quality traits. Properties of cooked rice are controlled by amylose content and gelatinization temperature. Varieties with intermediate values for both parameters remain nonsticky and tender after cooking and low values for both make the rice sticky on cooking.

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