Next-generation protein-rich potato expressing the seed protein gene *AmA1* is a result of proteome rebalancing in transgenic tuber

Subhra Chakraborty[a,1,2], Niranjan Chakraborty[a,1], Lalit Agrawal[a], Sudip Ghosh[a], Kanika Narula[a], Shubhendu Shekhar[a], Prakash S. Naik[b], P. C. Pande[c], Swarup Kumar Chakrborti[b], and Asis Datta[a,2]

[a]National Institute of Plant Genome Research, New Delhi 110067, India; [b]Central Potato Research Institute, Shimla, Himachal Pradesh 171001, India; and [c]Central Potato Research Institute Campus, Modipuram, Uttar Pradesh 250110, India

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Protein deficiency is the most crucial factor that affects physical growth and development and that increases morbidity and mortality especially in developing countries. Efforts have been made to improve protein quality and quantity in crop plants but with limited success. Here, we report the development of transgenic potatoes with enhanced nutritive value by tuber-specific expression of a seed protein, AmA1 (Amaranth Albumin 1), in seven genotypic backgrounds suitable for cultivation in different agro-climatic regions. Analyses of the transgenic tubers revealed up to 60% increase in total protein content. In addition, the concentrations of several essential amino acids were increased significantly in transgenic tubers, which are otherwise limited in potato. Moreover, the transgenics also exhibited enhanced photosynthetic activity with a concomitant increase in total biomass. These results are striking because this genetic manipulation also resulted in a moderate increase in tuber yield. The comparative protein profiling suggests that the proteome rebalancing might cause increased protein content in transgenic tubers. Furthermore, the data on field performance and safety evaluation indicate that the transgenic potatoes are suitable for commercial cultivation. In vitro and in vivo studies on experimental animals demonstrate that the transgenic tubers are also safe for human consumption. Altogether, these results emphasize that the expression of AmA1 is a potential strategy for the nutritional improvement of food crops.

allergenecity | essential amino acids | nutritional health

Humans require a diverse and nutritionally well-balanced diet to maintain optimal health and depend largely on plants for their daily nutritional requirements. Moreover, a large proportion of the world's 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. Proteins, one of the principal constituents of a balanced diet, impart nutritional value to food due to their structural constituents, amino acids. However, it is very rare in nature to find all of the essential amino acids in a single food crop. Protein malnutrition is essentially caused by poor quality diets that include a high intake of staple crops with less protein and/or low-quality proteins in terms of amino acid composition. Protein deficiency lowers resistance to disease, delays physical growth and development, and may cause permanent impairment of the brain in infants and young children. A major effort has been to improve the amino acid composition of plant protein because animals, including humans, are incapable of synthesizing 10 of the 21 amino acids required for protein synthesis, and these "essential amino acids" must therefore be obtained from the diet (1). Because of the importance of dietary protein and the fact that plants are its major source, development of strategies to increase protein levels and the concentration of essential amino acids in food crops is of primary importance in a crop improvement program. 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 (2), but with limited success (3). A promising strategy is the genetic engineering of genes encoding proteins with high nutritional value into food crops (1, 4–6). However, despite promises that genetically modified (GM) crops could make a significant contribution to achieving global food security, the new-generation GM varieties are primarily used for industrial crops, such as cotton and animal fodder (7), and few have been commercialized.

Although cereals and starchy food crops contribute to more than 80% of the calories in a diet, noncereal crops are becoming more popular throughout the world with the continuing change in food habits. The demand for noncereal crops will continue to increase as a consequence of the expanding human population. Potato is the most important noncereal food crop and ranks fourth in terms of total global food production. It is also used as animal feed and in industrial products. Today, potatoes are grown in nearly 125 countries and more than a billion people worldwide consume them on a daily basis (8). 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 (9). The United Nations 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 (10). Although in developing economies the majority of potato is used for direct consumption, a shift toward the use of potato in convenience foods, for example, in potato chips and fries, has dramatically increased in developed countries (11). 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 (12). To guarantee a sufficient supply of quality protein in a diet consisting mainly of staple foods such as potato, specific interventions in genetic engineering are an absolute necessity. However, there is currently public concern about the use of genetically engineered foods in contemporary agriculture, particularly when genes are from a nonplant source. During the past decade, several potential candidate genes have been targeted for the nutritional improvement of protein content in crops, namely: Brazil nut 2S albumin (13), *AmA1* (Amaranth Albumin 1) (14), β-phaseoline (15), HS-7 zein (3), cruciferin (16), sunflower seed albumin (17), and S-rich zein (18). However, excepting *AmA1* (14, 19, 20), introduction of these genes in target plants has often resulted in an increase in one of the amino acids at the expense of others, leading to an imbalance of the amino acid profile in transgenic crops.

AmA1 has great agricultural importance because it is a well-balanced protein in terms of amino acid composition, possessing even better values than recommended by the World Health Or-

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[1]S.C. and N.C. contributed equally to this article.

[2]To whom correspondence may be addressed. E-mail: subhrac@hotmail.com or asisdatta@hotmail.com.

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ganization for a nutritionally rich protein. More importantly, because it is a nonallergenic protein that originated from an edible crop, the transgenic crops expressing AmA1 would have greater acceptability. In a previous study, we showed that the *AmA1* cDNA can be expressed functionally in fission yeast (21) and in food crops (1). The name "protato" has been coined to describe the genetically modified potatoes that contain an increased quantity of protein with improved quality in terms of amino acid composition.

Geographical distributions of potato plants are greatly influenced by different agro-climatic conditions. In this work, we translated the approach of genetic modification into commercial potato varieties suitable for cultivation in different agro-climatic regions to overcome protein deficiency in potato tubers. Here, we describe the tuber-specific expression of *AmA1* into seven economically important potato genotypes and the results obtained from 2-y field trials in an advanced generation of the transgenic varieties. This study provides unequivocal evidence on the biosafety assessment and benefits of the genetically modified tubers, which would accelerate the platform for rapidly bringing products to consumers. This is a comprehensive report of translational research toward protein improvement programs in crop plants.

Results

Transgenic Plants Display a Wild-Type Growth and Developmental Phenotype. To develop new-generation potato cultivars, which might be suitable for practical use, the pSB8G construct (1) having full-length *AmA1* under the control of a tuber-specific, granule-bound starch synthase promoter, *GBSS*, was used for large-scale transformation into seven commercial potato cultivars by *Agrobacterium tumefaciens*-mediated transformation. We developed a robust, genotype independent, and reproducible transformation system using internodes as explant on the basis of our earlier protocol (1) with few modifications. Because the copy number and the site of integration greatly influence the transgene expression, dozens of primary independent transformants were developed and screened in each category to obtain the desired phenotype. Moreover, the genetic differences between potato varieties and the growing environment could also contribute to differences in performance. The putative transformants were selected on the basis of their growth on medium containing kanamycin as a selectable marker. We then transferred 1–2 dozen independent transgenic events from each category to the green house. There were no visible phenotypic changes in morphology either in the primary transgenic populations or in the subsequent generations.

Integration, Tissue-Specific Expression, and Accumulation of AmA1 in Transgenic Tubers. To identify the successful transgenic events, genomic PCR analysis was carried out on all kanamycin-positive transformants that revealed an amplicon of 1.02 kb, corresponding to the size of the *AmA1*-coding region. Furthermore, the transgene copy number in the PCR-positive plants was determined by real-time PCR using Taqman chemistry, which revealed the presence of a single copy of the transgene in most of the transgenic events with very few having two to three copies. In a step further, all of the kanamycin-selected, PCR-positive, and copy-number–detected transgenic events across the populations were transferred to the experimental plot for generating potato tubers. RT–PCR analysis revealed that the *AmA1* transcript was most abundant in tubers followed by the stolon and stem; the leaf showed the least *AmA1* transcript (Fig. S1*A*), suggesting the tissue-specific expression of the transgene. Our data support the fact that the *GBSS* promoter is most active in tubers, as reported earlier (22). Although the transcript was found in different organs, the protein products could be detected only in the field-grown tuber. Immunodetection of AmA1 in tubers showed a 35-kDa band in all of the transgenic events but at varying levels (Fig. S1*B*), suggesting that the transgene was actively producing the desired protein in the alien environment.

Overexpression of AmA1 Results in Increased Tuber Protein and Amino Acids. The protein content in crops is determined on the basis of nitrogen content, and the Kjeldahl method has been almost universally applied to determine total nitrogen content. We determined the amount of total protein on the basis of the nitrogen content in transgenic tubers and compared that with the respective control background. A detailed chemical analysis revealed that the overexpression of AmA1 leads to an increased level of tuber protein compared with wild type. There was a 35–60% increase in total protein content in moderately expressed transgenic events of all varieties (Table 1), which was higher than we had reported earlier (up to 35%) for a diploid cultivar (1). It was expected that the increased levels of protein in modified tuber could have a direct bearing on the amino acid ratio. The balance of amino acid is a key determinant for nutritive proteins because plant proteins typically do not provide the optimum amino acid ratios required for efficient protein synthesis in animals, including humans. Analysis of the pool sizes of the various amino acids revealed a significant increase in amino acids, notably lysine, tyrosine, and sulfur amino acids, which are otherwise limited in potato tubers. Also, there was an increase in aspartic acid, glutamic acid, arginine, leucine, and isoleucine levels (Table S1). These changes were notably more prominent in transgenic events exhibiting a higher level expression of AmA1 (Fig. S1*B*).

It seems likely that the increase in nitrogen storage in sink tissue may result in alteration of total biomass production. Nitrogen storage and carbohydrate metabolism are closely related. It is postulated that carbohydrate distribution within the plant is affected by the nitrogen supply, which strongly influences the processes of carbon metabolism, and thus nitrogen status has a great impact on the postharvest performance of plants (23). To confirm this, the dry weight of transgenic as well as wild-type plants was determined. Indeed, the dry weight of most transgenic plants significantly increased when compared with their wild-type counterparts. On average, the increases in biomass of most promising transgenic events were found to be in the range of 7–20% (Fig. S2*A*), in-

Table 1. Comparison of total protein content of wild-type and *AmA1*-transgenic tubers

Genotype		Year I	Year II
K Chipsona 1	C	87.90 ± 1.30	83.60 ± 5.10
	21	131.70 ± 3.30	127.90 ± 5.60
		(49.83)	(52.99)
K Chipsona 2	C	103.0 ± 1.0	103.40 ± 10.9
	15	138.50 ± 2.10	138.70 ± 6.00
		(34.47)	(34.13)
K Jyoti	C	106.30 ± 1.00	102.70 ± 1.60
	16	131.70 ± 1.70	129.6 ± 9.8
		(23.89)	(26.19)
K Sutlej	C	94.80 ± 3.70	88.00 ± 2.10
	3	148.60 ± 5.80	140.10 ± 7.50
		(56.75)	(59.20)
K Badsah	C	89.40 ± 1.90	84.40 ± 7.70
	9	121.20 ± 0.80	115.10 ± 2.30
		(35.57)	(36.37)
K Bahar	C	97.60 ± 5.70	98.20 ± 3.80
	5	124.90 ± 2.30	126.90 ± 12.50
		(27.97)	(29.22)
K Pukhraj	C	95.00 ± 5.30	86.90 ± 7.20
	1	126.60 ± 7.70	118.00 ± 3.90
		(33.26)	(35.79)

Potato plants, in field trials, were grown to maturity and tubers were harvested. Three tubers from each replication for each wild-type and transgenic events were randomly collected. The pooled tubers were divided into three groups, oven-dried at 100 °C, and powdered. Total protein content (mg protein g^{-1} dry weight) of tubers was determined by standard Kjeldahl method in duplicate from three independent groups and compared. Values are presented as the mean ± SE. Numerical represents the number of transgenic event against its corresponding wild-type background. The values in parentheses represent percent increase in protein in transgenic tubers when compared with that of wild-type tubers (C).

dicating a positive correlation between the increased nitrogen storage and the biomass in the transgenic plants.

2D Electrophoresis Analysis Shows Increase in Protein Content. Earlier it was reported that transgene expression may lead to a rebalancing of the proteome toward an increase in protein content (24). To examine the impact of AmA1 toward an increase in protein content, the changes in the tuber proteome of transgenic potatoes were monitored using 2D electrophoresis (2-DE) analysis. The gels showed more than 90% high-quality protein spots and had a correlation coefficient of variation above 0.8 (Fig. S3), suggesting high reproducibility among the replicates (Fig. 1, Table S2). PDQuest analysis showed an average of 323 and 362 spots in wild-type and transgenic events, respectively, substantiating the biochemical findings of increased protein content in transgenic events by Kjeldahl analysis (Table 1).

Field Performance of AmA1 Plants in Different Agro-Climatic Conditions. Field trials of transgenic plants provide a relevant test for understanding the *in planta* biological role of transgenes and proteins and also for verifying their potential use in commercial applications. The planning and execution of these trials are labor intensive but a useful task because agronomic performance of the transgenic crops in the field does not necessarily match the conditions maintained in the greenhouse or experimental plot. To assess the field performance of transgenic plants expressing AmA1 in different agro-climatic conditions, 3–10 independent transgenic events from each genotypic background were selected for further evaluation. The AmA1 plants from size-normalized seed tubers were grown in the field alongside the wild type and randomly replicated three, five, and seven times for four seasons in different field experiments. We selected the best independent transgenic event from each category in which the *AmA1* was stably expressed over generations. In all field trials, determination of tuber yield was carried out on fully senescent plants at about 100 d from the date of plantation. There were no significant changes in the earliness of the crop in any of the transgenic events. However, a moderate increase (≈15–25%) in the tuber yield was found in a few of the promising transgenic events (Table S3). These results are of great importance, given the twin problems of environmental deterioration and the progressive increase in world population.

Because of the increased tuber yield in the transgenic plants and the fact that photosynthetic carbon metabolism is one of the major determinants for crop growth and yield (25), we measured the photosynthetic efficiency of the transgenic and wild-type plants under standard atmospheric (360 ppm CO_2) and light (750 $\mu mol \cdot m^{-1} \cdot s^{-1}$) conditions. Compared with wild-type plants, photosynthetic CO_2 fixation of the most promising transgenic plants was up to 27% higher (Fig. S2*B*). This was corroborated by the corresponding increase in total biomass of the transgenic plants (Fig. S2*A*). Indeed, these results represent a unique example in which expression of a seed storage protein results in improved carbon fixation and growth in transgenic plants.

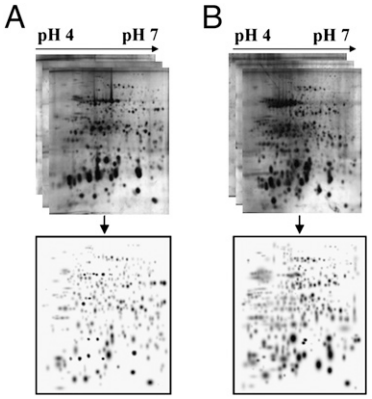

Fig. 1. Proteomic analysis of the mature potato tubers of (*A*) wild-type (KC1) and (*B*) the transgenic line (KC1/21). Proteins were extracted from same mass of mature tuber, and an equal volume (250 μL) of protein was separated by 2-DE as described in *SI Materials and Methods*. Three replicate silver-stained gels for each stage were computationally combined using PDQuest software, and four representative standard gel images were generated.

AmA1 Potato Tubers Are Nontoxic, Nonallergenic, and Safe for Consumption. In recent years, the use of animal models has become an integral part of the safety evaluation process for genetically modified crops. To examine the toxicity and allergenicity, if any, associated with the AmA1 potato, tubers of transgenic events along with the corresponding wild-type tubers were tested in two different animal models (rat and rabbit) following the recommendation laid down by the Food and Agriculture Organization and WHO (2006) (26). The potato tubers of transgenic events were found to be nontoxic to rats when administered orally as a single dose at a maximum dose level of 5 g of test substance per rat. The given samples were also found to be nonirritating to the skin of the rabbits. Furthermore, the samples of transgenic tubers were found to be nonirritating to the vaginal mucous membrane of the rabbits.

Mean values of hematological and serum biochemical parameters obtained from both male and female rats fed with wild-type and AmA1 potato for 21 and 90 d are presented in Table S4 and Table 2. No differences were observed in a blood biochemistry analysis. Counts of WBC, RBC, and hemoglobin were at same level in both male and female rats of each group. Total plasma protein, sugar, and urea levels were similar for transgenic and control group rats. Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels were comparable in both groups in AmA1 and wild-type potato, indicating normal liver function. Furthermore, the heart and kidney functions of transgenic and wild-type potato-fed rats were also comparable, suggesting normal cellular metabolism.

Histopathological analysis of hematoxylin- and eosin-stained gut tissues of rat-fed AmA1 potato revealed normal structure with no distortion in gut lining. The stomach layer showed no inflammation and/or infiltrations of inflammatory cells into surrounding tissues (Fig. 2 *A* and *B*). Intestinal villi of AmA1 potato-fed rats showed normal surface area with numerous absorptive enterocytes and mucous-secreting cells and no sign of ulcer, bleeding, or disruption-like symptoms and were comparable to that of the control group (Fig. 2 *C* and *D*). Furthermore, no abnormal structures were observed in liver and kidney cells of rats fed with wild-type and AmA1 potato (Fig. 2 *E* and *F*). Transgenic potato-fed rats showed normal lung structure with defined bronchial epithelial lining and alveoli. No histopathological differences were observed in brain and heart tissues (Fig. S4). Thus, under the conditions of this study, long-term oral feeding for 90 d did not show any detectable clinical and histopathological changes or observable toxic effects in any groups of the animals tested.

It is recognized that most allergenic proteins tend to have characteristic sequence stretches. The amino acid sequence of AmA1, however, did not show any homology with known allergenic candidates when searched against the allergen online database (www.allergenonline.com). In a previous study, we had shown that the AmA1 protein in its purified form does not evoke any IgE response, negating the possibility of the protein being allergenic (1). To further confirm whether potato tubers expressing AmA1 are allergenic or not, the hypersensitivity test of the transgenic tubers was carried out. In this experiment, serum from the laboratory animals fed with genetically modified AmA1 tubers was subjected to ELISA study. Although the IgG level was quite high, the IgE level could not be detected, suggesting that the transgenic tubers did not evoke any allergenic response in the animals. These results conclusively proved that AmA1 potato tubers are nontoxic and nonallergenic.

Intraperitoneal (i.p.) sensitization with wild-type and AmA1 potato protein produced low IgE titer on day 15, which remained unchanged until day 45 (Fig. 3*A*), whereas ovalbumin immunization produced high IgE response by the i.p. route. Compared with ovalbumin, i.p. sensitization with both wild-type and AmA1 potato

Table 2. Serum biochemistry indices of rats fed with control potato and AmA1 potato for 21 and 90 d

	90 d				21 d			
	Male		Female		Male		Female	
Parameter	C1	T1	C1	T1	C1	T1	C1	T1
Blood sugar (mg %)	103.60 ± 6.64	106.60 ± 6.06	103.60 ± 6.60	104.40 ± 7.20	103.60 ± 6.35	101.00 ± 6.20	103.60 ± 6.35	102.40 ± 5.46
BUN (mg %)	27.70 ± 4.15	28.48 ± 4.06	26.15 ± 4.42	26.77 ± 2.82	25.92 ± 3.81	26.98 ± 2.71	20.06 ± 1.57	26.42 ± 2.46
Total protein (g %)	6,546 ± 0.34	6.196 ± 0.19	6.484 ± 0.43	6.218 ± 0.19	6.86 ± 0.25	7.03 ± 0.48	6.90 ± 0.28	7.14 ± 0.22
SGPT (IU)	38.58 ± 7.67	33.96 ± 4.82	35.98 ± 8.05	26.58 ± 4.20	29.90 ± 3.54	31.90 ± 2.94	30.36 ± 1.78	29.46 ± 2.13
SGOT (IU)	39.38 ± 12.83	42.55 ± 13.49	34.12 ± 12.57	37.20 ± 9.30	33.98 ± 2.54	33.74 ± 2.81	33.32 ± 3.44	33.82 ± 2.93
Albumin (g %)	4.02 ± 0.22	4.05 ± 0.25	4.00 ± 0.25	4.02 ± 0.19	4.01 ± 0.25	4.13 ± 0.12	4.10 ± 0.08	4.05 ± 0.17
SAP (U/L)	86.00 ± 8.81	91.37 ± 6.72	88.75 ± 7.96	86.86 ± 7.42	92.80 ± 7.46	88.58 ± 10.73	93.78 ± 6.53	91.56 ± 7.64

BUN, blood urea nitrogen; SAP, serine alkaline phosphatase.

showed a high IgG1 response (Fig. 3*B*). Both wild-type and AmA1 potato produced a similar IgG2a level on day 15, which increased by day 30 (Fig. 3*C*). No significant difference ($P > 0.05$) was observed between the IgG1 and IgG2a antibody response induced by wild-type and AmA1 potato proteins.

Simulated Gastric Fluid- and Simulated Intestinal Fluid-Induced Digestibility of AmA1 Potatoes. The potent food allergens are known to be very stable in in vitro pepsin digestion, whereas most dietary proteins are readily digestible (27). The pepsin digestibility assay is thus considered one of the major ways to identify food allergens (28–30). To determine the relative stability of AmA1 to the extremes of pH and pepsin protease encountered in the mammalian digestive tract, the protein extracts of transgenic tubers were subjected to pepsin digestibility. The in vitro digestibility of tuber protein was confirmed by immunodetection assay. The results revealed that the high-molecular-weight proteins, including AmA1, were readily digested within 20 min in simulated gastric fluid (SGF) and also were completely degraded within 15 min in simulated intestinal fluid (SIF). The SGF- and SIF-mediated digestibility altogether suggests that the transgenic potatoes may not be allergenic when consumed as food.

Discussion

A global scientific emphasis has been laid on food security and on improving the nutritional qualities of food crops through contemporary science. Thus, the International Food Policy Research Institute launched 2020 Vision, a call for a new "Green Revolution." A total of 1.02 billion people, residing mostly in the developing world, suffer from chronic under-nutrition, and 200 million children are affected due to lack of essential energy and protein (31). Therefore, improvement of nutritional value in food crops, particularly improvement in protein content, poses a major challenge in developing countries where plants provide most of the protein in the human diet and in animal feed. The biotechnology-based strategies to modify proteins and/or amino acids have always focused either on increasing the concentration of the direct precursors for biosynthesis of the targeted product (2) or on the concentration of some unique proteins (1, 32, 33). Although many of these strategies have yielded positive results, many others have failed despite changes in some or all of the required metabolic precursors (34). Our approach of using *AmA1* to increase the nutritional quality in tuber crops, particularly in potato, has been successful. We have developed an efficient, rapid, and genotype-independent transformation method for potato. The protocol involves a single medium composition throughout, and transformants were obtained within 6–8 wk. To our knowledge, the increase in protein content of genetically modified tubers in this study is one of the highest increases observed in any transgenic crop. Although the expression level of AmA1 in transgenic tubers was not high enough to be directly correlated with the protein increase, comparative proteome analysis showed a positive correlation with the finding of biochemical analysis. The increase in total protein content as shown by Kjeldahl and 2-DE analyses may be explained as due to de novo synthesis and accumulation of unique proteins and/or quantitative change in the expression level or redistribution of proteins as a result of AmA1 expression. It is known that synthesis of seed storage protein depletes free amino acid in the storage organ that leads to an increase in the rate of photosynthesis (35). *AmA1*, a seed storage protein gene, when engineered in potato tuber might lead to the depletion of the endogenous free amino acid pool for its synthesis and accumulation. It is likely that the depletion of endogenous free amino acids in the transgenic tuber is then sensed by the photosynthetic machinery, causing an increase in the rate of photosynthesis. Earlier studies have also shown that protein synthesis increases as a consequence of photosynthesis, which is the ultimate basis of yield (35, 36). Thus, the increase in photosynthesis in transgenic potato, as shown in Fig. S2*B*, in turn enhances the total protein content as well as the yield. Seed storage

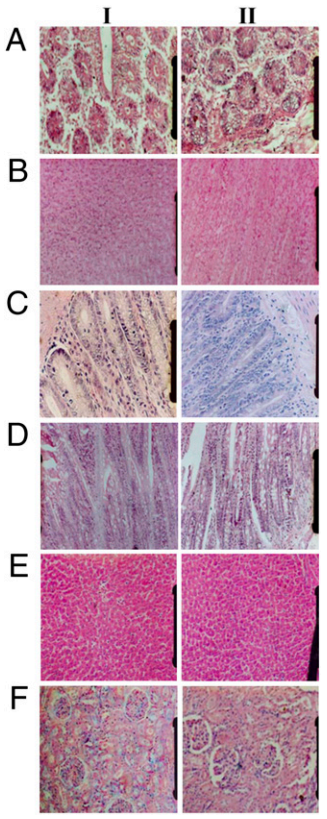

Fig. 2. Histopathological analysis of gut tissues. (*I*) Rats fed wild-type potato and (*II*) rats fed AmA1 potato: sections showing hematoxylin and eosin staining of (*A*) stomach (nonglandular), (*B*) stomach (glandular), (*C*) small intestine, (*D*) large intestine, (*E*) liver, and (*F*) kidney.

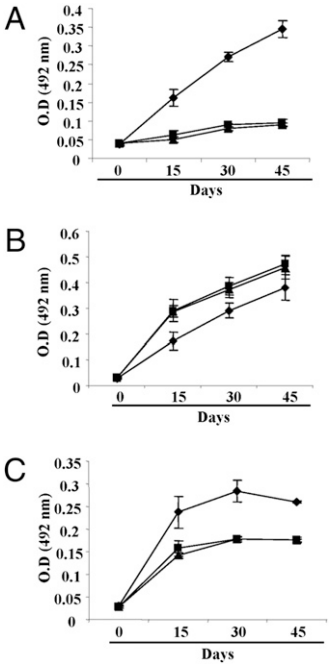

Fig. 3. Specific antibody response in Balb/C mice: serum antibody following i.p. administration of ovalbumin (◆), wild-type (■), and transgenic (▲) potato protein extract. (*A*) specific IgE, (*B*) specific IgG1, and (*C*) specific IgG2a. A preimmune serum was taken as the control, and the values were subtracted before plotting. Data are reported as mean ± SD.

proteins are known to serve as the sink to regulate the movement of photosynthate into developing organs (35). It is conceivable that AmA1 as a storage protein might act as a sink protein in transgenics, thereby regulating the movement of metabolites, including the amino acids, into the developing tuber where they are fixed into newly synthesized proteins and consequently enhance the level of essential amino acids.

Potatoes are grown as vegetable for consumption and also as raw material for many processed foods. It is well documented that dry matter, specific gravity, and viscosity of the tuber are the major parameters that determine the cooking quality and viscosity and firmness reflect the processing grade of potato (37, 38). To find the cooking quality, processing, and palatability of AmA1 potato, we performed the comparative physio-chemical and rheological analyses of the transgenic and the corresponding wild-type tubers. Specific gravity of most of the transgenic lines was found to be 1.68–6.86% higher (Fig. S5*A*), indicating better quality of the processed product. With reference to dry matter, KC1/21 and KC2/15 showed the maximum increase, having 23.7 and 19.5%, respectively, as compared with 12.5–18.8% in the transgenic lines of other genetic backgrounds (Fig. S5*B*). Furthermore, transgenic potatoes upon frying showed less browning (Fig. S6 *A* and *B*) with higher firmness after boiling (Fig. S6 *C* and *D*), suggesting better palatability. Proteins are known to be major water absorbers, and they increase viscosity significantly (39). Viscoamylograph analyses revealed that transgenic potatoes reach pick viscosity either more slowly or at the same time in comparison with their respective wild types, maintaining a similar pasting temperature (Fig. S6 *E* and *F*). These results demonstrate that enhanced protein content in transgenic potatoes neither changed pasting temperature nor increased peak viscosity to affect crispiness and texture of fried potatoes. Altogether, analyses of these variances imply better cooking and processing quality of the transgenic tubers. Our strategy thus allows the achievement of developing nutritionally enhanced potato with quality traits by judicious manipulation of storage protein.

Recently, there have been efforts to express 2S albumin from Brazil nut and sunflower seed albumin to increase the nutritive value of transgenic crops. However, these attempts were not successful because, when introduced in target plants, the transgene resulted in a dramatic increase only in methionine along with a significant decrease in cysteine (13, 17). Other examples include the soybean glycinin, expression of which in potato tubers did not lead to any remarkable increase either in protein content or in amino acid concentration (40). Furthermore, plans for commercialization of these transgenic crops were abandoned because the donor proteins were allergenic (41). On the contrary, an intensive literature search did not reveal any allergenicity associated with the source of the transgene, amaranth grain or amaranth forage (*SI Text*). Feeding trials of the AmA1-transgenic tubers in rat models did not show any toxicity. Blood and serum biochemical indices indicated normal functioning of metabolic organs in the test animals. Postmortem examination demonstrated normal appearance of stomach, intestine, liver, kidney, and other organs. Thus, it seems that consumption of AmA1 potato has no detrimental effect on the growth and metabolic function of rat. The hypersensitivity test in animal models also showed that potato tubers expressing AmA1 do not evoke any IgE response, which negates the possibility of the protein being allergenic. The in vitro digestibility test showed that the accumulated AmA1 is readily degraded by SGF, confirming that the transgenic tubers are nonallergenic. The findings are significant because the ability of the food allergens to reach the intestinal mucosa is the prerequisite to allergenicity. It is apparent that the ability of food allergens to reach the intestinal mucosa necessarily implies their survival to gastric digestion by pepsin secreted in the stomach. Because the expressed protein is efficiently digested and degraded, the AmA1 protein as a whole would not reach the intestinal mucosa and cause allergenic reaction.

In summary, the tuber-specific expression of *AmA1* was associated with increased protein and a concomitant increase in several of the essential amino acids that are otherwise limited in potato. This study represents a major technological advance in translational research in which the engineering of a seed storage protein has led to nutritional improvement with essentially no negative collateral effects on crop yield or quality. The commercial potential of genetically modified plants depends on stable integration and expression of the transgene under the different genotypic backgrounds of the host species, on their wider environmental applicability, and on sustainable production, including food safety. Therefore, our strategy may prove to be more acceptable to the general public than currently used genetically modified crops because *AmA1* is an edible crop-derived sequence. The benefits are magnified particularly considering that this occurs without any yield penalty, but rather in combination with increased harvestable biomass. Because potato constitutes an important part of the diet of many people in developed as well as developing countries, it is apparent that this can add value to potato-based products with enhanced benefits for better human health. Our strategy also offers unique opportunities for the genetic engineering of unique traits into the next-generation crop to accrue nutritional benefits.

Materials and Methods

For detailed descriptions of materials and methods, see *SI Materials and Methods* and associated references. Transgenic potato lines were developed and molecular analyses of transgenics were performed as described in *SI Text*. Expression of AmA1 protein was detected and confirmed at maturity of potato tubers using anti-AmA1 antibody as described in *SI Text*. Chemical analysis and proteomic study of transgenics were carried out using extracts from wild-type and transgenic tubers as described in *SI Text*. Field trials, morphological characterization, measurement of photosynthetic activity, digestibility and allergenecity analyses, food safety assessment and toxicity studies, palatability, and cooking and processing quality analyses are also described in *SI Text*.

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