



International Journal of Herbal Medicine

Biotechnological Intervention for Biofortification in Crop Plants – A Brief Review

Y. J. Patel¹, R. S. Ingalhalli^{2*}

1. C.G.Bhakta Institute of Biotechnology. UKA Tarsadia University, Maliba Campus, Gopal vidyanagar, Bardoli- Mahuva Road, Ta. : Mahuva, Dist. Surat- 394 350. Gujarat, India.
2. Assistant professor C. G. Bhakta Institute of Biotechnology. UKA Tarsadia University, Maliba Campus, Gopal vidyanagar, Bardoli- Mahuva Road, Ta. : Mahuva, Dist. Surat- 394 350. Gujarat, India.
[E-mail: rajashekhar@utu.ac.in; Tel: +91-9448371037]

Over half of the global population is estimated to be suffering from various food deficiency diseases. A large portion of the population in the developing countries relies mainly on one or more staple food crops for their nutrition. In this context, it has become imperative to supplement the staple crops with important necessary nutritional factors. Biotechnological approaches have played an important role in crop improvement and can be a helping tool in developing staple food crops with sufficient nutritional supplements. The scientists from all over the world are engaged in biofortifying various staple food crops to fight different nutritional disorders among poorer populations.

Keyword: Biofortification, Golden Rice, Nutrient Deficiency, Genetic Engineering.

1. Introduction

A major challenge of our time is that one sixth of the world's population suffers from hunger, a situation which is totally unacceptable. In addition, over half of the global population, is afflicted by a different form of food deficiency^[1]. A significant portion of the developing world's population relies largely on one or more staple food crops for their nutrition and these are the subjects of biofortification projects, both by conventional breeding and by modern biotechnology tools. Agricultural biotechnology, in specific genetic engineering represents therefore a complementary strategy for the development of more nutritious crops. Biofortification may be defined as the development of micronutrient-dense staple crops using the

best traditional breeding practices and modern biotechnology^[2,3].

1.1 Biofortification for vitamin A

Vitamin A deficiency is particularly prevalent among children of Africa and Southeast Asia. It causes irreversible blindness and increased susceptibility to disease and mortality. Rice plant produces β -carotene (provitamin A) in green tissues, but not in the seeds. A public-private partnership to produce rice varieties rich in provitamin A culminated in the development of Golden Rice, in which two genes were introduced by genetic engineering.

Beaton & his associates^[4] and Sommer and West^[5]. have cited that a number of randomized controlled trials in developing

countries have shown that administration of vitamin A capsule for infants and pre-school children helps to reduce the mortality rates from all causes by 23 percent and administration of capsules with vitamin A and beta-carotene among women during child-bearing period can reduce maternal mortality related to pregnancy by 40 percent and 49 percent, respectively.

Ye & others^[6] used *Agrobacterium* – mediated transformation technique to introduce the entire β -carotene (pro – vitamin A) biosynthetic pathway in which they introduced *psy*, *crt I* and *lcy* gene into rice endosperm which produces 1.6 $\mu\text{g/g}$ carotenoids.

Villamor & his associates^[7] while studying subclinical trials on vitamin A deficiency e often observed anemia, impaired linear growth and morbidity from common childhood infections such as respiratory and diarrheal diseases^[5], measles^[8], and malaria^[9].

Paine & others^[10] produced a new variety of rice (*Oryza sativa*) called Golden rice-2 through genetic engineering to biosynthesize beta-carotene, *psy* gene from *Zea mays* and *crt I* from *Erwinia uredovora*, which produces 23 fold (maximum 37.1 $\mu\text{g/g}$) more beta-carotene compared to Golden Rice-1.

1.2 Biofortification for Iron and Zinc

Fe and Zn deficiencies are the sixth and fifth highest health risk factors respectively, in developing countries, and they have high mortality rates. Fe deficiency causes anemia and Zn deficiency causes impaired growth and immune dysfunction. Fe and Zn-biofortified rice varieties offer a promising potential solution. Nicotianamine, the precursor of phytosiderophores, chelates of Fe^{2+} and Zn^{2+} and plays an important role in

transporting these metal ions to both vegetative and reproductive organs within the plant.

Scientists from four Future Harvest Centers of the Consultative Group on International Agricultural Research (CGIAR) and partner organizations (1995) have been evaluating the feasibility of using modern breeding techniques to produce new varieties of staple crops with high zinc, iron, and beta carotene content. Zinc deficiencies have equally serious consequences for health. Recent randomized controlled trials show that zinc supplementation can reduce morbidity from a number of common childhood infections, especially diarrhea, pneumonia, and possibly malaria, by one-third^[11,12,13].

Gregorio & his associates^[14] has evaluated the amounts of iron that may be added through biofortification to diets for selected food staples for the indicated sub regions. These minimum increments are based on average levels of consumption of these food staples (data available from the Food and Agriculture Organization of the United Nations) and conservative estimates of the extent to which conventional breeding might increase iron content in the consumed portions of these staple foods.

It is also estimated that zinc density can be increased substantially through conventional breeding, perhaps by 100% in rice and wheat and by 75% in beans^[14,15,16].

Lucca & others^[18] introduced three genes *pfe*, *rgMT* and *phy A* into rice grains to produce iron- rich rice. The iron content in mature T1 seeds varied between 11.53 ± 0.16 and 22.07 ± 0.70 $\mu\text{g/g}$ per seed. Two-fold increases in the iron content of seeds from the transgenic plant with the highest iron level were also observed.

Masuda & others¹⁹¹ cultivated three transgenic rice lines possessing barley genome fragments containing gene for MAs synthesis (i.e., *HvNAS1*, *HvNAA1-A* and *IDS3*) in a paddy field. Polished rice seeds with *IDS3* inserts had up to 1.40 and 1.35 times higher Fe and Zn concentrations, respectively, compared to non-transgenic rice seeds. Similarly, Masuda & others²⁰¹ reported increase in Fe and Zn contents of three and two fold respectively, in polished T1 rice grains by over expressing the barley nicotianamine synthase *HvNAS1*.

Wirth & his associates²¹¹ studied the effect of the increased expression of *AtNAS1*, *Pvferritin* (ferritin gene from *Phaseolus vulgaris*) and *Afphytase* (phytase gene from *Aspergillus fumigatus*) in rice (*Oryza sativa* sp. *Japonica*) on the concentration of iron in the endosperm strongly increased upto 6.3-fold. Along with iron content amount of zinc in transform endosperms increased by 1.3 to 1.5 fold over wild type.

1.3 Biofortification for Multivitamin (β -Carotene, Ascorbate and folate)

Ascorbate is synthesized in plants from the precursor D-glucose, and its deficiency causes scurvy while Folic acid act as a coenzyme for enzymatic activity and its deficiency causes Megaloblastic anaemia. The absence of Key vitamins in cereal grains reflects the fact that the corresponding metabolic pathways are absent, truncated, or inhibited in the endosperm. Therefore, used suitable strategy to enhance these pathways is to introduce genes encoding Key enzymes.

Naqvi & others²²¹ increased the levels of 3 vitamins. (β -carotene, ascorbate, and folate) with modification of 3 separate metabolic pathways using multigene engineering. In the best-performing line, the endosperm accumulated ~60 $\mu\text{g/g}$ β -carotene comparatively most successful then GR1 (1.6 $\mu\text{g/g}$ dry weight) and GR2 (31 $\mu\text{g/g}$

DW). Ascorbate and Folate content was found to be increased 6 fold (~110 $\mu\text{g/g}$ DW) and 1.94 $\mu\text{g/g}$ DW respectively in transgenic corn endosperm.

1.4 Biofortification for Increased Protein Content

Nutritional value of seed storage proteins is often limited. It may lack one or more amino acids essential to human health e.g. legume seeds lack cysteine and methionine; other seeds might lack lysine. Thus, amino acid balance in seeds has been manipulated in laboratory experiments using number of strategies.

Chakraborty & his associates²³¹ have studied that increase in nutritive value of potato by expressing a non allergenic seed albumin gene *AmA1* from *Amaranthus hypochondriacus*. They reported 4-8 fold higher essential amino acids in pSB8G transgenic potatoes.

Taber & others²⁴¹ has found out that Tg, soil-grown seeds expressing a foreign Met-and Cys-rich protein, decreased pools of free Met, free Cys, and glutathione indicated that the rate of synthesis of sulfur amino acids in the cotyledon had become limiting.

1.5 Biofortification for Oil Quality

Vegetable oil and fats constitute important components in human diet. They are very rich source of energy and an important source of certain vitamins and hormones and form structural components of cell. Mammals cannot synthesize linoleic (18:2) and linolenic(18:3) fatty acids. Therefore, they must be supplied in their diet, and they are called essential fatty acids^{25,261}.

Deborah & his associates²⁷¹ modified Brassica seed oil by antisense expression of a stearyl-acyl carrier protein desaturase gene which showed dramatically increased state levels in the seeds.

2. Conclusion

From the foregoing discussion, it can be concluded that, through genetic engineering now a complete biosynthetic pathway can be engineered for desirable nutrients in crop plants so that we supply all the necessary nutrients to the poor people in their daily food. By biofortification, micronutrient, vitamins, protein and oil quality of these energy rich staples can be increased among the poor in particular. Biofortification is also fairly cost effective after an initial large research investment.

5. References

1. <http://www.fao.org/docrep/006/Y5160E00.htm>. FAO. (2004).
2. Nestel P, Bouis H, Meenakshi J, and Pfeiffer W, Symposium, Am. Soc. Of Nut. 2006; 1064-1066.
3. Nestel P, Bouis HE., Meenakshi JV and Pfeiffer W. Biofortification of Staple Food Crops. J. Nutr. Washington DC and Harvest Plus, Centro International de Agricultural Tropical, Cali, Colombia 2006; 136: 1064-1067.
4. Beaton G, Martorell HR, Aronson KJ, Edmonston B, McCabe G, Ross AC, and Harvey B, Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. ACC/SCN State-of-the-Art Series, Nutrition Policy Discussion Paper No. 13. Geneva: United Nations Administrative Committee on Coordination/Sub-Committee on Nutrition. 1993
5. Sommer A, West KP (eds.) Vitamin A deficiency: health, survival and vision NY: Oxford University Press, . New York, 1996.
6. Ye X, Babili S, Kloti A, Zhang J, Lucca P, Beyer P, and Potrykus I, Science 2000; 287: 303-305.
7. Villamor E, Fawzi WW, Vitamin A supplementation: implications for morbidity and mortality in children. J Infect Dis 2000; 182 (Suppl 1) : 122-133.
8. West CE, Vitamin A and measles. Nutr Rev 2000; 58 (2 Pt 2) : S46-S54.
9. Shankar AH, Genton B, Semba RD, et al. Effect of vitamin A supplementation on morbidity due to Plasmodium falciparum in young children in Papua New Guinea: a randomised trial. Lancet 1999; 354: 203-209.
10. Paine J, Shipton C, Chaggar S, Howells R, Kennedy M, Vernon G, Wright S, Hinchliffe E, Adams J, Silverstone A, and Drake R, Nat. Biotechnol 1999; 1 :1-3.
11. Bhutta ZA, Black RE, Brown KH, Gardner JM, Gore S, Hidayat A, Khatun F, Martorell R, Ninh NX, Penny ME, Rosado JL, Roy SK, Ruel M, Sazawal S, and Shankar A, Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Zinc Investigators' Collaborative Group. J Pediatr. 1999; 135: 689-697.
12. Bhutta, ZA, Bird SM, Black RE, Brown KH, Gardner JM, Hidayat A, Khatun F, Martorell R, Ninh NX, Penny ME, Rosado JL, Roy SK, Ruel M, Sazawal S, and Shankar A, Therapeutic effects of oral zinc in acute and persistent diarrhoea in children in developing countries: pooled analysis of randomized controlled trial. Zinc investigators' collaborative group. American Journal of Clinical Nutrition 2000; 72: 1516-1522.
13. Black R. E. Therapeutic and preventive effects of zinc on serious childhood infectious diseases in developing countries. American Journal of Clinical Nutrition 1998; 68: S476-S479.
14. Gregorio GB, Senadhira D, Htut H, and Graham RD, Breeding for trace mineral density in rice. Food and Nutrition Bulletin 2000; 21 (4): 382-386.
15. Monasterio I, and Graham RD, Breeding for trace minerals in wheat. Food and Nutrition Bulletin 2000; 21 (4): 392-396.
16. Beebe S, Gonzalez AV, and Rengifo J, Research on trace minerals in the common bean. Food and Nutrition Bulletin 2000; 21 (4) : 387-391.
17. Bhargava A, Bouis H, and Schrimshaw N, Dietary intakes and socioeconomic factors are associated with the hemoglobin concentration of Bangladeshi women. Journal of Nutrition . 2001; 131 (3) : 758-764.
18. Lucca P, Hurrell R, and Potrykus I, Theor. Appl.Genet. 2001; 102 : 392-397.

19. Masuda H, Suzuki M, Morikawa C, Kobayashi T, nakanishi H, and Takahashi M, Rice. 2008; 1 : 100-108.
20. Masuda H, Higuchi K, Nakanishi H, Mori S, and Nishizawa N, Rice. 2009; 21 : 55-166.
21. Wirth J, Poletti S, Aeschlimann B, Yakandawala N, and Drosse, Bpl. Biotechnol. J 2009; 7 : 1-14.
22. Naqvi. S, Zhu C, Farre G, Ramessar K, Bassie L, Breitenbach J, and Conesa D, Proc. Nat. Acad. Sci. 2009; 106 : 7762-7767.
23. Chakrabort S, chakraborty N, and Datta A, Proc. Nat. Acad. Sci. 2000; 97 : 3724-3729
24. Tabe L, and Droux M, Plant Physiol. 2002; 128 : 1137-1148.
25. Friesen L, Nelson G, and Ackeer V, Agron. 2003; 95(5) : 1342-1347.
26. Singh BD, Biotechnology Expanding Horizons. Kalyani publ. 2009; pp 493-516.
27. Deborah S, Gregory A, Sharon E, Knauf C, and Jean C, Proc. Nat. Acad. Sci. 1991; 88 : 2510-2514.