Frontiers in Crop Improvement, Vol. 5 (Spl.) : 311-315 (2017) **ASTHA FOUNDATION**
Print ISSN : 2393-8234, Online ISSN : 2454-6011 *Meerut (U.P.) INDIA*

BIOFORTIFICATION OF WHEAT GRAIN TO ENHANCE ZINC AND IRON CONCENTERATION : PROGRESS AND FUTURE PROSPECTS

Ravi Ranjan Saxesena[1], Lal Bahadur Gaur[1], O.P. Yadav[2], S.C. Gaur[3*] and S.P. Singh[4]

[1]*Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.)*

[2]*Department of Plant Pathology and* [3]*Department of Genetics and Plant Breeding, B.R.D. (P.G.) College, Deoria (U.P.)*

[4]*CSAUAT-Agricultural Research Station, Kalai, Aligarh (U.P.)*

**Email : scgaur101@gmail.com*

ABSTRACT

Importance of wheat as staple cereal crop in the world is well documented, relatively rich in micronutrients but like many other staple cereals, contains low levels of the essential micronutrients mainly zinc and iron. Dietary deficiencies of essential micronutrients such as zinc and iron are a major health concern in developing countries especially for pregnant women and children below the age of five years who suffer from severe acutemalnutritionleads to stunted growth.Therefore, biofortification is of great importance in enriching wheat grain with mineral micronutrient levels. The agricultural strategies which are used to improve the nutritional value of crop plants are knownas biofortification strategies that include genetic biofortification, which is based on conventional as well as molecular plant breeding, and agronomic biofortification through optimized fertilizer applications. In recent past,International Maize and Wheat Improvement Center (CIMMYT) have initiated research to determine the available genetic variability for grain micronutrients from a wide range of germplasm and screened more than 3000 wheat accessions under Harvest Plus program to use them in future wheat- micronutrients breeding programs. During the past decades, traditional breeding programs have successfully incorporated novel alleles into elite germplasm, which has had significant impacts on production globally, alsohas developed biofortified wheat 'Zinc Shakti' commonly called Chitraby introgressing synthetic hexaploid (*A. tauschii*) with elite germplasm with 40%highergrain zinc. Modern breeding technique *viz.* Genomic Selection and Next Generation Sequencing are promising technologies that could help harness large numbers of favorable exotic alleles and lower the level of the anti-nutrients like phytic acid.

Key words : *Wheat, Biofortification, micronutrients, zinc, iron.*

Wheat is the widely cultivated cereal in the world, relatively rich in micronutrients, including minerals and vitamins, and supplies up to 20% of the energy intake of the global population but like many other staple cereals, contains low levels of the essential micronutrients such as zinc and iron (Shewry, 2009; Cummins and Roberts-Thomson, 2009). An adequate intake of a number of minerals in the daily diet is essential for human health (FAO, 2012).Zinc and iron deficiency are the most common and widespread, victimizing more than half of the human population (White and Broadley, 2009). The dietary deficiency of zinc and iron, also known as 'Hidden hunger',results in major health concern in developing countries especially for pregnant women and children below the age of five years who suffer from severe acute malnutrition leads to stunted growth(Stein, 2010). The problem can be addressed by dietary diversification, supplementation and biofortification. Dietary diversification and modification suffers from difficulty in the change of food habits of people and high costs of diets with readily bioavailable zinc and iron content (Zimmerman and Hurrel, 2007; Rawat *et al.,* 2013), whereas supplementation refers to the oral delivery of micronutrients in the forms of syrups or tablets but this strategy has been used in chronic deficiencies.The most acceptable approach is biofortification refers to the process for increasing the bioavailable concentrations of essential elements in the edible portions of crops through genetic selection or agronomic intervention (White and Broadley, 2005; Xu *et al.,* 2011). Genetic biofortification is based on conventional as well as molecular plant breeding approach, a long-term process requiring substantial effort and resources, whereas agronomic biofortification through optimized fertilizer applications.The strategy of biofortification requires the identification of relevant donors as well as an understanding of how micronutrients are accumulated in the grain (Ladizinsky, 1998; Srinivasa *et al.,* 2014). In recent past, International Maize and Wheat Improvement Center (CIMMYT) have initiated research to determine the available genetic variability for grain micronutrients from a wide range of germplasm and screened more than 3000 wheat accessions under Harvest Plus program to use them in future wheat quality breeding programs. Classical breeding and genetic engineering techniques are the two approaches that may be used to biofortify the crops with minerals like iron and zinc (Pfeiffer and McClafferty, 2007; Tiwari *et al*., 2010). During the past decades, traditional breeding programs have successfully incorporated novel alleles into elite germplasm, which has had significant impacts on production globally, alsohas developed biofortified wheat 'Zinc Shakti' commonly called Chitra by introgressing synthetic hexaploid (*A. tauschii*) with elite germplasm with 40% higher grain zinc. Several anti-nutritional factors like phyticacid, fibres and tannins also reduce the bioavailability of these micronutrients from dietary intakes by preventing their absorption in the intestine as well as milling and polishing of cereals make them even deficient for zinc and iron (Pfeiffer and McClafferty, 2007; Borg *et al.,* 2009).

Genetic Variations at Micronutrients level in Wheatm : A substantial genetic variation is present at micronutrients level in wheat either naturally or by the crop improvement through plant breeding strategies (Xu *et al.,* 2011). Both tetraploid (*Triticum turgidum*ssp. *durum*) and hexaploid (*T. aestivum* L.), primary gene pool of wheat, harbors little genetic variation for grain Zn and Feaccumulation, but some of their close wild relatives, inparticular the diploids *T. urartu*tum*., T. boeoticum* L. and *Aegiliopstauschii* (Coss.) Schmal., as well as the tetraploid(*T. turgidum* ssp. dicoccoides) are considered promising aspotential breeding parents for this trait (Cakmak *et al.,* 2004) Among the hexaploids, spelt wheat (*T. aestivum* var. *spelta*) typically has a high grain Zn and Fe content (Velu *et al.,* 2011a; Velu *et al.,* 2014). Few genotypes show significantly higher Zn and Fe concentrations than the most widely grown cultivars, *e.g.,* wild species, landraces and lines from pre-breeding program at CIMMYT (Monasterio and Graham, 2000). Synthetic hexaploid lines [*T. turgidum* ssp. durum (Desf) Husn_Ae. tauschii] had 25 to 30% more Fe and Zn concentrations in their grains than common wheat cultivars (Calderini and Ortiz-Monasterio, 2003; Xu *et al.,* 2011). All these studies indicate that these species could be potential germplasm resources for the genetic improvement of Zn and Fe concentrations. In past decades, CIMMYT have initiated research under the Harvest-Plus project to determine the available genetic variability for grain mineral elements from a wide range of germplasm in the genus *Triticum*, and to study the feasibility of using them in future wheat-breeding programs.

Bioavailability and associations study of micronutrients (Zn and Fe) : The terms, bioavailability is defined as the amount of a nutrient in a meal that is absorbable and utilizable for metabolic processes in the body (Welch and Graham, 2004; Goudia and Hash, 2015). Only a small portion of accumulated minerals in edible parts is bioavailable. Thus, determining the bioavailability of Zn and Fe in the genetically enhanced new lines is an important aspect of wheat biofortification programs. The levels of bioavailable Fe and Zn in staple food crop seeds and grains are as low as 5% and 25%, respectively (Bouis and Welch, 2010; Goudia and Hash, 2015). Thus, bioavailability of micronutrients is an important factor despite the total concentration in the grain; hence plant breeders should therefore consider the bioavailability of micronutrients and the micronutrient concentration while breeding (Goudia and Hash, 2015).

The associations between micronutrients have indicated that grain Zn and Fe are positively correlated in wheat, implying that the alleles for Zn and Fe deposition in the grain co-segregate or pleiotropic, and therefore that Zn and Fe can be improved simultaneously (Peleg *et al.,* 2009; Velu *et al.,* 2012). Some reports showed slightly negative associationbetween Zn and grain yield in wheat (Zhao *et al*., 2009). A significant positive correlation wasalso found between grain protein with Zn and Fe concentrations (Velu *et al.,* 2011a). A very strong significantly positive correlation between grain Zn, Feand protein concentration were detected in many studies. This indicates that high Zn, Fe and grain protein traits might have the same genetic base to some extent, and could be combined andsimultaneously improved by breeding strategies (Welch and Graham 2004; Xu *et al.,* 2011).The Gpc-B1gene is also known to be effective in improving Zn and Fe concentrations (Distelfeld *et al*., 2007). Therefore, enhancing wheat quality to increase protein, Zn and Fe concentrations can be conducted simultaneously in wheat biofortification breeding programs.

Harnessing Existing Genetic Variation for Biofortification of Wheat Grain : Genotypic variation for micronutrient accumulation in grain has been documented in wheat and successful exploitation of genetic variation is the basis for crop improvement through plant breeding methods.Breeding to introgress beneficial genomic regions into wheat is conditioned by the relatedness between the species (Friebe *et al.,* 1996). The various technique used for introgression are direct hybridization, homologous recombination, back crossing and selection if the donor species belongs to the primary gene pool, e.g., hexaploid landraces, cultivated tetraploids **(***T. turgidum*), wild emmer wheats (*T. dicoccoides*) or diploids *T. monococcum*and *A. tauschii*. If the donor species belongs to the secondary gene pool(e.g.,polyploid*Aegilops*and *Triticum* species, and the S-genome species of the genus *Aegilops*) homologous recombination is possible if the loci of interest are transferred in homologous chromosomes, whereas species belonging to the tertiary gene poo l(*e.g., Elymus*species),gene transfer can be achieved (Mondal *et al*., 2015). Velu *et al.* (2011a) reported that among a diverse range of wheat core-collection accessions of diverse origin revealed the existence of large variability for Zn and Fe concentrations. In case of *T. dicoccoides* genetic variation for Zn was substantial. In a *T. dicoccoides* germplasm including 518 lines, Zn concentrations ranged between 30 and 98 mg/kg (Cakmak *et al.,* 2004a). Several hundreds of CIMMYT core-collection accessions were screened for Zn and Fe nearly a decade ago, resulting in the identification of

Table-1: Various level of Zn concentration documented for bread wheat and durum wheat in different studies.

Germplasm/Lines	Number of genotypes studied	Zn concentration (mg/kg)		References
		Mean	Range	
Bread wheat	132	35.5	25.2-53.3	Graham et al., 1999
Bread wheat	150	21.4	13.5-34.5	Zhao et al., 2009
Bread wheat (advanced)	20	33.6	32.6-34.4	Joshi et al., 2010
Bread wheat (advanced)	1300	30.5	23.0-52.0	Velu et al., 2011a
Bread wheat (advanced)	600	30.4	16.9-60.8	Velu et al., 2011b
Bread wheat (advanced)	40	32.5	29.0-39.5	Velu et al., 2012
Bread wheat (BC1F6)	165	48.30	26.1 -74.2	Srinivasa et al., 2014
Bread wheat (BC1F6)	105	48.28	30.1 -74.7	Srinivasa et al., 2014
Durum wheat	65	21	17.0-28.0	Cakmak et al., 2001
Durum wheat	10	21.4	14.0-26.9	Zhao et al., 2009
Durum wheat (old)	10	36.4	33.7-41.4	Ficco et al., 2009
Durum wheat (modern)	57	33.9	28.5-46.3	

Triticum turgidum ssp. *dicoccum* accessions with elevated Zn and Fe levels. These tetraploids were used to develop synthetic hexaploid wheats in the CIMMYT wide-crossing unit (Ortiz-Monasterio *et al.*, 2007). Scientists reported different level of Zn concentration in their studies using significant number of lines of bread wheat and durum wheat (Table-1).

Quantitative Trait Loci (QTL) Analysis And Marker-Assisted Selection (MAS) : Grain Zn and Fe concentrations were controlled by many genes, which make the accumulation of minerals in seeds a complex polygenic phenomenon. The advent of molecular marker enables to dissect such complex traits via analysis of quantitative trait loci (QTLs).The identification of QTLs for grain mineral nutrients can accelerate crop improvement through marker assisted selection (MAS) (Srinivasa et al., 2014). Quantitative trait locus (QTL) analysis provides a powerful approach to unravel the genes underlying the natural variation for Znand Feconcentrations (Ghandilyan *et al.,* 2006). By the use of recombinant inbred lines (RILs) or doubled haploid populations, many QTLs for micronutrient concentration in wheat grain have been mapped in recent years (Distelfeld *et al.*, 2007; Peleg *et al.,* 2009; Tiwari *et al.,* 2009; Srinivasa *et al.,* 2014). In a study conducted in wheat, nine additive and four epistatic QTLs were identified for Fe and Zn, among which six and four, respectively, were effective at the two environments (Xu *et al.,* 2012). The identification and tagging ofmajor QTLs for the traits in relation to micronutrients with large effects will be helpful in the selection ofthe QTLs in early generations with MAS technique (Ortiz-Monasterio *et al.,* 2007). Marker assisted backcrossing is being applied at CIMMYT to improve grain Zn and Fe concentrations. Various studies have reported QTL for high grain Fe and Zn concentrations on chromosomes 1A, 2A, 2B, 3D, 4B, 6A, 6B, and 7A in different species of diploid, tetraploid, and hexaploid wheat (Tiwari *et al.,* 2009; Xu *et al.*, 2012; Hao *et al.,* 2014; Srinivasa *et al.,* 2014). A recombinant inbred line (RIL) population developed from the cross between 'PBW343' and 'Kenya Swara' was used to identify QTL and markers associated with Zn. Two novel large effect QTL on chromosomes 2B and 3A were successfully converted into usable form for marker assisted introgression of this QTL in to an elite background (Mondal *et al.,* 2015).

Reducing rhe Micronutrients Inhibitors and Promoters in Wheat Grain : Most of the grains of cereal crops contain various anti-nutrient factors, such as phytic acid and tannins (Guttieri *et al.,* 2006), which can reduce the bioavailability of micronutrients (Xu *et al.*, 2011) Among inhibitors, phytic acid, tannins, dietary fiber and calcium are the most potent, while organic acids are known to promote iron absorption (Hambidge *et al.,* 2010). Phytate, a complex of phytic acid and mineral elements, decreases the bioavailable concentration ofnutrient elements, and thus leads to health problems, such as Znand Fe deficiency, for populations whose diets arebased mainly on cereals and legumes (Liu *et al.,* 2006; Goudia and Hash, 2015). Significant genetic variation has been reported for grain phytate concentration in wheat (Raboy *et al.,* 1991; Welch *et al.,* 2005) but attempts to use genetic variability for lowering phytic acid in crops are limited. Promoter like- vitamin C, pro-vitamin A, hemoglobin and various organic and amino acids that can stimulate the absorption of micronutrients also exist in cereal crops. Amounts of both anti-nutrients and promoters depend on genetic and environmental factors (Welch and Graham, 2004; Goudia and Hash, 2015). Phytic acid concentration in wheat grain has shown considerable genetic variation, i.e., 7-12 mg/g with Zn fertilization and 8-13 mg/g without Zn fertilization (Erdal *et al.,* 2002). Significant genetic variation within-species for phytate concentration has been detected in wheat grain (Welch *et al.,* 2005; White and Broadley, 2009). Fewer genes are involved in the biosynthesis and metabolism of inhibitors and promoters compared with the uptake, transport, and deposition of Fe and Zn. Thus, improving the bioavailability of Fe and Zn shouldbe much easier than increasing their concentrations in grains (Bouis and Welch, 2010). Enhancing the promoters and decreasing the inhibitors could improve micronutrient bioavailability (Welch and Graham, 2004). However, breeders should be cautious of the possiblenegative consequences of changing anti-nutrients because of the important roles phytate and some other anti-nutrients play in plant metoblism and human diets (Goudia and Hash, 2015).

Future Strategy for Breeding High-Yielding Wheat Varieties With Improved Zinc and Iron Concentration : Landraces and wild relatives of wheat have high levelof Zn and Fe concentrations that might be important genetic resources for enhancing micronutrientsthrough conventional and molecular breeding; also by agronomic strategies(optimized fertilizer application) andreducing the anti-nutritional compounds (Fig.-1). The major QTLs that are consistently expressed under various environments can be used in wheat MAS breeding and in positional cloning. Furthermore, the genes that have impacts on absorption, transportation, and accumulation of micronutrients in grains must be detected, and the biosynthetic pathways producing micronutrient inhibitors and promoters must be further dissected to manipulate the amounts and bioavailability of micro nutrients in grains (Xu *et al.,* 2011).

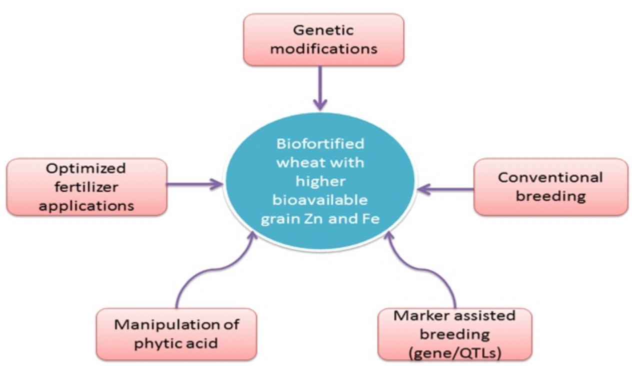

Fig.-1: Future prospects for breeding high-yielding wheat varieties with improved levels of bioavailable Zn and Fe concentration, Source : Goudia and Hash, 2015.

Combining conventional breeding with MAS and genetic engineering strategies will be more effective and feasible for breeding desirable cultivars with higher yield, concentrated micronutrients, and improved micronutrients bioavailiability in biofortification breeding programs. Agronomic strategies can serve as a quick solution to micronutrient deficiency and a desirable complementary approach to breeding strategy.The most favorable work plan to successfully alleviate micronutrient malnutrition will be to increase mineral content in the crops and simultaneously enhance their bioavailability by reducing anti-nutritional compounds or enhancing concentration of mineral absorption promoters (Rawat *et al.,* 2013).

CONCLUSION

Sufficient intake of a number of minerals in the daily diet is essential for human health but significant population in developing countries, suffers from a dietary deficiency in zinc and iron.Therefore, biofortification of wheat is of great importance in enriching seeds with mineral micronutrient for overcoming 'hidden hunger.'There is large genetic variation present for Zn and Fe concentration in wheat grain in different part of the world. Therefore, conventional breeding and modern breeding technique *viz.* Genomic Selection and Next Generation Sequencing are promising technologies that could help harness large numbers of favorable exotic alleles and reducing the level of the anti-nutritional compounds.

REFERENCES

1. Borg, S., Brinch-Pedersen, H., Tauris, B. and Holm, P.B. (2009). Iron transport, deposition and bioavailability in the wheat and barley grain. *Plant Soil* 325: 15-24.
2. Bouis, H.E. and Welch, R.M. (2010). "Biofortification—A Sustainable agricultural strategy for reducing micronutrient malnutrition in the global south," *Crop Sci.* 50: 20-32.
3. Cakmak, I., Torun, A., Millet, E., Feldman, M., Fahima, T., Korol, A., Nevo, E., Braun, H.J. andOzkan, H. (2004a.). Triticum dicoccoides: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Sci. Plant Nutr.* 50: 1047-1054.
4. Cakmak, O., Ozturk, L., Torun, B., Ozkan, H., Kaya, Z. and Cakmak, I. (2001). Tolerance of 65 durum wheat genotypes to zinc deficiency in a calcareous soil.*J. Plant Nutr.* 24: 1831-1847.
5. Calderini, D.F. and Ortiz-Monasterio, I. (2003).Are synthetic hexaploids a means of increasing grain element concentrations in wheat? *Euphytica*134: 169-178.
6. Cummins, A.G. and Roberts-Thomson, I.C. (2009). Prevalence of celiac disease in the Asia-pacific region.*J. Gastroenterol. Hepatol.* 24: 1347–1351.
7. Distelfeld, A., Cakmak, I., Peleg, Z., Ozturk, L., Yazici, A.M. and Budak, H. (2007). Multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations.*Physiol. Plant* 129: 635-643.
8. Erdal, I., Yilmaz, A., Taban, S., Eker, S., Torun, B. and Cakmak, I. (2002). Phytic acid and phosphorus concentrationsin seeds ofwheat cultivars grown with and without zinc fertilization. *J. Plant Nutr.* 25: 113-127.
9. FAO.(2012). FAOSTAT.FAO. faostat.fao.org/site/ 339/ default.aspx (accessed 1 Nov. 2013).
10. Ficco, D.B.M., Riefolo, C., Nicastro, G., De Simone, V., Di Gesu, A.M., Beleggia, R., Platani, C., Cattivelli, L. and De Vita, P. (2009). Phytate and mineral elements concentration in a collection of Italian durum wheat cultivars. *Field Crops Res.* 111: 235-242.
11. Friebe, B., Jiang, J., Raupp, W.J., McIntosh, R.A., and Gill, B.S. (1996). Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica* 91: 59–87.doi:10. 1007/BF00035277

12. Ghandilyan, A., Vreugdenhil, D. and Aarts, M.G.M. (2006). Progress in the genetic understanding of plant iron and zincnutrition. *Physiol. Plant*. 126: 407-417.
13. Goudia, B.D and Hash, C.T (2015).Breeding for high grain Fe and Zn levels in cereals.International.*J. of Innovation and Applied Studies* 12: 342-354.
14. Graham, R.D., Senadhira, D., Beebe, S., Iglesias, C. and Monasterio, I. (1999). Breeding for micronutrient density in edible portions of staple food crops conventional approaches.*Field Crops Res*. 60: 57-80.
15. Guttieri, M. J., Peterson, K.M. and Souza, E.J. (2006). Agronomic performance of low phytic acid wheat.*Crop Sci.* 46: 2623-2629.
16. Hambidge, K.M., Miller, L.V., Westcott, J.V., Sheng, X. and Krebs, N.F. (2010). Zinc bioavailability and homeostasis,*Am. J. Clin. Nutr.* 91: 1478-1483.
17. Hao, Y., Velu, G., Peña, R.J., Singh, S. and Singh, R.P. (2014).Genetic loci associated with high grain zinc concentration and pleiotropic effect on kernel weight in wheat (*Triticum aestivum* L.). *Mol. Breed.* 34: 1893–1902.doi: 10.1007/s11032-014-0147-7
18. Joshi, A.K., Crossa, I., Arun, B., Chand, R., Trethowan, R., Vargas, M. and Ortiz-Monasterio, I. (2010). Genotype environment interaction for zinc and iron concentration of wheat grain in eastern Gangetic plains of India.*Field Crops Res.* 116: 268-277.
19. Ladizinsky, G. (1998). Plant evolution under domestication. Kluwer, Dordrecht, the Netherlands.
20. Liu, Z.H., Wang, H.Y., Wang, X.E., Zhang, G.P., Chen, P.D. and Liu, D.J.(2006). "Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron, and zinc contents in wheat (*Triticum aestivum* L.)," *J. Cereal Sci.* 44: 212-219.
21. Mondal, S., Rutkoski, J.E., Velu, G., Singh, P.K., Crespo-Herrera, L.A.,,Guzmán C., Bhavani, S., Lan, C., He, X. and Singh, R.P. (2016). Harnessing Diversity in Wheat to Enhance Grain Yield, Climate Resilience, Disease and Insect Pest Resistance and Nutrition Through Conventional and Modern Breeding Approaches. *Front. Plant Sci.* 7: 991. doi: 10.3389/ fpls.2016.00991
22. Ortiz-Monasterio, I., Palacios-Rojas, N., Meng, E., Pixley, K., Trethowan, R. and Pena, R.J. (2007). "Enhancing the mineral andvitamin content of wheat and maize through plant breeding," *J. of Cereal Sci*. 46: 293-307.
23. Peleg, Z., Cakmak, I., Ozturk, L., Yazici, A., Jun, Y., Budak, H., Korol, A.B., Fahima, T., Saranga, Y. (2009). Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat x wild emmer wheat RIL population. *Theor. Appl. Genet.* 119: 353-369.
24. Pfeiffer, W.H. and McClafferty, B. (2007). Harvest Plus: breeding crops for better nutrition. *Crop Sci.* 47: 88-105.
25. Raboy, V. (2007). Seed phosphorus and the development of low-phytate crops. In: Turner B.L., A.E. and Mullaney E.J. (eds), Inositol Phosphates: Linking Agriculture and Richardson, the Environment, 111-132. CABI, Wallingford, UK.
26. Rawat, N., Neelam, K., Tiwari, V.K, and Dhaliwal, H.S. (2013). Biofortification of cereals to overcome hidden hunger.*Plant Breed.* 132: 437–445.
27. Shewry, P.R. (2009). The health grain programme opens new opportunities for improving wheat for nutrition and health. *Nutr. Bull.* 34: 225–231.
28. Srinivasa, J., Arun, B., Mishra, V.K., Singh, G.P., Velu, G. and Babu, R.(2014). Zinc and Iron concentration QTL mapped in a *Triticum spelta*x *T. aestivum* cross. *Theor. Appl. Genet.* 127:1643–1651.doi:10.1007/ s00 122-014-2327-6
29. Stein, A.J. (2010). Global impacts of human mineral malnutrition," *Plant and Soil* 335: 133-154.
30. Tiwari, V.K., Rawat, N.,Neelam, K., Kumar, S., Randhawa, G.S. and Dhaliwal, H.S. (2010). Substitution of 2S and 7U chromosomes of Aegilopskotschyi in wheat enhances grain iron and zinc concentration. *Theor. Appl. Genet.* 121: 259-269.
31. Tiwari, V.K., Rawat, N., Chhuneja, P., Neelam, K., Aggarwal, R., Randhawa, G.S., Dhaliwal, H.S., Keller, B. and Singh, K. (2009). Mapping of quantitative trait loci for grain iron and zinc concentration in diploid genome of wheat. *J. Hered.* 100: 771-776.
32. Velu, G., Singh, R.P., Huerta-Espino, J. and Peña, R.J. (2011b). Breeding for enhanced zinc and iron concentration in CIMMYT spring wheat germplasm.*Czech J. Genetics Plant Breed*. 47:174-177.
33. Velu, G., Ortiz-Monasterio, I., Singh, R.P. and Payne, T. (2011a.). Variation for grain micronutrients concentration in wheat core-collection accessions of diverse origin. *Asian J. Crop Sci.* 3: 43-48.
34. Velu, G., Singh, R.P., Huerta-Espino, J., Peña- Bautista, R.J., Arun, B., Mahendru-Singh, A., YaqubMujahid, M., Sohu, V.S., Mavi, G.S., Crossa, J., Alvarado, G., Joshi, A.K. and Pfeiffer, W.H. (2012). Performance of biofortified spring wheat genotypesin target environments for grain zinc and iron concentrations. *Field Crops Res*. 137: 261-267.
35. Welch, R.M. and Graham R.D. (2004). "Breeding for micronutrients in staple food crops from a human nutrition perspective," *J. Exp. Bot*. 55: 353-364
36. Welch, R.M. and Graham, R.D. (2005) Agriculture: the real nexus for enhancing bioavailable micronutrients in food crops. *J. Trace Element Med. Biol*. 18: 299-307.
37. White P.J. and Broadley M.R. (2009). "Biofortification of crops with seven mineral elements often lacking in human diets -iron, zinc, copper, calcium, magnesium, selenium and iodine," *New Phytol.* 182: 49-84.
38. Xu, Y., An, D.G., Li, H. and Xu, H. (2011). Review: Breeding wheat for enhanced micronutrients. *Can. J. Plant Sci*. 91: 231-237.
39. Xu, Y.F., An, D.G., Liu, D.C., Zhang, A.M., Xu, H.X. and Li, B. (2012).Molecular mapping of QTLs for grain zinc, iron and protein concentration of wheat across two environments. *Field Crops Res.* 138: 57–62.doi:10.1016/ j.fcr.2012. 09.017
40. Zhao, F.J., Su,Y.H., Dunham, S.J., Rakszegi, M., Bedo, Z., McGrath, S.P. andShewry, P.R., (2009). Variation in mineral micronutrient concentrations in grain of wheat linesof diverse origin. *J. Cereal Sci.* 49: 290-295.
41. Zimmerman, M.B. and Hurrel, R.F. (2007). Nutritional iron deficiency. *Lancet* 370: 511-519.