DEVELOPMENT OF IRON-RICH CROPS BY GENETIC ENGINEERING

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Keywords: Iron accumulation, iron metabolism, ferritin, seed specific promoter, glutelin, bioavailability

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Summary

The genetic improvement of crops to contain high levels of iron is one of the ways to overcome the malnutrition problems in the world. This paper reviews the concepts underlying such genetic improvement and the results of experiments using the soybean ferritin gene system transferred into three species of plant: rice (*Oryza sativa* L. cv. Kita-ake), lettuce (*Lactuca sativa* L. cv. Green Leaf) and tobacco (*Nicotiana tabacum* L. cv. Petit-Havana SR1). The introduced ferritin gene was expressed under the control of a rice seed-storage protein glutelin promoter, *GluB-1* (-1302/+18), to specifically increase iron content in the rice grain. Another construct containing the *CaMV 35S* promoter was also used for expressing the ferritin gene constitutively in lettuce and tobacco. Results indicated that soybean ferritin could be highly expressed and accumulated in the endosperm tissue of rice and in leaves of lettuce and tobacco. These exogenously produced ferritins increased iron content up to three fold in rice seed, 1.7 fold in lettuce leaf and 1.3 fold in tobacco leaf. Tolerance to oxidative damage and growth stimulation were also described as additional distinctive features of the

transformants. Finally, future work to store more iron in ferritin and to increase the bioavailability of iron has been discussed.

1. Introduction

Iron is an important mineral for human health and is often deficient where inadequate plant-based diets are the primary food source. Iron deficiency is still a major problem afflicting an estimated 30% of the world population (see *Agronomic Approaches for Increasing Iron Availability to Food Crops*). Anemia caused by iron deficiency triggers more serious disorders, such as abortion, brain damage in infants, increased susceptibility to infection and chronic exhaustion (see *Iron Nutrition in Man: Global Perspectives on Iron Deficiency and Malnutrition*). To overcome iron deficiency, direct oral administration of iron as a food additive or in tablet form is most effective. However, this is difficult to implement in developing countries mainly because of the cost. Crops containing high iron concentrations have also contributed to overcoming the deficiency. Some crops, such as spinach and leguminous plants, are known for their high iron content. However, they also contain oxalic acid and phytate-like harmful substances that decrease the bioavailability of iron, whereas, various agronomic approaches, such as modifying culture media, have been attempted to increase iron content in crops, such approaches are also costly and it is not possible to target iron accumulation to a preferable part of the plant by this method. In addition, the excess iron and/or chelator in the media not only suppress plant growth, but also reduce commercial value and productivity. Given such problems, the molecular genetic approach is a potential way to overcome these problems.

The concept, strategies and problems of iron content improvement are reviewed herein, using an experimental system of the soybean ferritin gene transferred into rice (*Oryza sativa* L. cv. Kita-ake), lettuce (*Lactuca sativa* L. cv. Green Leaf) and tobacco (*Nicotiana tabacum* L. cv. Petit-Havana SR1). The practical value of the transformants as high-level iron containing crops is estimated. The qualities of the transformants other than increasing iron storage capacity, such as tolerance to oxidative damage and growth stimulation, are also described as targets for further investigation. Finally, future work to store more iron in ferritin and to achieve the bioavailability of iron are discussed.

2. Overview of the Genetic Improvement for Iron Content in Crops

During the past decade, gene manipulation technologies have been started, to breed crops with improved nutritional quality (see *Molecular Genetic Approaches to Improve the Nutritional Quality of Staple Food Crops*). The first target was protein composition, especially in seeds. It is called seed storage proteins and is utilized as an essential nutrient source for the plant seedling upon germination by nature. These are synthesized only in developing seeds and usually in large amounts. They do not demonstrate any enzymatic activity. Thus, improvement can be achieved simply by introducing and over expressing a target storage protein gene or modifying it to contain the nutritionally deficient amino acids (i.e., cysteine and methionine to the dicot seed; lysine, tryptophan and threonine to the monocot seed). This work has been somewhat successful and, as a result, interest has expanded to modify other kinds of nutritional content, e.g., carbohydrates, vitamins, fatty acids and minerals.

At the primary steps, the factors to be considered for genetic improvement are common for improving seed storage proteins and other nutritional contents (e.g., transcription, translation and assembly of the peptides). To increase the transcriptional level, promoter activity, gene numbers, chromosomal position and codon usage of the introduced gene should be designed well. To increase the translational level, although the methodology has never been established, polysome activity and mRNA stability should be considered. Sometimes, avoiding gene silencing may be important. Eliminating repeated elements from transgene constructs and regulating the moderate transcription rates may alleviate such problems. Furthermore, the most important factor in the primary steps is peptide assembly, because many proteins are produced as immature polypeptides. These polypeptides must be transported to specific organelles, cleaved to a certain form, must then interact with another peptide and then assembled to the mature form. Success in these sequential processes is needed to obtain the functional protein. The stability of the protein is also essential. Some proteins are degraded at a specific developmental stage, or in a specific tissue or organelle. Certain types of degradation could be regulated by environmental stress.

On the other hand, just being an introduced gene product is not enough for the nutritional content improvement, other than the case of seed storage proteins. In other words, the products of the introduced gene must function as an enzyme and must create the expected nutritional content. In addition, the introduced gene has to be well organized at not only the level of function, but also at the level of cooperation with other enzymatic activities and/or metabolites, in order not to corrupt the endogenous homeostasis. Sometimes, the ectopic expression of the exogenous gene can induce a preventive competition with native enzymes both at the substrate and product levels. Accordingly, the target trait and the related metabolism for the modification should be well characterized, both in the physiological and molecular levels. From this point of view, iron metabolism is a candidate for modification in plants, even though there are still some missing links in its physiology. Analysis of the effect of iron over-accumulation controlled by exogenous gene expression provides not only practical value as a human food source but also further knowledge about iron metabolism in plants.

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