Supporting Information

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SI Text

AmA1 Potatoes Are Nontoxic, Nonallergenic, and Safe for Consumption. Amaranth meal or flour has been used in the production of unleavened bread in many countries. Grain amaranth is also used in many other foods throughout the world. Furthermore, the fact that amaranth forage has been an important component of the human diet and perhaps the most widely consumed vegetable in the humid tropics for centuries also suggests its nonallergenic nature. Moreover, animal feeding trials with a rat population using amaranth seed grain have shown that the grains are suitable as animal feed (1).

SI Materials and Methods

Plant Transformation and Selection of Transgenic Plants. The commercial potato (*Solanum tuberosum* L.) cultivars (K. Chipsona 1, K. Chipsona 2, K. Sutlej, K. Jyoti, K. Badshah, K. Bahar, and K. Pukhraj) were obtained from Central Potato Research Institute, India. The potato plants were maintained as in vitro shoot cultures, and the internodal stem segments were transformed with pSB8G (LBA4404) as described (2) with the following modifications. The potato regeneration medium contained Murashige and Skoog (MS) basal medium with 3 $mg{\cdot}L^{-1}$ each of zeatin and GA3, 0.5 mg L^{-1} indoleacetic acid (IAA), and 2.5–3.0% sucrose (pH 5.6). The selection of transformants was done by lowering the concentration of kanamycin to 50 $mg{\cdot}L^{-1}$.

Molecular Analysis. DNA from transgenic plants was isolated (3), and the copy number of the *AmA1* gene was determined by real-time PCR using Taqman chemistry per the manufacturer's protocol (Applied Biosystems). The intactness of the *AmA1* gene in transgenic plants was determined by PCR using the gene-specific primers F51 and R1044, and tissue-specific expression was monitored by RT–PCR as described earlier (2).

Expression of AmA1 and Immunoassay. Total protein was extracted from 250-mg tuber tissues in 0.5 mL of extraction buffer (25 mM Tris–acetate, pH 8.5; 0.5 M NaCl, and 5 mM PMSF). The homogenate was centrifuged at 12,000 × *g* for 10 min. Protein concentration was determined using the Bradford protein assay kit (Bio-Rad). An aliquot of 50 μg protein from each sample was denatured by boiling for 5 min in loading buffer (4) and resolved on a uniform 12.5% SDS/PAGE. The proteins resolved in SDS/PAGE were immobilized onto Hybond-C membrane as described earlier (5) at 150 mA for 2 h. The membranes were subsequently blocked with 5% (wt/vol) nonfat milk for 1 h, and AmA1 was detected with a rabbit polyclonal anti-AmA1 antibody in combination with alkaline phosphatase-conjugated goat anti-rabbit IgG. Signals were detected using the nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate, toluidine salt (NBT/BCIP) method.

Chemical Analysis. Total protein content in wild-type and transgenic tubers was determined by the standard Kjeldahl method. The amino acid composition of the tubers was determined using a C18 HPLC column equipped with an online Pico Tag amino acid analyzer (Waters). Acid hydrolysis and derivatization of lyophilized tuber tissue with phenylisothiocyanate (PITC) was done as per the Pico tag manual. The PITC derivative of each amino acid was detected at A_{254}.

Isolation of Tuber Proteins and 2D Gel Electrophoresis. Different potato cultivars along with their transgenic lines were grown from size-normalized seed tubers in randomized plots. Samples were collected from three randomized plots and pooled to normalize the effect of variations in the biological replicates, if any, and stored at −80 °C after quick-freezing in liquid nitrogen. The tuber-specific soluble proteins were isolated from equal weight (5 g) of mature tuber as described (6). Three replicate 2D gel electrophoresis (2-DE) gels with equal volume from each wild-type and transgenic sample were run and then computationally combined into a representative standard gel, the first-level match set (Fig. 1). The replicates had a correlation coefficient of variation above 0.8 as displayed in the scatter plots (Fig. S3). Each spot included on the standard gel met several criteria: it was present in at least two of the three gels and was qualitatively consistent in size and shape in the replicate gels. Protein spot detection and quantification were obtained using normalized spot volumes given by PDQuest software (Version 7.2.0, Bio-Rad) using the total spot volume normalization procedure to discard experimental variations in 2-DE gels.

Structural Design of Field Trials. The field trials of transgenic potato plants expressing AmA1 were approved by the Department of Biotechnology, the Government of India [permit no. BT/BS/17/09/99-PID (2001) and BT/BS/17/09/99-PID (2002)]. The preproduction trials of AmA1 potato were conducted for four seasons. Initially, the trials were conducted at the Central Potato Research Institute campus (Modipuram, India) in three randomly distributed replication plots consisting of 30 and 145 tubers, respectively. Tubers were multiplied (in subsequent field trails) in two different locations (Modipuram, and New Delhi) in five replication plots consisting of 150 tubers. A similar trial was conducted in both these locations in seven replication plots consisting of 420 tubers. The distance between the rows in the replication trials was 60 cm and between the plants was 20 cm. The isolation distance was 5 m from all sides of the trial plots.

Morphological Characterization and Photosynthetic Activity. The leaf area was measured using a LI-3100C area meter (LI-COR Bioscience). Biomass was determined on a dry weight basis after incubation at 70 °C until no further decrease in weight could be detected. The photosynthesis rate of plants was measured with a portable photosynthesis measurement system (GFS3000; Waltz). The photosynthetic capability of plants determined on the basis of single leaf measurements of five to seven different leaves of each plant was recorded under standard atmospheric (360 ppm CO_2) and light conditions (750 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$). Leaves were held in the chamber for 2–3 min until the rates of photosynthesis were in a steady-state condition.

Food Safety Assessment and Toxicity Studies. The toxicological effect of potato tubers was determined at 21 and 90 d of oral feeding in rats. Three groups of 10 rats from each sex were used for each individual transgenic event as follows: (1) first group was fed pellets only; (2) second group was given 5.0 g $animal^{-1}$ of the wild-type tubers; and (3) third group was given 5.0 g $animal^{-1}$ of the transgenic tubers. The potato tubers were fed to the rats every day, and the leftover tubers were removed and weighed to determine the amount of potato consumed by the rats. Diets and drinking water were provided ad libitum. During the experimental period, all animals were clinically observed daily for activity, fur color, food consumption, and excretion. On completion of the feeding trials, detailed studies on hematology, serum biochemistry, urine analysis, and histopathology were carried out using standard protocols.

Blood Biochemistry. Blood samples from test animals and controls were collected on day 22 and day 91 for hematological analysis.

Serum biochemical indices were determined by standard clinical method. Total plasma protein, urea, serum glutamic oxaloacetic transaminase, and serum glutamic pyruvic transaminase levels were determined for liver and kidney functioning by using commercial kits.

Histological Studies. The gut tissues were collected from the killed animals and fixed in 10% neutral-buffered formalin (vol/vol) overnight. The tissues were embedded in paraffin, sliced into 4-μm sections, and stained with hematoxylin and eosin for analysis of antigen-induced inflammation. Sections were scanned under a light microscope, and images of these fields were captured by an in-line camera.

Statistical Analysis. Student's *t* test (unpaired) was used for statistical analysis of the data, and $P < 0.05$ was considered as statistically significant.

Allergenicity Assessment. ***Sequence analysis.*** The introduced sense strand of the *AmA1* gene was translated into protein sequence using expasy tool (http://www.expasy.ch/tools/dna.html). The translated sequence was studied in all of the three frames for homology with allergen database (http://allergenonline.com).

Allergenicity testing in mice. Six-week-old BALB/c mice were segregated into four groups of six mice each. Group 1 mice were injected daily with phosphate buffer saline (PBS) and group 2 with ovalbumin for 45 d. Group 3 and 4 mice were given wild-type and transgenic potato protein extract, respectively, via an i.p. route. Mice were injected (i.p.) with 100 μg protein in 100 μL PBS once a week for 6 wk. Blood samples were collected to measure serum IgE, IgG1, and IgG2a antibodies on days 15, 30, and 45.

Measurement of ovalbumin, wild-type, and AmA1 potato-specific antibodies. Serum antibodies specific for ovalbumin, wild type, and AmA1 potato were measured by ELISA. Briefly, the microtiter plates were coated with 250 ng of ovalbumin or with 1 μg of wild-type and transgenic potato extracts in carbonate buffer (pH 9.6). The plates were incubated with mice sera for IgE (1:10), IgG1, and IgG2a (1:500) estimation. After washing, rat anti-mouse IgE (1:1,000), rat anti-mouse IgG1 (1:1,000), and rat anti-mouse IgG2a (1:1,000) in PBS were added and developed.

In vitro digestibility of the expressed AmA1. Protein extracts (225 μg) from transgenic tubers were incubated at 37 °C with simulated gastric fluid (porcine pepsin 0.03 M NaCl at pH 1.2) for 60 min and then adjusted to pH 8.0 with 100 mM Tris·HCl, pH 9.5, 2 mM $CaCl_2$ for simulated intestinal fluid (porcine trypsin and bovine chymotrysin) at a 1:100 ratio (digestive enzyme: protein). The reaction was terminated with buffer (50 mM Tris·HCl, pH 9.5, 100 mM NaCl). At the desired times, 20-μg aliquots were taken, and the reaction mixture was subjected to SDS/PAGE and then to Western blotting as previously described (7).

Cooking Quality, Processing, and Palatability Assessment. ***Determination of dry matter.*** The dry matter (DM) content of wild type and AmA1 potato was calculated by weighing 50 g of mature tuber both before and after drying of the samples in an oven at 110 °C as described earlier (8, 9). Dry matter was calculated using the following formula: % DM = (dry weight/fresh weight) × 100. The experiments were done in triplicate.

Determination of specific gravity. The specific gravity of wild type and AmA1 potato was determined as described earlier (9). Five kilograms of mature tuber from each line were weighed in air and then in water. The weight measured is the difference between the weight of the sample and the weight of the equal volume of water. The specific gravity was calculated using the following formula: Specific gravity = weight in air/(weight in air − weight in water). The experiments were carried out in triplicate.

Texture analyses. The texture of wild type and AmA1 potato was determined as described earlier (10). Tubers were cut into cylinders (20 mm diameter × 20 mm height), boiled in water for 10 min, and cooled immediately. The texture of cooked samples was evaluated by firmness analysis using a TA-XT plus Texture Analyzer (Stable Microsystems) equipped with a 50-N load cell. Cooked potato cylinders were compressed in a single-cycle compression–decompression test, using a flat plate (50 mm diameter) at a cross-head speed of 1 $mm{\cdot}s^{-1}$ up to 7 d.

Rapid viscosity measurement. Viscocity of wild type and AmA1 potato was determined as described earlier (11). Mature potato tubers were washed in distilled water, peeled, and lyophilized. For each sample, 2 g of lyophilized powder was taken in 25 mL distilled water and subjected to a Rapid Visco-Analyzer (RVA Techmaster, Newport Scientific) for evaluation of pasting properties. A programmed heating and cooling cycle was used at a constant shear rate, and the sample was equilibrated at 50 °C for 1 min, heated to 95 °C in 3.42 min, held at 95 °C for 10 min, cooled to 50 °C in 4 min, and then held at 50 °C for 2 min. The speed was 960 rpm for the first 10 s followed by 160 rpm for rest of the experiment.

Surface browning test. Size normalized mature wild-type and AmA1 potato tubers were sliced uniformly and fried in refined vegetable oil. Excess oils were soaked and the chips were photographed.

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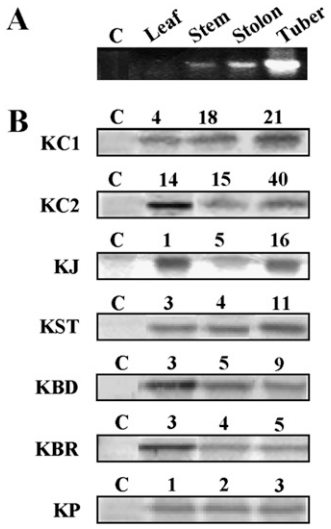

Fig. S1. Molecular analyses of transgenic plants expressing *AmA1*. (*A*) Organ-specific expression of the *AmA1* transcript in the transgenic plants. "C" represents the control in RT–PCR. (*B*) Immunodetection of AmA1 protein in transgenic tubers. Aliquots of 50 μg soluble protein, each from wild-type and transgenic tubers, were separated by 12.5% SDS/PAGE and electroblotted onto Hybond-C membrane. AmA1 protein was detected with a polyclonal anti-AmA1 antibody and alkaline phosphatase-conjugated anti-rabbit IgG antibody. The lane numbers represent the individual transgenic event, and "C" represents the wild-type tuber. KC1, Kufri Chipsona-1; KC2, Kufri Chipsona-2; KJ, Kufri Jyoti; KST, Kufri Sutlej; KBD, Kufri Badshah, KBR, Kufri Bahar; KP, Kufri Pukkraj.

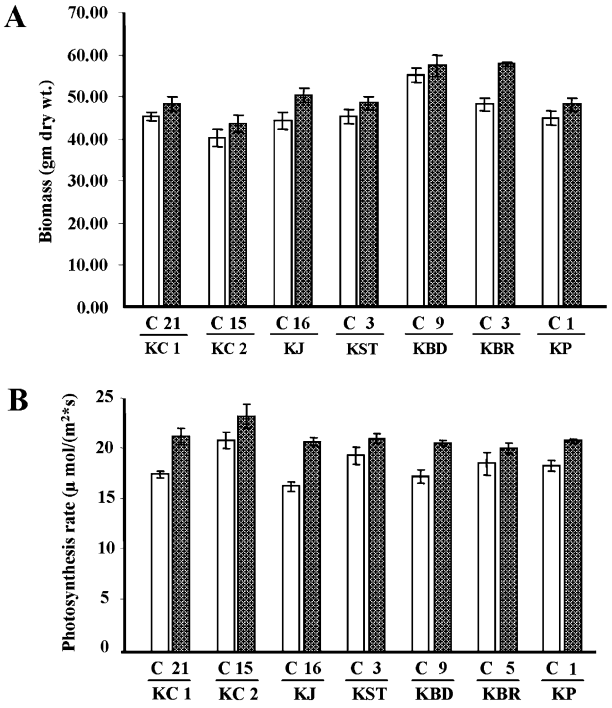

Fig. S2. Comparison of (*A*) biomass and (*B*) net photosynthetic rate between wild-type and the corresponding AmA1 plants. The photosynthetic capability was determined by a photosynthesis measurement system (GFS3000) on the basis of single leaf measurements and the gas exchange of at least five to seven different leaves of each plant. Light intensity in the study was 750 μmol photons $m^{-2}{\cdot}s^{-1}$. The total photosynthetic capacity of each plant was estimated in relation to the leaf area. White and patterned bars indicate wild-type and the transgenic events, respectively. Numbers below the bars for each variety represent the number of individual transgenic events, and "C" represents the wild-type plants. KC1, Kufri Chipsona-1; KC2, Kufri Chipsona-2; KJ, Kufri Jyoti; KST, Kufri Sutlej; KBD, Kufri Badshah, KBR, Kufri Bahar; KP, Kufri Pukkraj.

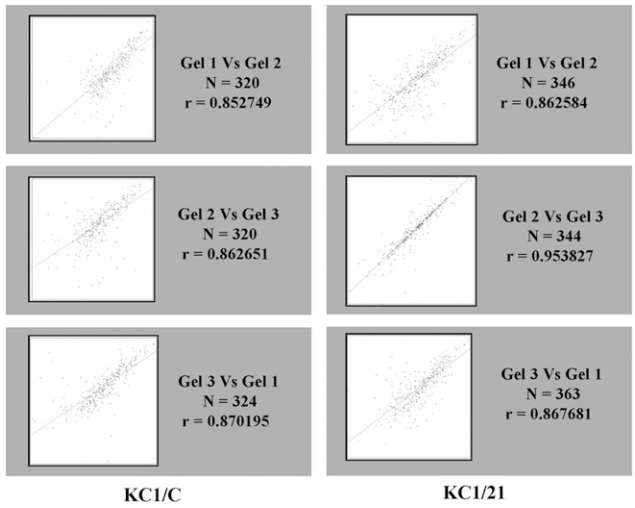

Fig. S3. Scatter plots displaying a correlation coefficient of variation above 0.8 among the three replicates of a transgenic line (KC1/21) and the corresponding wild-type (KC1).

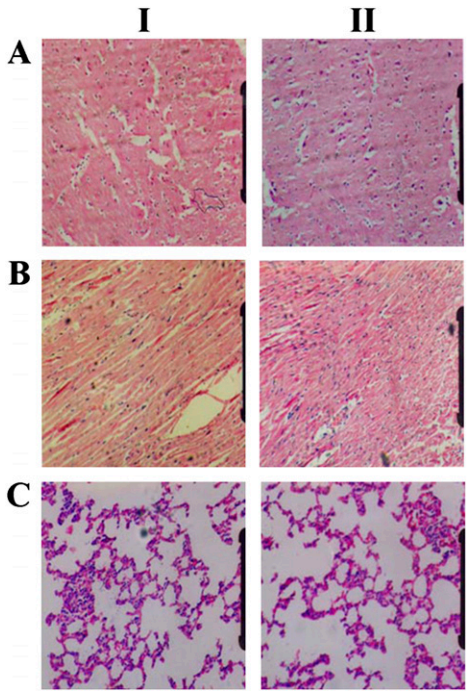

Fig. S4. Histopathological analysis from the organs of (*I*) rats fed with wild-type potato and (*II*) rats fed AmA1 potato. Sections showing hematoxylin and eosin staining of (*A*) brain, (*B*) heart, and (*C*) lung.

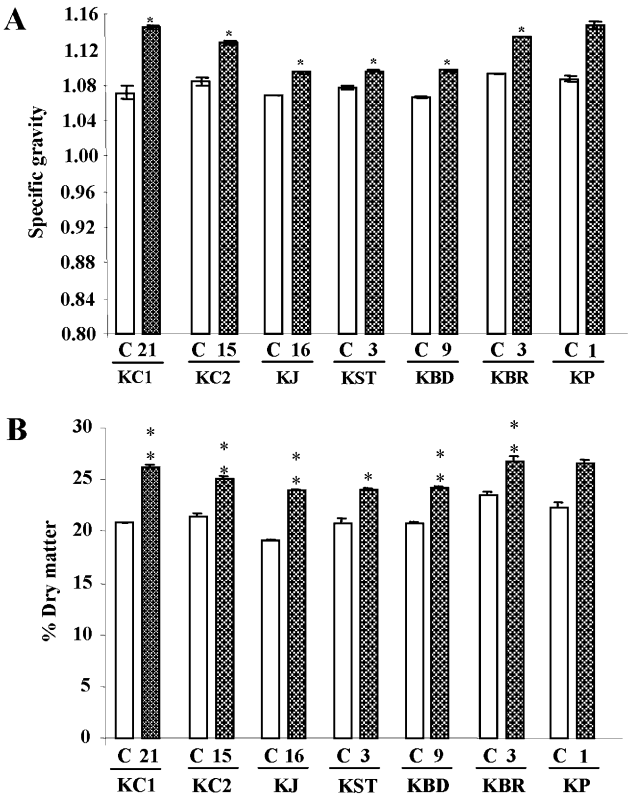

Fig. S5. Analyses of dry matter and specific gravity. (*A*) Specific gravity of wild-type and AmA1 potato was measured by the ratio of weight in air and weight in water method. (*B*) Dry matter content of wild-type and AmA1 potato was measured by the ratio of dry weight and fresh weight. Numbers below the bars for each variety represent the number of individual transgenic events, and "C" represents the wild-type plants. KC1, Kufri Chipsona-1; KC2, Kufri Chipsona-2; KJ, Kufri Jyoti; KST, Kufri Sutlej; KBD, Kufri Badshah; KBR, Kufri Bahar; KP, Kufri Pukkraj. Values are represented as mean SD for three replicates. Student's *t* test was performed to evaluate statistical significance. $*P \leq 0.05$; $**P \leq 0.005$.

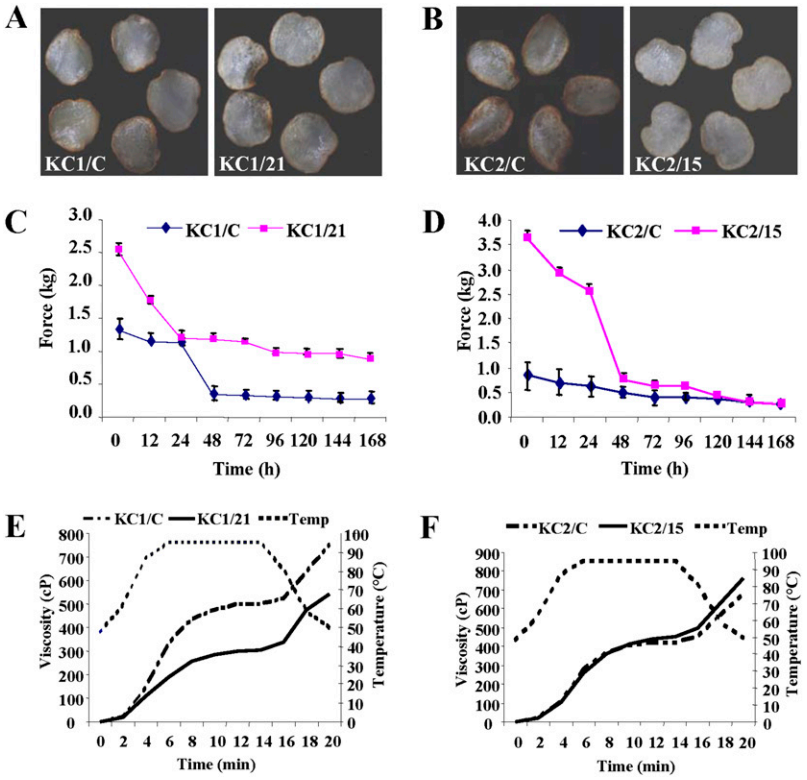

Fig. S6. Comparative evaluation of browning, texture and viscosity in wild-type and transgenic potato tubers. (*A*, *B*) Photographs showing surface browning. (*C*, *D*) Textural changes of cooked potatoes. Values are represented as mean ± SD for three replicates. (*E*, *F*) Viscoamylograph showing time kinetics of pasting properties of wild-type and transgenic potato tubers. The reported values are the means of duplicate measurements. KC1 and KC2 denote wild-type while KC1/21 and KC2/15 represent their transgenic counterparts, respectively.

Table S1. Amino acid profile of potato tubers of wild-type and AmA1 transgenic events

Amino acid (μmole/g dry wt)	K Chipsona 1		K Chipsona 2		K Jyoti		K Sutlej		K Badshah		K Bahar		K Pukhraj	
	C	21	C	15	C	16	C	3	C	9	C	5	C	1
D	84.5 ± 5.3	127.9 ± 7.0	53.9 ± 3.6	110.9 ± 6.5	94.4 ± 6.0	132.5 ± 7.3	128.2 ± 7.9	169.4 ± 7.9	50.3 ± 4.0	77.8 ± 5.3	82.2 ± 4.6	84.7 ± 4.6	64.3 ± 3.3	63.4 ± 3.3
E	67.9 ± 4.0	84.3 ± 5.3	52.8 ± 3.6	98.8 ± 6.4	121.5 ± 6.4	163.7 ± 7.9	92.5 ± 6.0	141.2 ± 7.3	53.6 ± 3.3	95.0 ± 6.0	92.1 ± 6.0	97.7 ± 6.0	72.5 ± 4.0	75.0 ± 4.0
S	14.0 ± 1.3	17.2 ± 1.5	10.9 ± 1.1	17.3 ± 1.5	13.6 ± 1.3	19.4 ± 2.0	19.4 ± 1.3	26.0 ± 2.0	8.8 ± 1.3	12.1 ± 1.3	15.0 ± 1.3	16.9 ± 1.3	9.7 ± 1.3	10.8 ± 1.3
G	22.5 ± 2.0	28.2 ± 2.5	17.8 ± 1.5	28.7 ± 2.3	20.9 ± 1.5	28.0 ± 2.7	28.5 ± 2.0	38.3 ± 2.7	12.7 ± 1.6	18.2 ± 2.0	22.5 ± 2.0	30.4 ± 3.3	17.0 ± 2.0	19.4 ± 1.7
H	5.7 ± 0.7	7.8 ± 0.8	6.8 ± 0.7	9.0 ± 0.9	6.8 ± 1.2	7.3 ± 1.3	7.9 ± 1.7	8.7 ± 0.7	2.7 ± 0.9	3.8 ± 0.7	6.2 ± 1.3	10.0 ± 1.3	3.3 ± 1.2	4.5 ± 1.3
R	20.3 ± 1.7	33.5 ± 2.7	22.2 ± 2.1	26.9 ± 2.2	35.1 ± 2.1	40.9 ± 2.0	30.6 ± 2.3	43.4 ± 3.3	22.0 ± 1.7	32.8 ± 3.3	25.5 ± 3.3	38.8 ± 2.0	18.0 ± 1.6	20.9 ± 2.0
T	8.9 ± 0.8	12.6 ± 1.3	6.7 ± 0.7	9.9 ± 1.0	7.8 ± 0.9	8.7 ± 1.0	9.9 ± 3.0	11.9 ± 1.3	6.1 ± 1.2	7.7 ± 1.3	7.0 ± 0.7	7.5 ± 1.3	4.6 ± 0.7	4.6 ± 0.7
A	3.6 ± 0.4	37.6 ± 2.8	2.0 ± 0.2	5.0 ± 0.5	5.0 ± 0.8	5.5 ± 0.8	4.3 ± 1.3	4.2 ± 0.7	2.1 ± 0.7	2.6 ± 1.0	2.2 ± 0.3	2.7 ± 0.3	1.9 ± 0.5	1.7 ± 0.3
P	9.1 ± 0.9	10.3 ± 1.0	6.4 ± 0.8	11.6 ± 1.1	10.2 ± 1.3	14.4 ± 1.3	14.5 ± 2.7	17.8 ± 1.3	6.4 ± 0.5	9.9 ± 1.3	11.1 ± 1.3	15.3 ± 1.3	7.6 ± 1.3	7.9 ± 0.9
Y	4.6 ± 0.5	5.3 ± 0.7	3.3 ± 0.4	8.7 ± 0.9	4.7 ± 0.7	9.5 ± 1.0	5.0 ± 1.1	6.6 ± 1.0	2.4 ± 0.7	3.7 ± 1.4	5.3 ± 0.7	10.0 ± 1.3	4.7 ± 1.2	5.3 ± 1.1
V	18.7 ± 1.5	20.8 ± 1.6	13.4 ± 1.3	22.5 ± 2.0	27.5 ± 3.8	33.6 ± 2.0	27.7 ± 2.3	36.0 ± 2.0	13.0 ± 1.9	17.6 ± 2.8	21.7 ± 2.0	23.2 ± 2.0	15.1 ± 1.3	15.0 ± 1.3
M	4.4 ± 0.6	5.6 ± 0.7	2.6 ± 0.4	5.1 ± 0.5	1.4 ± 0.4	3.1 ± 0.3	2.1 ± 0.5	3.4 ± 0.9	1.9 ± 1.2	2.4 ± 0.7	2.7 ± 0.3	3.9 ± 0.3	1.8 ± 0.6	2.1 ± 0.3
C	0.5 ± 0.1	2.1 ± 0.3	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	0.9 ± 0.4	1.0 ± 0.5	0.2 ± 0.1	0.3 ± 0.3	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.3	0.3 ± 0.1
I	12.5 ± 1.2	15.4 ± 1.4	8.3 ± 0.9	14.4 ± 1.4	16.2 ± 1.4	20.6 ± 2.0	17.9 ± 1.4	22.3 ± 1.7	6.6 ± 1.3	9.1 ± 1.3	8.9 ± 1.3	9.9 ± 1.3	6.6 ± 1.0	6.4 ± 0.7
L	25.2 ± 2.1	28.5 ± 2.3	15.7 ± 1.4	29.6 ± 2.3	19.9 ± 2.1	27.3 ± 3.3	32.2 ± 2.3	40.9 ± 2.4	11.1 ± 1.3	15.3 ± 2.0	14.7 ± 2.0	18.2 ± 2.7	10.4 ± 1.2	13.1 ± 1.3
F	11.7 ± 1.1	12.4 ± 1.2	9.2 ± 1.0	13.7 ± 1.3	14.6 ± 1.3	17.8 ± 2.7	16.1 ± 1.9	18.8 ± 1.7	7.6 ± 2.0	10.7 ± 1.3	10.8 ± 1.3	13.3 ± 2.0	7.5 ± 1.3	7.8 ± 1.2
K	21.0 ± 2.1	23.0 ± 2.1	16.1 ± 1.5	29.0 ± 0.3	20.5 ± 1.7	24.9 ± 4.6	24.6 ± 2.1	29.2 ± 2.5	11.7 ± 1.5	14.5 ± 2.7	20.6 ± 2.3	20.8 ± 1.8	13.8 ± 2.0	14.3 ± 1.3

Three tubers from each replication for each wild-type and transgenic event were randomly collected at maturity. The pooled tubers were lyophilized and used for amino acid analysis. Values are presented as the mean ± SE for three, each of wild-type and transgenic tubers. Numerical below each variety represents the number of transgenic event and C represents the corresponding wild-type plants.

Table S2. Reproducibility of 2-DE gels

Sample	Average no. of spots*	High-quality spots†	Reproducibility (%)
KC1	323	311	96.28
KC1/21	362	335	92.54

*Average number of spots present in three replicate gels at each time point.
†Spots having a quality score of more than 30 assigned by PDQest (Ver.7.2.0).

Table S3. Yield, tuber number, and estimated productivity of AmA1 potato plants

	K Chipsona 1		K Chipsona 2		K Jyoti		K Sutlej		K Badsah		K Bahar		K Pukhraj	
	C	21	C	15	C	16	C	3	C	9	C	5	C	1
Field trial I														
No. of tubers per plant	11.38 ± 0.33	16.33 ± 1.06	13.13 ± 2.41	15.92 ± 10.26	15.28 ± 0.73	10.50 ± 2.47	11.60 ± 1.36	15.53 ± 2.33	13.40 ± 0.96	11.94 ± 2.75	12.48 ± 2.27	11.65 ± 1.24	11.02 ± 0.19	16.51 ± 4.55
Tuber fresh weight per plant (g)	434.42 ± 13.85	490.21 ± 5.94	405.33 ± 9.29	424.64 ± 57.17	389.66 ± 45.38	475.08 ± 58.09	463.54 ± 19.86	495.74 ± 22.14	647.67 ± 25.08	644.67 ± 17.13	498.26 ± 15.98	593.97 ± 31.16	486.75 ± 21.87	501.48 ± 25.01
Mean fresh weight per tuber (g)	38.06	30.01	30.83	26.10	24.63	44.33	40.08	32.03	48.33	54.04	40.68	51.19	44.07	30.76
Estimated yield (tons/ha)	36.20	40.85	33.78	34.62	31.30	39.88	39.00	41.31	53.97	53.72	41.89	49.36	40.44	41.75
Field trial II														
No. of tubers per plant	11.44 ± 1.67	13.66 ± 1.05	7.51 ± 0.95	8.03 ± 0.55	7.29 ± 0.79	7.42 ± 0.66	11.31 ± 0.41	11.53 ± 1.32	10.19 ± 0.72	8.68 ± 1.67	9.40 ± 1.50	10.17 ± 2.15	9.48 ± 0.51	10.28 ± 1.45
Tuber fresh weight per plant (g)	350.33 ± 5.25	346.37 ± 3.80	326.99 ± 4.19	342.94 ± 2.95	333.30 ± 1.26	380.19 ± 10.67	404.14 ± 3.19	379.14 ± 3.52	462.76 ± 6.95	446.60 ± 2.54	355.35 ± 2.64	430.02 ± 13.19	426.27 ± 13.86	424.94 ± 4.10
Mean fresh weight per tuber (g)	30.60	25.47	43.45	42.49	46.00	51.21	35.78	32.90	45.42	51.21	37.80	42.12	44.96	41.37
Estimated yield (tons/ha)	26.81	25.85	25.24	25.81	25.30	30.06	33.17	31.36	37.71	36.39	29.61	34.53	34.75	33.58

Potato plants were grown under field conditions in Modipuram and New Delhi, India (field trial I and field trial II). In both trials, determinations were carried out on fully senescent plants. Data represent the mean ± SE of determinations on the plants as described in *SI Materials and Methods*. Numbers below each variety represent the number of transgenic events, and C represents the corresponding wild-type plants.

Table S4. Blood 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
WBC count	9.43 ± 2.41	8.58 ± 1.24	8.87 ± 2.70	7.25 ± 1.96	8.20 ± 1.14	8.20 ± 0.68	8.42 ± 0.64	8.28 ± 0.76
Lymphocyte (%)	84.90 ± 2.92	84.60 ± 2.17	84.70 ± 2.91	83.30 ± 1.77	81.20 ± 1.92	81.40 ± 1.14	80.00 ± 0.84	80.60 ± 2.07
Neutrophil (%)	13.70 ± 2.71	13.90 ± 1.66	13.80 ± 2.62	14.50 ± 3.68	18.40 ± 1.82	82.20 ± 0.84	18.00 ± 0.84	19.20 ± 1.92
Eosinophil (%)	0.30 ± 0.48	0.30 ± 0.48	0.30 ± 0.48	0.40 ± 0.52	0.20 ± 0.45	0.20 ± 0.45	0.20 ± 0.45	0.20 ± 0.45
Monocyte (%)	1.10 ± 1.30	1.20 ± 1.23	1.20 ± 1.47	1.80 ± 1.87	0.20 ± 0.45	0.20 ± 0.45	0.20 ± 0.45	0 ± 0
Basinophil (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Rectic (%)	2.14 ± 0.25	2.03 ± 0.22	2.08 ± 0.18	2.05 ± 0.25	2.41 ± 0.44	2.23 ± 0.24	2.26 ± 0.21	2.30 ± 0.22
RBC count ($\times 10^6$)	7.23 ± 0.42	7.45 ± 0.52	7.02 ± 0.29	6.93 ± 0.25	8.34 ± 0.88	8.72 ± 0.63	8.58 ± 0.39	8.54 ± 0.45
Hb (g %)	14.34 ± 0.64	14.21 ± 0.45	13.90 ± 0.21	13.94 ± 0.34	13.66 ± 0.98	14.00 ± 0.78	13.50 ± 0.98	13.86 ± 0.40
HCT (%)	38.06 ± 2.26	38.97 ± 2.51	37.54 ± 1.51	37.20 ± 1.41	41.20 ± 3.26	42.12 ± 2.91	48.44 ± 3.02	41.78 ± 1.69
Platelet count	827.60 ± 101.63	792.90 ± 77.30	780.30 ± 75.34	777.90 ± 82.79	711.20 ± 54.02	778.20 ± 56.16	746.00 ± 77.06	768.00 ± 51.77

g %, gram percent; HCT, hematocrit.