Engineering Crop Plants With Seed Storage Protein For Improved Nutritional Quality

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Abstract

The improvement in nutritional quality of crop plants, particularly in terms of protein and amino acid, is an urgent global health issue. Proteins represent one of the principal constituents of a balanced diet, which impart nutritional value to the food and fodder. Plants are the major source of dietary protein. Increased protein levels and the concentration of essential amino acids in food crops are of primary importance in crop improvement program. Efforts have been made to create protein-rich crops, but with limited success so far. A promising strategy is the genetic engineering of genes encoding proteins with high nutritional value into food crops. Towards this, we have developed nutritionally enriched potato and rice by engineering a gene encoding a seed storage protein, AmA1. Further, we have elucidated the molecular mechanism of AmA1 function in transgenics.

Keywords: Nutritional quality, crops, seed storage protein, genetic engineering

Introduction

Humans require a diverse and nutritionally well-balanced diet in order to maintain optimal health and depend largely on plants for their daily nutritional requirements. Moreover, a large proportion of the world population is undernourished. Thus, nutritional improvement of crop plants is an urgent worldwide health issue, as basic nutritional requirements for much of the world's population are still not met (Chakraborty et al 2010). Basic nutrients include vitamin A, iron, iodine, zinc, quality protein. In many parts of world people are restricted to a diet, based exclusively on staple food. Deficiencies of these nutrients in staple food do cause micronutrient and protein-energy malnutrition throughout much of the developing world. Thus enhancing the nutritional quality of crops is an important undertaking for future food security and the nutritional well-being of world population (IAPTC&B2006). Protein deficiency is the most crucial factor that affects physical growth and development, and increases morbidity and mortality especially in developing countries. It lowers resistance to disease and may cause permanent impairment of the brain in infants and young children. Protein malnutrition is essentially caused by poor quality diets, characterized by high intake of staple crops either with less protein and/or low quality proteins in terms of amino acid composition. It is one of the principal constituents of a balanced diet, which impart nutritional value to the food due to its structural constituents, amino acids. However, an ideal situation exists very rarely in nature where one finds all the essential amino acids in a single food crop. A major effort has been to improve the amino acid composition of plant protein, since animals including humans are incapable of synthesizing 10 of the 21 essential amino acids for protein synthesis and these must therefore be obtained from the diet (Chakraborty et al 2000).

There have been several attempts through mutant selection, and engineering genes encoding key amino acid biosynthesis pathway enzymes, to increase free essential amino acids in crop plants (Matthews and Hughes 1993). However, an increase in the free essential amino acids does not lead to an increase in the fixed content and could be leached out from the plant tissue and lost during boiling and other processing (Falco et al 1995). A promising strategy then is the genetic engineering of genes encoding proteins with high nutritional value into food crops (Chakraborty et al 2000; Stoger et al 2001; Yang et al 2002). However, despite promises that genetically modified (GM) crops could make significant contribution to achieve global food security, the new generation varieties are primarily used for industrial crops, such as cotton and animal fodder (Qaim and Zilberman 2003) and a few have been commercialized.

More than 80% of the calories in a diet are derived from cereals and starchy food crops, of which rice and potato are the primary cereal and non-cereal staple crops. The demand for cereal and starchy crops will continue to increase as a consequence of the expanding human population, which could add >1.5 billion people by 2025. Potato is the most important non-cereal food crop and ranks fourth in terms of total global food production. It is also used as animal feed and other industrial products. Potatoes are grown in nearly 125 countries and more than a billion people worldwide consume them on a daily basis (Mullins et al 2006). The total value of the crop is estimated at 40 billion dollars for the top 10 producing countries, which account for two-thirds of global potato production (http://fao.org). The United Nations had declared 2008 as the "International Year of the Potato", affirming the need to focus on the role that the potato can play in providing food security (UN 2005, Session 60). Unfortunately, the nutritional quality of potato tubers is greatly compromised because they contain less protein and are deficient in lysine, tyrosine and the sulfur containing amino acids (Jaynes et al 1986). On the other hand, rice is the most important staple food crop for half of the world's population. It is grown in at least 114 countries and more than 50 countries have an annual production of 100,000 tons or more. Asian countries account for 90% of world's area and 92% production for rice. Rice provides 20% of the world dietary energy supply, while wheat supplies 19% and maize 5% (FAO 2004). Rice, a carbohydrate rich food contains 85% carbohydrate, 7% fat, and 8% protein. In comparison to legumes which contain 30-40% protein, rice has much less protein. Moreover, the amino acids composition of rice storage proteins having low levels of threonine, lysine, and methionine is not balanced with respect to all essential amino acids as recommended by WHO, (Takaiwa 1999).

To guarantee sufficient supply of quality protein in diet, mainly consisting of cereal and starchy staple foods, specific interventions in genetic engineering is an absolute necessity although there is currently public concern about their use in contemporary agriculture, particularly when genes are from non-plant source. Introduction of an appropriate storage protein gene in these food crops would help to improve the nutrient balance. During the last decade, several potential candidate genes have been reported for the nutritional improvement of crops in terms of protein that include Brazil nut 2S albumin (Altenbach et al 1989), AmA1 (Raina and Datta 1992), β-phaseoline (Zhenweiz et al 1995), HS-7 zein (Falco et al 1995), cruciferin (Kohno-Murase et al 1995), sunflower seed albumin (Molvig et al 1997), and S-rich zein (Bellucci et al 1997) (Table 1). However, except AmA1 (Raina and Datta, 1992; Datta et al 1997, 1998) introduction of these genes in target plants often resulted in the increase in one of the amino acid at the expense of others leading to imbalance of amino acid profile in transgenic crops. Thus, only AmA1 has emerged as a potential donor protein for the nutritional quality of carbohydrate-rich food crops.

The seed storage protein AmA1 from *Amaranthus hypochondriacus* (Raina and Datta 1992) has great agricultural importance as it is a well-balanced protein in terms of amino acid composition and even better than the values recommended by the World Health Organization for a nutritionally rich protein. More importantly, it is a non-allergenic protein (Chakraborty et al 2000). As this problem originated from an edible crop, the transgenic crops expressing AmA1 would have larger acceptability. *AmA1* gene is developmentally regulated and expressed only in seed. It is known to be synthesized during early embryogenesis, reaching a maximum by mid-maturation. Although AmA1 transcript is not found in 1-day-old seedlings but the protein showed a delayed breakdown on germination (Raina and Datta 1992). It has been shown that the AmA1 cDNA can be expressed functionally in fission yeast (Chakraborty et al 2002) and in food crops (Chakraborty et al 2000; Tamas et al 2009).

Table 1. List of seed storage proteins used in plant genetic engineering and protein design

No.	Transgenic plant	Protein/Source
1.	Potato	AmAI of *Amaranthus*
2.	Wheat	AmAI of *Amaranthus*
3.	Rice	Phaseolin of bean
4.	Yeast	Phytohaemeagglutelin of beans
5.	Barley	Zein of maize
6.	Petunia	Zein of maize
7.	Soybean	Zein of maize
8.	Wheat	Vicilin of pea
9.	Tobacco, Wheat	Legumin of pea
10.	*Brassica*	Cruciferin (rapeseed)
11.	*Vicia. narbonensis*	2S protein (Brazil nut)
12.	Canola	2S albumin from sunflower
13.	Rice	2S protein of *Brassica*
14.	Petunia	Conglycinin of soybean

To develop new generation tetraploid potato cultivars, which might be suitable for practical use, we have carried out large scale transformation using pSB8G construct having full-length *AmA1* under the control of tuber-specific granule-bound starch synthase promoter, *GBSS* (Chakraborty et al 2010). Further, the gene was transferred into different commercial rice cultivars under seed specific promoter. The detailed chemical analysis revealed that the over-expression of *AmA1* resulted in increased tuber and seed protein in transgenic potato and rice. There was 35-60% increase in total protein content in the transgenic events.

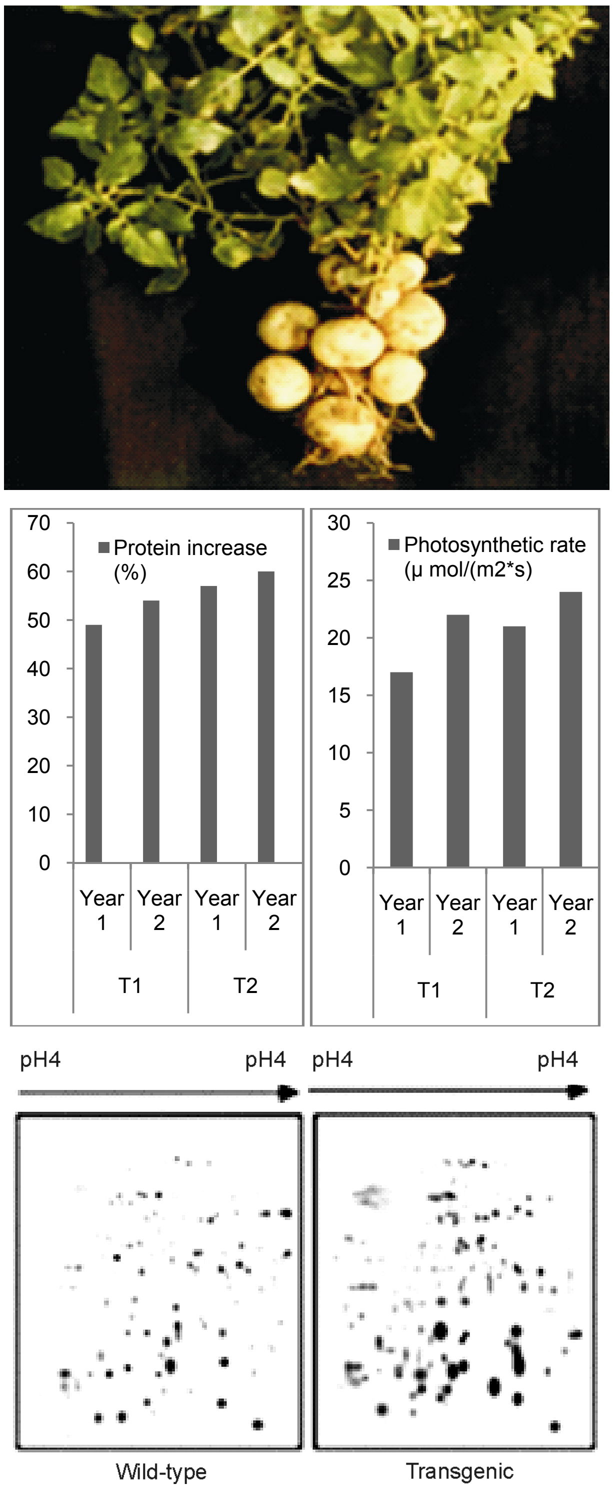

Figure 1. Molecular analyses of transgenic plants AmA1-potato (top); increase in protein content (mid-left); increase in photosynthetic rate (mid-right); proteomic analysis of mature potato tubers (bottom)

The results obtained from a 2-yr field trial in an advanced generation of the transgenic potatoes resulted in a marked increase in protein and several essential amino acids. We have also used stable transgenic lines to test the effects of *AmA1* on tuber protein and the amino acid composition and carried out biosafety trials in laboratory animals. Moreover, the in vitro digestibility altogether suggests that the transgenic potatoes may not be allergenic when consumed as food (Chakraborty et al 2010).

Our study provides unequivocal evidence on the biosafety assessment and benefits of the genetically modified tubers, which would accelerate platform for rapidly bringing products to the consumers. This would also offer unique opportunities for genetic engineering of novel traits into the next generation of crop plants to accrue nutritional benefits. To our knowledge, this is the first comprehensive report of translational research towards protein improvement programmes in crop plants.

The expression of AmA1 into potato under constitutive and tissue specific promoters, led to a high increases in protein content, several of the essential amino acids and tuber yield in transgenic plants. Thus, we hypothesized that induction of protein synthesis or mitogenic activity might be the result of overexpression of AmA1 or its turned-over products as signal molecule. Complex biological processes, development and metabolism, in particular are often regulated, at least, in part by change in the protein expression profile. To understand the transgene induced molecular mechanism affecting the increased protein synthesis and reserve accumulation in transgenic tuber, a comparative proteomic approach has been applied in potato during tuber life-cycle using two-dimensional gel electrophoresis (2-DE) coupled with LC/MS/MS (liquid chromatography-tandem mass spectrometry). The proteomic analyses led to the identification of tuber proteins that could be assigned with function, impinging upon the role of *AmA1* in transgenics.

Our results highlight the importance of expressing seed storage protein as a useful strategy to develop protein rich food crops. In addition, the differential expression profiles of the candidate proteins may provide new insight into the underlying mechanism of AmA1 function.

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