**Strategies for breeding nutritional quality in major cereal crops**

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**Abstract:**

Cereals are the edible grains of family Gramineae, 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 also contains a range of micronutrients, vitamin E, some of the vitamin B complex, non-starch polysaccharides and bioactive substances which are required for proper functioning & essential metabolic activities in human beings and provide proper health benefits. Deficient micronutrient uptake in over half of all people globally, notably women & children is identified by WHO. Out of 7 billion world population, more than 2 billion people suffer from micronutrient deficiency. Major cereal crops viz. wheat, maize & rice are the staple food providing wholesome nutrition. However, these crops are associated with some deficiencies of micronutrients & nutritional components like inadequate bioavailability of Fe & Zn in rice & wheat, deficiency of vitamin A 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 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

**Introduction:**

The word Cereal is derived from Latin word “Cerealis” or “Ceres” which means the ‘Goddess of Grain’. From ancient times to till date, Cereals are the staple foods worldwide. Cereals has historical significance in human civilization. It is the edible seeds or grains of the grass family Gramineae. These are the important source of nutrients in both developed and developing countries viz. energy, carbohydrate, protein & fibre, starch, a range of micronutrients such as vitamin E, some of the B vitamins, magnesium & zinc, various amino acids and bioactive substances (Ram and Mishra, 20101). Wholegrain cereals are considered as the healthy food and have significant role in the prevention of many chronic diseases including coronary heart disease, diabetes, colorectal cancer (colon & rectum cancer) etc.

**Nutritional value of crops:**

**Macronutrients:**

**Carbohydrate**: Cereals are often classified as carbohydrate rich foods, as they are composed of approximately 75 per cent of carbohydrate. Within common varieties of cereals, 25-27 per cent of starch is present as amylase, while in waxy varieties (e.g. rice & corn) most of the starch is amylopectin. Amylopectin is not digested in human body and get absorbed in the small intestine. This is referred to as resistant starch. Resistant starch goes all the way to small intestine without being digested. Resistant starch is used for fuel by the bacteria present in colon when it reaches there. 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, 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. Increased consumption of whole grain cereal foods can reduce the risk of non-infectious chronic diseases. Resistant starch (RS1) refers to starch that is physically inaccessible for digestion as it is trapped (e,g. intact whole grains and partially milled grains).RS2 refers to native resistance starch granules (e.g. found in high amylase maize starch). RS3 refers to retrograded starch (e.g. found in cooked & cooled potatoes, bread & some types of cornflakes. RS4 refers to chemically modified starch (e.g. commercially manufactured starch) (Baghurst et al. 19962).

**Protein**: Cereals contain about 6-15 per cent protein. The major storage protein in wheat are gliadins & glutenins, while in rice it is glutelin (oryzenin), in maize it is prolamin (zein).

**Micronutrients**

**Vitamins**: Cereals are important source of vitamin E & most B vitamins especially thiamin, riboflavin and niacin (Kulp & Ponte, 20003).

**Minerals**: Cereals are low in sodium content. They are a good source of potassium. Wholegrain cereals also contain considerable amounts of iron , magnesium & zinc as well as lower levels of many trace elements e.g. selenium. Among the cereal grains, rice have the highest level of selenium.

**Non-starch polysaccharides**: All cereals are rich source of non-starch polysaccharides and contain insoluble & soluble polysaccharides which help in weight control e.g. Arabinoxylans.

**Phytochemicals**: Cereals contain a range of substances which have health promoting effects that are often referred to as plant bioactive substances or phytochemicals.

**Nutritional Quality**: Quality refers to the suitability or fitness of an economic plant product in relation to its end use. Definition of quality varies according to our needs from the viewpoint of seeds, crop growth, crop product, post harvest technology, consumer preferences, cooking quality, keeping quality, transportability etc (Gupta and Govindarajan, 20014). A trait that defines suitability & fitness of nutrient present in food crop is called nutritional quality trait. Each crop has a specific & often somewhat to completely different set of nutritional quality traits.

**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, inadequate amount of essential amino acids like lysine, methionine & tryptophan, micronutrient like iron and zinc.

**Wheat:** Wheat contains a lot of starch & carbohydrate, 11 per cent of protein i.e. albumins, globulins, gliadins & glutenins, 2 per cent of fat, 13 per cent of fibre, 1 per cent of mineral i.e. iron, phosphorus, potassium, magnesium, calcium, zinc, manganese, high amount of entire vitamins of B complex except vitamin B12. Wheat germs are rich in vitamin E & enzymes. Albumin is water soluble, globulin salt soluble, gliadins ethanol soluble and glutenins are acid soluble. But bioavailability of micronutrient in wheat based diet is reduced by the content of anti nutritional factors such as phytic acid in the grain which inhibits 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 and zinc but in wild species it has wider variation upto 110 mg/kg (Chhuneja et al. 20065). Thus, wild progenitors can be used in bio-fortification for enhanced micronutrient density. Also the bio availability of micronutrients can be improved by reducing the phytic acid content as well as increasing phytase activity in the grain. Therefore, identifying genotypes having low phytic acid content & high phytase activity along with higher iron & zinc content is necessary before we proceed to improve nutritional quality of wheat. There is high vitamin E content in wheat germ which cures many diseases. Nutrients are generally found in highest concentrations in germ or embryo and in the aleurone cells surrounding the starchy endosperm followed by bran, whole wheat flour and wheat flour. Gluten present in wheat is the viscoelastic complex mainly formed of glutenins & gliadenins. Adverse reaction to gluten causes allergy due to naturally occurring constituent namely COELIAC disease. Patient of celiac develops gliadenin specific igA & igG antibodies. However, this disease is less prevalent in India.

**Maize:** The corn grains contains 60-70 per cent of starch, lot of roughage, proteins, vitamins from the group B, vitamins D, E, K, pro vitamin A and a source of omega-3 acids. It is a good source of minerals like iodine, zinc, phosphorus, potassium, sodium, calcium, magnesium, iron, copper, manganese & selenium. Zeins usually account for 50-70 per cent of the endosperm proteins & are characterized by a high content of glutamine, leucine and proline, however they lack lysine and tryptophan. In normal maize, proportions of various endosperms storage protein fractions are albumins (3 per cent), globulins (3 per cent), zeins (60 per cent) & glutelins (34 per cent). All fractions other than zeins are balanced in amino acid content and are quite rich in lysine & tryptophan. Suppression of lysine deficient zein fraction without drastically altering the contribution of other fractions could be thus seen as feasible approach to bring about improvement in the amino acids balance in maize grains. In 1963, researchers at Purdue University, USA discovered that a mutation designated opaque-2, made grain proteins in the endosperm nearly twice as nutritious as those found in normal maize. Opaque 2 mutation was first discovered by Jones and Singleton in the early 1920s, but the nutritional significance of mutation was first discovered by Mertz and coworkers. This was soon followed by the discovery of 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 were not easily overcome. Breeders at the CIMMYT Mexico have continued working for improvement of protein quality in maize converting opaque-2 maize into varieties that have high nutritional quality, high yields, appearance of normal maize, greater hardness, equal or superior pest & diseases resistance. This enhanced opaque-2 is called Quality Protein Maize (QPM), it contains nearly twice the lysine & tryptophan, higher amounts of histidine, arginine, asparatic acid& glycine and lower amount of glutamic acid, alanine & leucine.

**Need for nutritional quality improvement strategy**: More than 2 billion people of the world suffer from micronutrient malnutrition. About 1.5 billion suffer from zinc deficiency, 1.6 billion suffer from iron deficiency, about 1 billion people reside in iodine deficient regions, 400 million people have vitamin A deficiency, 800 million people have calorie deficiency. 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.

**Iron deficiency**: Iron is important co factor of various enzymes for basic functions in humans. Affecting mainly children under 5 years and poor women of child bearing age. Deficiency symptoms are poor mental development & depressed immune function to anaemia. In childhood it impairs 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.

**Zinc deficiency**: Its deficiency is fifth major cause of diseases & deaths in developing countries. Deficiency causes health problems like anorexia, dwarfism, weak immune system, skin lesions, hypogonadism & diarrhea.

**Vitamin A deficiency:** A serious public health problem in developing world, particularly in Africa & South East Asia. Annually an estimated 2.5-5 lakh preschool children go blind and about 3 milion 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.

**Foliate (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 die from common illness that could have been avoid through adequate nutrition.

 India is also suffering from malnutrition problem. More than 50 per cent of women, 46 per cent of children below 3 years are underweight & 38 per cent are stunted.

**Breeding Strategies for Quality Improvement:**

* **Nutritious crops-rich in nutrients**
* **Biofortified Crops**
* Conventional Breeding techniques/methods
* Molecular Marker Assisted breeding approach
* Genetic Engineering Approach
* Transgenic Approach
* Agrobacterium mediated transformation
* RNAi technique

Bio-fortification: The word Bio-fortification is derived from Greek word ‘bios’ means life and Latin word ‘fortificare’ means make strong. Bio-fortification is a 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 the malnutrition in human beings, increment of nutritional quality in daily diets, improvement of crop quality & increment of variability in germplasm. It is a cost effective & sustainable solution for alleviating malnutrition.

**Pathways for Bio-fortification:**

**Discovery**

* Identify target population
* Set nutrient target level
* Screen germplasm & gene discovery

**Development**

* Breed bio-fortified crops
* Test the performance of new crop varieties
* Measure nutrient retention in crop
* Evaluate nutrient absorption & impact

**Dissemination**

* Develop strategies to disseminate the seed
* Promote marketing & 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:** The rice endosperm is deficient in many nutrients including vitamins, proteins, micronutrients etc. The aleurone layer of dehusked rice grains is nutrient rich but is lost during milling & polishing. 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.

**Breeding for High Zinc Rice**: BRRI Dhan 62, the world’s first zinc rich variety has been released in Bangladesh in 2013. The scientists of Bangladesh Rice Research Institute developed a variety BRRI Dhan 62 using conventional breeding methods contains 20 to 22 ppm of zinc content whereas the normal rice has 14 to 16 ppm zinc content.

**Methods used for Rice Bio-fortification:**

**Marker Assisted Selection:** Fine mapping population have been developed & purified. Molecular marker associated with genes for iron uptake, transport & accumulation are identified.

**Genetic Engineering:** Genetic engineering is the obvious alternative to enhance the beta carotene levels in crop plants. The scientist developed the ‘Golden Rice’ using genetic engineering of multiple genes encoding key enzymes by redirecting the complete biosynthetic pathway of carotenoids. They did this by inserting genes from daffodil the produce functioning versions of the first & last enzymes of the pathway. In addition, a single bacterial gene that provides the same function as the second & third enzymes of the pathway was also introduced. 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.

**How does it work:** Addition of two genes in rice genome will complete the biosynthetic pathway.

1. Phytoene synthase (*psy*): This is derived from daffodils (*Narcissus pseudonarcissus*). It is a transferase enzyme which is involved in the biosynthesis of carotenoids and help in catalyzing the conversion of GGPP to phytoene.
2. Lycopene cyclase (*crt1*): Isolated from soil bacteria *Erwinia uredovora*. It produces enzyme & catalysts for the synthesis of carotenoids in the endosperm of rice.

**Iron & Zinc bio-fortification**: Iron is abundant in mineral soils and the major problem with its acquisition is solubility, thus application of soil Fe as fertilizer is not an effective strategy for increasing seed Fe. However, the application of Zn 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.

* **Bio-fortification through increasing the amount of metal chelators:** Graminaceous plants, for acquiring micronutrients from soil & transporting them from roots to shoots and grains secrete small molecules called mugineic acid family phtosiderophores (MAs) having potential to solubilize Fe, Zn, Cu and Mn. So, increasing these mugineic compounds would increase iron & zinc concentration in 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 in rice grains. It is found that Ferretin, a globular protein has ability to store and keep iron in soluble and non-toxic forms. In plants ferretin may be localized to plastids. So, overexpression of ferretin was first attempt to increase iron on rice grains. Pusa Basmati (Pusa Sugandh) overexpresses rice ferretin under control of endosperm specific promoter (glutelin A2). The expression of ferretin in these plants was 7.8 times more than the wild types & these plants accumulated iron & zinc at level 2.1 & 1.4 fold respectively as compared to wild types.

**Zinc bio-fortification:** Zinc content in rice can be increased by fertilization. Zinc fertilization had little effect on rice grain yield with the exception of increases of upto 10 per cent in some locations in China & India. As an average of all trials, Zn application increased grain yield by about 5 per cent. On average, Zn concentration in rice was increased by 25 per cent & 32 per cent by foliar and foliar + soil Zn applications respectively and only 2.4 per cent by soil Zn application.

**Breeding Strategies for Wheat**

Low genetic variation in cultivated wheat for zinc & iron. Wild relatives (*Triticum spelts*, *Aegilops tauschii*, emmer wheat & landraces) are known to have upto 190 ppm of zinc & iron. Recreated synthetics, wild & landraces are being used as progenitor for high zinc & iron. For introgressing high zinc into elite wheats, selected bulk scheme & backcross approach are the most effective approaches. CGIARs initiated a program to breed nutrient dense staple foods. They used synthetic hexaploid wheat from *T.dicoccum* & *Aegilops tauschii* with high micronutrient content & developed agronomically superior wheat with 100 per cent more zinc & 30 per cent more iron than the modern cultivars. 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 the control wheat. Department of Biotechnology, Government of India launched a program called “Bio-fortification of wheat for enhanced micronutrients using conventional & molecular breeding” Phase I (2005) & Phase II (2011) 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. But they are devoid of lysine & tryptophan. Quality protein maize has less zein fraction & more non-zein fraction so that it has more lysine & tryptophan content. QPM contains a naturally occurring mutant opaque 2 that increases the amount of two essential amino acids. Due to 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 opaque 2 maize into variety. 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 upto 30-40 per cent along with high lysine & tryptophan content which prevents from Pellagra.

**Conclusion:** People are suffering from malnutrition problem worldwide. 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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