

BREEDING TECHNIQUES FOR ENHANCING THE NUTRITIONAL QUALITY OF MAJOR FOOD CROPS

Abstract

Cereals belonging to family Gramineae are edible grains and are dominant crops in world agriculture. They are the major source of energy, carbohydrate (nearly 75 per cent), starch (25-27 per cent), fibre (13 per cent), fat (2 per cent) and protein (6-15 per cent) for humans & livestock. Cereals are good source of micronutrients, vitamins such as vitamin A, B1, B2, B5, B6, B9, E & K, non-starch polysaccharides and bioactive substances which are required for proper functioning & essential metabolic activities in human beings and provide proper health benefits. WHO identifies deficient micronutrient uptake in over half of all people globally particularly in women & children. Out of 7 billion world population, micronutrient deficiency is reported in about 2.5 billion people. Major cereal crops viz. wheat, maize & rice are the staple food providing wholesome nutrition. However, these crops are associated with some deficiencies of the micronutrients & nutritional components like inadequate bioavailability of Fe & Zn in rice & wheat, vitamin A deficiency in rice endosperm, deficiency of essential amino acid like lysine & tryptophan in maize causes severe pellagra disease while gluten content in wheat causes severe allergic celiac disease. Availability of nutritious & bio-fortified food can overcome all these problems. Enhancing the bio availability of deficient nutrient content in these major cereal crops by bio-fortification is a feasible solution. Therefore, breeding techniques are efficient for enhancing nutrient content.

Keywords: Cereal; nutrients; iron; zinc; vitamin A; quality; breeding

Authors

Dr. Shailja Sharma

Department of Genetics and Plant
Breeding College of Agriculture
CSKHPKV Palampur
Palampur, Himachal Pradesh, India
Shailjasharma567@gmail.com

Rhitisha Sood

Department of Genetics and Plant
Breeding College of Agriculture
CSKHPKV
Palampur Himachal, Pradesh, India
soodrhitisha@gmail.com

Vivek Singh

Department of Genetics and Plant
Breeding College of Agriculture
CSKHPKV
Palampur Himachal, Pradesh, India
vivek.singh30796@gmail.com

I. INTRODUCTION

The word Cereal is derived from the Latin word “Cerealis” or “Ceres” which means the ‘Goddess of Grain’. From ancient times to till date, Cereals are staple foods worldwide. Cereals belonging to grass family Gramineae are edible grains & has historical significance in human civilization. These are the important source of energy, carbohydrate, protein & fibre, starch, vitamins, various amino acids, magnesium & zinc, certain micronutrients and bioactive substances (Ram and Mishra, 2010¹). Wholegrain cereals are considered as the healthy food and have a significant role in controlling diseases like coronary heart disease, diabetes, colon & rectum cancer etc.

II. NUTRITIONAL VALUE OF CROPS

1. Cereals contains both macro & micronutrients. These are:

- **Carbohydrate:** Cereals contains nearly 75 per cent carbohydrate, so they are called carbohydrate rich foods. In many varieties of cereals, starch is present as amylose (25-27 per cent), whereas in rice & corn varieties (waxy) starch is mostly amylopectin. This amylopectin is not digestible inside the human body which is referred to as resistant starch and goes all the way to the small intestine. Bacteria uses this resistant starch for energy when it reaches the colon. This process is called fermentation which produces a certain type of fat called short chain fatty acids which are the main products & produce most of the calories from resistant starch and has many benefits. Therefore, a modification of the starch composition of wheat by raising its resistant starch content presents an opportunity for a potentially large scale improvement in public health. The risk of non-infectious chronic diseases can be overcome by eating whole grain cereal foods. There are four types of resistant starch. The starch which is not accessible for digestion is RS1, whereas resistant starch granules refer to RS2. Retrograded starch is RS3 and starch which is chemically modified is RS4 (Baghurst et al. 1996²).
- **Protein:** The protein content in cereals is about 6-15 per cent. The gliadins & glutenin, glutelin (oryzenin) and prolamin (zein) are the main storage proteins found in wheat, rice & maize respectively.

2. Micronutrients

- **Vitamins:** Cereals contains vitamin B1, B5, B6, E and K in sufficient quantity (Kulp & Ponte, 2000³).
- **Minerals:** There are high levels of minerals like potassium, iron, magnesium & zinc and low amount of many trace elements e.g. selenium in whole grain cereal. These have low sodium content. Among the cereal grains, rice has the highest level of selenium.
- **Non-starch polysaccharides:** All cereals contain insoluble & soluble non- starch polysaccharides which help in weight control e.g. Arabinoxylans.

- **Phytochemicals:** These are bioactive substances present in cereals which have health promoting effects.

III. NUTRITIONAL QUALITY

The suitability or fitness of an economic plant product in relation to its end use is referred to as quality. The definition of quality varies in terms of the consumer's need. From the nutrition point of view, it varies in terms of seeds, crop growth, crop product, post harvest technology, consumer preferences, cooking quality, keeping quality, transportability etc (Gupta and Govindarajan, 2001⁴). Nutritional quality trait is any trait that defines suitability & fitness of nutrient present in food crop. Each crop has a specific & a completely different set of nutritional quality traits.

1. **Rice:** Rice is superior to other cereal crops in terms of protein quality with higher lysine (3.8-4.5 per cent). It contains high starch i.e. 94 per cent. However, it lacks vitamin A, B12, essential amino acids like lysine, tryptophan & methionine, micronutrient like iron and zinc.
2. **Wheat:** There are high amounts of carbohydrate, starch, all vitamins of B complex & enzymes present in wheat grains. It contains 11 per cent of protein like albumins, globulins, gliadins & glutenins, 2 per cent of fat, 13 per cent of fibre, 1 per cent of mineral like magnesium, calcium, zinc, manganese, iron, phosphorus & potassium. Albumins are water soluble, globulins are salt soluble, gliadins are ethanol soluble and glutenins are acid soluble. But bioavailability of micronutrients in wheat based diet are reduced by the content of phytic acid, a anti-nutritional substance present in the grain. It is responsible for inhibiting the release of iron & zinc in the intestine, thus their absorption is reduced. Therefore, iron & zinc are major micronutrient deficiency among the people where cereals are consumed as major foods. The enhancement of their density & bioavailability by reducing anti nutritional factors can lead to improved nutritional status of vast sections of human beings throughout the world. Recent reports indicate that sufficient variability does not exist in cultivated hexaploids for the traits of iron & zinc, but in wild species, it has wider variation up to 110 mg/kg (Chhuneja et al. 2006⁵). Thus, wild progenitors can be used in bio-fortification for enhanced micronutrient density. By lowering the amount of phytic acid and increasing the activity of phytase enzyme in the wheat grain, we can improve the bio- availability of micronutrients. Thus, before proceeding to improve the quality of wheat, the important step is to identify the genotypes having low phytic acid content & high phytase activity along with higher iron & zinc content. There is high vitamin E content in wheat germ which cures many diseases. There is generally higher concentration of nutrients in germ or embryo and in the aleurone cells surrounding the starchy endosperm followed by bran, whole wheat flour and wheat flour. The gluten present in wheat is the viscoelastic complex mainly formed of glutenins & gliadins. Adverse reaction to gluten causes allergy due to naturally occurring constituent namely COELIAC disease. The patient of celiac develops gliadin specific igA & igG antibodies. However, this disease is less prevalent in India.
3. **Maize:** There is a lot of roughage, all the vitamins of B complex, vitamin D, E, K, pro vitamin A, about 60-70 per cent starch present in maize grain. It is a good source of omega-3 acids & minerals like iodine, zinc, phosphorus, potassium, sodium, calcium,

magnesium, iron, copper, manganese & selenium. The main protein present in maize is Zeins which usually account for 50-70 per cent of the endosperm proteins. Zeins have high content of glutamine, leucine and proline, however they lack lysine and tryptophan. In normal maize, zeins (60 per cent), glutelins (34 per cent), albumins & globulins (3 per cent) are the proportions of various endosperm storage protein fractions. All the proteins except zeins are balanced in amino acid content and are quite rich in lysine & tryptophan. Thus, the feasible option to bring the improvement in the amino acid balance of maize grains is suppression of the lysine deficient zein fraction without drastically altering the contribution of other fractions. Mutation called opaque-2 that was responsible for making grain proteins in the endosperm nearly twice as nutritious as those found in normal maize was discovered by the scientists of Purdue University in 1963. This mutation was first discovered by Jones and Singleton in the early 1920s, but Mertz and coworkers discovered its nutritional significance. Soon after this, researchers discovered another mutation floury-2 (fl2) that also has the ability to alter endosperm nutritional quality. However, utilization of opaque 2 in breeding programmes was soon tempered due to the realization of the pleiotropic effects of this mutation namely a soft endosperm that results in damaged kernels, an increased susceptibility to pests & fungal diseases, inferior food processing & reduced yields. Continuous efforts of the breeders of CIMMYT Mexico for the improvement of protein quality of maize resulted in conversion of opaque-2 maize into varieties that have high nutritional quality as it contains twice the lysine & tryptophan, higher amounts of histidine, arginine, asparatic acid & glycine and the lower amount of glutamic acid, alanine & leucine, high yields, appearance of normal maize, greater hardness, equal or superior pest & disease resistance. This enhanced opaque-2 is called Quality Protein Maize (QPM).

4. **Need for nutritional quality improvement strategy:** More than 2 billion people of the world suffer from micronutrient malnutrition. Zinc deficiency is found in about 1.5 billion, 1.6 billion have iron deficiency, about 1 billion people reside in iodine deficient regions, 400 million people suffers from vitamin A deficiency, and calorie deficiency is found in 800 million people. Nearly 2 billion adults are overweight or obese, one in 12 has type 2 diabetes. About 159 million children are stunted, 50 million are wasted, 41 million are overweight. 57 countries in the world have serious levels of both under nutrition and obesity. Malnutrition accounts nearly 30 million death per year.
5. **Iron deficiency:** In humans, iron is an important co factor of various enzymes which are required for basic functions. Its deficiency mainly affects children of below 5 years and poor women of child bearing age. Retarded mental development, anaemia and suppression of immunity system are the major deficiency symptoms. At childhood age, its deficiency weakens physical growth, mental development & learning ability. In adults, it reduces the ability to do physical labour and anaemia which increases the death for women in child birth.
6. **Zinc deficiency:** Its deficiency is the fifth major cause of diseases & deaths in developing countries. Deficiency causes health related issues like anorexia, dwarfism, weak immune system, skin lesions, hypogonadism & diarrhea.
7. **Vitamin a deficiency:** A serious public health problem in the developing world particularly in Africa & South East Asia. Annually, an estimated 2.5-5 lakh preschool

children go blind and about 3 million children suffers from eye damage. More than half of children which goes blind dies within a short period of time. Symptoms of deficiency are night blindness, increased susceptibility to infection & cancer, anaemia (lack of red blood cells or haemoglobin), deterioration of the eye tissue and cardiovascular diseases.

- 8. Folate (Vitamin B9) deficiency:** Deficiency of foliate results in serious disorders including neural tube defects such as spina bifida in infants and megaloblastic anaemia. A large proportion of children dies from a common illness that could have been avoided through adequate nutrition.

India is also suffering from the malnutrition problem, where 38 per cent children are stunted, more than 50 per cent of women are suffering from deficiency of micronutrients, and 46 per cent of children below 3 years are underweight.

IV. BREEDING STRATEGIES FOR QUALITY IMPROVEMENT

1. Nutritious crops-rich in nutrients

2. Bio-fortified Crops

- Conventional Breeding techniques/methods
- Molecular Marker Assisted breeding approach
- Genetic Engineering Approach
- Transgenic Approach
- Agrobacterium mediated transformation
- RNA interference technique

Bio-fortification: The word Bio-fortification is derived from the Greek word ‘bios’ means life and Latin word ‘fortificare’ means make strong. Bio-fortification is a cost effective method of breeding crops to increase their nutritional value. It refers to genetically increasing the bio-available mineral content of food crops (Brinch-Pederson et al. 2007⁶). This technique focuses on making plant foods more nutritious when the plants are growing whereas in ordinary fortification nutrients are added to the foods when they are being processed. Crop bio-fortification is important to overcome malnutrition in human beings, increment of nutritional quality in daily diets, improvement of crop quality & increment of variability in germplasm.

Steps in Bio-fortification pathway: There are three major steps in the pathway of bio-fortification- Pathway, Development & Dissemination. Under pathway, firstly the target population is identified. For this target population, nutrient target level is set. Screening of germplasm is done for gene discovery. Then in development, breeding of bio-fortified crops is done followed by testing. Nutrient retention level in crop is measured and evaluation of nutrient absorption and impact is done. Under dissemination, strategies are developed for dissemination of seed. Steps are taken to promote marketing and consumption of bio-fortified crops.

For iron bio-fortification in cereals, there is no direct breeding approach so transgenic approach is only option

Bio-fortification in rice: There are many vitamins, micronutrients and proteins are deficient in rice endosperm. During milling and polishing of rice, the aleurone layer of dehusked rice grains is lost which is nutrient rich. Rice plants produce beta carotene (provitamin A) in green tissues, but not in the endosperm (the edible part of the seed). To overcome the deficiency of vitamin A in human beings, bio-fortification is necessary.

Breeding for high zinc rice: The scientists of the Bangladesh Rice Research Institute developed a variety BRR1 Dhan 62, the world's first zinc rich variety using conventional breeding methods. It contains 20 to 22 ppm of zinc content whereas the normal rice has 14 to 16 ppm zinc content.

V. METHODS USED FOR RICE BIO-FORTIFICATION

- 1. Marker assisted selection:** A fine mapping population are developed & purified. The molecular marker associated with genes for iron uptake, transport & accumulation are identified.
- 2. Genetic engineering:** To enhance the micro nutrient levels in crop plants, genetic engineering is the best method to be used. The scientists developed the 'Golden Rice' using this method. They redirected the whole biosynthetic pathway of carotenoids formation by genetic engineering of multiple genes encoding key enzymes. Scientists inserted genes from daffodil that are able to produce functioning versions of the first & last enzymes of the pathway. Another single bacterial gene was also inserted that provides the functioning versions of second & third enzymes of the pathway. With a functioning pathway, the transgenic rice is able to produce the vitamin A precursor beta carotene. It is this product that gives "Golden Rice" its characteristic yellow colour.
- 3. How does it work:** The addition of two genes in the rice genome has completed the biosynthetic pathway of carotenoid formation. Phytoene synthase (*psy*) is a transferase enzyme derived from daffodils (*Narcissus pseudonarcissus*). This enzyme has a role in the biosynthesis of carotenoids and help in catalyzing the conversion of GGPP to phytoene. The second gene is Lycopene cyclase (*crt1*) which is isolated from *Erwinia uredovora*, a soil bacteria It produces enzyme & catalysts for the synthesis of carotenoids in the endosperm of rice.
- 4. Iron & zinc bio-fortification:** Iron is found in mineral soils in abundance but the major problem in its acquirement is solubility. Therefore, the application of iron to soil as fertilizer is not a good option for increasing iron content of seed. However, the application of zinc as fertilizer is effective in promoting plant growth & also in the fortification of crops with zinc. It is believed that, only endosperm is left over milling in rice. Hence bio-fortification would be effective only when the metal ion concentration is increased in the endosperm.
 - Exploiting metal chelators for bio-fortification:** Gramineous plants secretes small molecules called the mugineic acid family phytosiderophores (MAs) that have potential to solubilize Fe, Zn, Cu and Mn. They use these MAs for acquiring micronutrients from soil & transporting them from roots to shoots and grains. So, increasing these mugineic compounds would increase iron & zinc concentration of rice.

- **Exploiting metal transporters for bio-fortification:** Protein 1: OsIRT1, OsIRT2 are the iron regulated transporters & are responsible for transporting different metals. The rice line that over expresses more OsIRT1 accumulated more iron & zinc in the seeds. Also inclusion of tissue specific promoter such as OsSUT1 may increase zinc concentration of rice grains. It is found that Ferretin, a globular protein has the ability to store and keep iron in soluble and non-toxic forms. In plants ferretin may be localized to plastids. So, over expression of ferretin was the first attempt to increase iron on rice grains. Pusa Basmati (Pusa Sugandh) overexpresses rice ferretin under control of endosperm specific promoter (glutelin A2). In this rice variety, ferretin expresses itself 7.8 times more and showed 2.1 & 1.4 fold more accumulation of iron & zinc as compared to wild types.

5. **Zinc bio-fortification:** Zinc content in rice can be increased by fertilization. Studies had shown that in rice zinc concentration increased up to 25 per cent by foliar application and up to 32 per cent by foliar plus soil zinc application and there is only 2.4 per cent increased by soil zinc application. Application of zinc fertilizers to rice increased grain yield by about 5 per cent.

VI. BREEDING STRATEGIES FOR WHEAT

In cultivated wheat varieties, there is less genetic variation for zinc & iron. Studies had shown that there is up to 190 ppm of zinc & iron in landraces, wild relatives such as *Triticum spelts*, *Aegilops tauschii*, emmer wheat. Researchers used landraces, wild relatives & recreated synthetics as progenitor for transferring high zinc & iron into cultivated varieties of wheat. For introgression of high zinc into elite wheat, selected bulk scheme & backcross approach are the most effective approaches. Agronomically superior wheat with 100 per cent more zinc & 30 per cent more iron than the modern varieties was developed by CGIARs with the aim to breed nutrient dense staple foods by using synthetic hexaploid wheat from *T.dicoccum* & *Aegilops tauschii* with high micronutrient content. Zinc intake was 72 per cent higher from the bio-fortified wheat with 95 per cent extraction and 0.5 mg/d higher absorption than control. “Bio-fortification of wheat for enhanced micronutrients using conventional & molecular breeding” Phase I (2005) & Phase II (2011) program was launched by the Department of Biotechnology, Government of India in which PAU Ludhiana used progenitor A & B genomes & related species, IARI New Delhi used progenitor D genome and IIT Roorkee, GB Pantnagar university used non-progenitor species with S, U & M genome.

Breeding strategies for Maize: Maize lacks lysine & tryptophan necessary for protein synthesis. Zeins usually account for 50-70 per cent of the endosperm proteins & are characterized by a high content of glutamine, leucine & proline. Quality protein maize has less zein fraction & more non-zein fraction so that it has a more lysine & tryptophan content. QPM contains a naturally occurring mutant opaque 2 that increases the amount of two essential amino acids. Due to the pleiotropic effect of opaque 2 it was tempered to utilize it in breeding programme as it produces soft endosperm which leads to pest & disease susceptibility and low yield. Breeders at CIMMYT have continued working & converted maize containing opaque 2 into improved maize. It has high nutritional quality, high yields, greater hardness, pest & disease resistance, twice the lysine & tryptophan and other amino acids. Two studies showed that children consuming QPM had a growth rate in height 15 per

cent greater than that of children who ate conventional maize. It has an zein fraction up to 30-40 per cent along with high lysine & tryptophan content which prevents from Pellagra.

VII. CONCLUSION

People are suffering from malnutrition problem worldwide. The availability of nutritious & bio-fortified food can overcome all these problems. Thus, enhancing bioavailability of deficient nutrient content in major cereal crops by bio-fortification is a feasible solution. Hence, plant breeding is a potential economic approach to enhance the nutritional quality of crops. Both conventional and molecular breeding approach like marker assisted selection, genetic engineering, agrobacterium mediated transformation, tissue culture techniques & transgenic approaches are proven efficient for this purpose. Therefore, breeding techniques besides enhancing the productivity will go a long way in increasing the nutrient quotient of major cereals.

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