

Assessment of Genetically Modified Potato Lines Resistant to Potato Virus Y Using Compositional Analysis and Molecular Markers

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Abstract: Transgenic potato lines expressing the coat protein gene for potato virus Y (CP-PVY) were evaluated in field trials under artificial infection with the potato virus Y. Nine different transgenic potato lines of the Desiree cultivar and non-transgenic controls were screened with 11 different RAPD primers and five AFLP primer pairs for the identification of genomic changes associated with the transformation process. Results of field trials provided evidence both for the expression of the resistance trait and for the occurrence of limited but consistent morphological, physiological and yield variation, as compared with control plants regenerated from dedifferentiated culture without transformation (control). Four of the nine transgenic potato lines of the Desiree cultivar showed consistent resistance to potato virus Y. Most transgenic plants showed genomic homogeneity among controls and verified minor genomic changes within the transgenic lines. The results obtained in this study confirm those achieved in similar studies in other plant species regarding the abundance and use of molecular markers in identifying transgenic potato lines harboring the resistant gene. Another aim of this study was to estimate the substantial equivalence of genetically modified (GM) potato Desiree lines compared to that of conventional non-transgenic potato Desiree cultivars (control group) through determination of nutritional composition parameters such as: protein, ash, fiber, fat, moisture, amino acids profile. All tested chemical parameters showed that there were no significant differences between the results obtained from the tested samples and those of the control sample. From these results, it can be concluded that GM potato Desiree lines (PVY 4, PVY5, PVY23 and PVY31) harboring the Cp-PVY gene are equivalent in its effect as the non-GM potato Desiree cultivar.

Key words: Transgenic Potato, Cp-PVY, nutritional composition, AFLP, RAPD

INTRODUCTION

Potatoes are considered the second most important vegetable crop in Egypt in terms of crop value and total production. Potatoes are subjected to different pathogenic attacks during its growing season, which affect both the quantity and quality of the final crop production. One of these pathogens is potato virus Y (PVY), a member of potyviruses^[1,2]. Potyviruses are one of the most important virus groups all over the world. They represent 90% of the known RNA viruses with a single positive strand. PVY is transmitted by *Myzus persicae* which reduces crop production by 80% depending upon the viral strain and the presence of

other pathogens. Viral spread in the potato fields is hard to control due to the vector activity and the way of transmission^[3,4,5].

Transgenic potato lines resistant to potato virus Y produced by infecting leaves with *Agrobacterium tumefaciens* were developed at AGERI under the National Genetic Engineering Program. These transgenic potato lines expressing the coat protein gene for potato virus Y (CP-PVY) were artificially challenged with a strain of potato virus Y and were evaluated in the experimental station at Assiut University. Nine independent lines were screened, five of which were shown to be highly resistance to PVY infection. These were determined according to ELISA

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and Dot-blot analysis. Such evaluation included vegetative growth characteristics (plant height, leaves and stems number per plant), yield and yield components (tubers number, size, weight and total yield/plant). Field plantation confirmed data obtained at greenhouse experiments according to [6].

Food or feed derived from genetic engineering is to be compared with its conventional counterpart to establish the extent of equivalence. "Substantial equivalence," or SE, is an internationally recognized standard that measures whether a genetically modified food or crop shares similar health and nutritional characteristics with its conventional counterpart. Biotech foods that are substantially equivalent have been determined to be as safe as their conventional counterparts. Products that are not substantially equivalent may still be safe, but must undergo a broader range of tests before they can be marketed. New foods or food components must be assessed for their safety and wholesomeness (nutritional adequacy) regardless of the method by which they are produced or processed. Safety considerations for biotech foods are essentially the same as those for products produced by traditional methods.

Substantial equivalence evaluations are conducted to assess whether the key nutrients or anti-nutrients in the plant components used for feed or food have been changed. If a biotechnology product is found not to have any differences in the composition of nutritional or anti-nutritional components from its conventional counterpart, it is considered substantially equivalent. In its basic form, SE is an analytical evaluation that compares the composition of the food/feed component under review with an existing food/feed or food/feed component that humans or animals already safely consume. The assessment relies on validated methods. If no counterpart exists, additional safety considerations are warranted. The comparison is based on the normal range of composition of key groups of reference components: nutrients like amino acids, protein, ash, fiber, fat, moisture, vitamins,etc. A determination that a biotech food or crop is not substantially equivalent does not mean the product is unsafe. However, a product that is determined to not be substantially equivalent would be subject to a broader analysis on a case-by-case basis, with the safety assessment focusing on established differences between the product and its conventional counterpart [7].

Molecular techniques are easily applied to determine variation levels in plant material at all stages, from single protoplasts to regenerated plants. Molecular markers such as RAPD, AFLP and SSR

analysis are often used to differentiate between particular plant lines, and sensitive enough to enable discrimination not only between tissues at different culture stages, but more importantly, between regenerated plants, even when there is no ostensible morphological abnormality [8].

This study was carried out to assess and evaluate the transgenic potato lines resistant to potato virus Y by using two approaches: the Substantial Equivalency of the genetically modified potato lines with its non modified counterpart using different nutritional composition parameters, and the molecular marker approach to assess whether the genomic changes that occurred in the transgenic potato lines produced by *Agrobacterium tumefaciens* was significantly different from controls or not.

MATERIAL AND METHODS

Plant Material: Transgenic Desiree potato lines (PVY 1, 2, 4, 5, 15, 23, 24, 25 and 31) and controls used in this study were developed at AGERI [6] by transforming 4 week-old leaves of *in vitro* grown potato (cv. Desiree) with *Agrobacterium tumefaciens*, containing pGA-PVY-CP construct. Lines (PVY4, 5, 23 and 31) were found to be highly resistant to PVY infection according to ELISA and Dot-blot analysis [6].

Chemical Composition Evaluation: Protein, ash, fat, fiber, starch, total carbohydrates, fatty acids composition according to [9], Iron, Zinc, Sodium, Potassium, Manganese according to [10,11], amino acids profile according to [12] and Vitamins B1, B2 and C according to [13,14] were all performed on the examined samples.

Statistical Analysis: Results of the tested samples were represented as mean values and were compared with the control sample results using t-test. Analysis of variance of the recorded data was performed according to the method described by [15].

Allergenicity test: Allergenicity test was performed against the FARRP allergen protein database version 8.0 released January 2008 using the search for 80 amino acid alignments tool which is available on Allergen Online webpage <http://www.allergenonline.com>. Allergen Online provides access to a peer reviewed allergen list and sequence searchable database intended for identifying proteins that may present a potential risk of allergenic cross-reactivity.

Specific algorithm was added to Allergen Online in January, 2009 to perform a search with every possible 80 amino acid segment of the query protein. The rationale is based on the recommendation by the FAO/WHO 2001 expert panel recommended using a criteria of >35% identity over any segment of 80 or more amino acids as an indication of possible cross-reactivity for allergens which was adopted by [16] as the primary sequence search criteria for use in flagging proteins that might be of some concern of cross-reactivity for genetically modified plants [16]. This comparison is done by sequential FASTA3 searches of amino acid segments of 1-80, then 2-81, 3-82, etc., until the end of the query sequence is reached. The identity score is adjusted to compensate for segments less than 80 amino acids due to inserted gaps, or aligned segments less than 80 amino acids that calculate to >35% identity if adjusted to 80 amino acids total.

DNA Extraction: Samples of nine randomly selected independent transgenic plants and individual non-transgenic control plants were frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. Total genomic DNA was isolated from transgenic and control plants using DNeasy kit (Qiagen Inc.).

RAPD Analysis: Eleven 10-mer primers were used in this study (Table 1). PCR reactions were carried out in a volume of 50 µl containing 25 ng of the genomic DNA, 40 pmoles of each primer, 200 µM of each dNTP, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM MgCl₂, 0.001 gelatin and 1.5 units of Taq DNA polymerase. DNA Amplification was carried out in a Perkin Elmer Gene Amp System 9600 thermocycler. It was programmed for 45 cycles as follows: 94°C for 4 min (1 cycle), 94°C for 1min, 36°C for 1 min, 72°C for 2 min, (45 cycles), 72°C for 7 min (last cycle) followed by soaking at 4°C. Amplified products were analyzed by electrophoretic separation in 1.4 % agarose gels, stained with ethidium bromide. The gels were run at 100 volts for about three hours. After electrophoresis the RAPD patterns were visualized under UV light (Gel Doc 2000, Biorad).

AFLP Analysis: AFLP analysis was performed as described by [17] with some modifications [18] using the Gibco BRL AFLP™ analysis system I and the AFLP starter primer kit (Cat. # 10544-013 and 10483-014, respectively). Amplified products from the selective amplification were separated on a 6% denaturing polyacrylamide (sequencing) gel. The DNA Silver

Staining System (Promega Cat # DQ 7050) was used for band visualization. A total of five *EcoRI*-*MseI* primer combinations (Table 1) were screened. Results were visually scored for the presence or absence of a given band.

Statistical Analysis: For the RAPD and AFLP analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) resulting in two binary data matrices. Each band was interpreted as one allele. Bands with the same mobility were assumed to be homologous. Each marker was treated as an independent unit character.

The polymorphism information content (PIC) and the effective numbers of alleles per locus were calculated as reported by [19]. The total number of effective alleles (Ne) surveyed by RAPD and AFLP analyses were calculated by summing the number of effective alleles of all the analyzed loci as $Ne = \sum (i)$. To compare the efficiency between both methods used in this study, where RAPDs and AFLPs detect two alleles and multiple bands per assay, an assay efficiency index (Ai) was calculated. Ai combines the effective number of alleles identified per locus and the number of the polymorphic bands detected in each assay as $Ai = Ne/P$, where Ne is the total number of effective alleles detected and P is the total number of assays performed for their detection. The genetic similarities (GSs) and similarity matrices from RAPD and AFLP data were calculated among all possible pairs of lines using Jaccard's coefficient.

Cluster analyses were based on similarity matrices obtained with the un-weighted pair group method using arithmetic averages (UPGMA) and relationships between inbred lines were visualized as dendrograms.

RESULTS AND DISCUSSION

Transgenic Desiree potato lines (PVY 1, 2, 4, 5, 15, 23, 24, 25 and 31) used in this study were developed at AGERI [6] through transforming four-week-old leaves of *in vitro* grown potato (cv. Desiree) with *Agrobacterium tumefaciens* containing pGA-PVY-CP construct. Genomic polymorphism was evaluated in the DNA extracted from the nine transgenic events. As control, DNA was also extracted from untransformed Desiree plants. The molecular tools employed for genome analysis in this study were RAPD and AFLP.

RAPD Analysis: RAPD analysis was performed with 11 random decamer primers listed in Table (1) on the nine transgenic lines and controls. The maximum

number of bands was found to be 104 with primer OPC-20, whereas the least number of bands was 53 with primer OPB-14 (Table 1). Altogether, 856 amplified bands were recorded, 53 of which were found to be polymorphic. Only four out of the 11 primers used showed polymorphism while the other seven showed no polymorphism which demonstrates a rather low genomic variation in the material tested.

AFLP Analysis: AFLP amplification reactions were performed with the same DNA preparations used for RAPD, by using five different pairs of DNA primers. The maximum number of bands was found to be 891 with primer combination E-AGG x M-CTT, whereas the least number of bands was 595 with primer combination E-AGG x M-CTG (Table 1), thus confirming the high multiplex ratio produced by AFLP markers. Altogether, 3605 amplified bands were scored (Table 1 and Fig. 1), 151 of which were polymorphic among the material tested. Only three out of the five primer pairs used showed polymorphism while the other two showed no polymorphism which demonstrates a rather low genomic variation in the material tested and confirms the RAPD results.

Genomic Similarity: All bands resolved in agarose (for RAPD) or polyacrylamide gels (for AFLP) were scored as a binary character for presence or absence and genomic similarity was calculated (Table 2). Data obtained was used to produce the UPGMA dendrograms representing the genomic distances between the nine selected potato lines (Fig. 2 A and B) using the Diversity Database Fingerprinting Software.

In the case of RAPD, the genomic similarity value ranged from 91.3-100% showing low genomic variability among the analyzed plants. While the AFLP data showed a similarity value ranging from 94.3-99.7% showing the same low genomic variability. In both cases the genomic similarity values were extremely high which indicated that there were no real detected differences between control and transformed plants. The dendrograms revealed no real differences between control and transformed plants in this study.

In order to facilitate selection of an appropriate technology for a given application, we used different metrics to compare the two marker systems used in this study. A good measure of information content, which is a function of a marker system ability to distinguish between genotypes, is the PIC value, which is a measure of allelic diversity at a locus. The second metric is the multiplex ratio of a marker system, which defines the number of loci (or bands) simultaneously,

analyzed per experiment. The assay efficiency index, is proposed as a universal metric to represent the amount of information obtained per experiment for a given marker system. For the quantitative comparison of data, values of polymorphism index (PI) may be used. This is defined as the ratio between polymorphic bands and total recorded bands produced within each analyzed transgenic population and with each specific analytical tool^[20]. The levels of polymorphism detected with each marker system and the index comparing their informativeness are reported in Table (3).

This work showed a PI value of 6.19 and 4.19 in the case of RAPD and AFLP, respectively (Table 3). The PIC value was almost the same for RAPDs (0.969) and AFLPs (0.954) because of low level of polymorphism detected by both assays. The assay efficiency index was (721) for AFLPs because of the large number of bands per gel and (77.8) for RAPDs because of the relatively small number of bands per gel in comparison to AFLPs (Table 4). In particular, for AFLPs the high assay efficiency index is due to simultaneous detection of several bands in a multiplex amplification per single reaction.

It has been proposed that genomic variability in transgenic plants may result from: (1) tissue culture induced mutagenesis that results in apparently random genomic changes^[21]; (2) pre-existing genomic variability in the original plant population, as documented in cross-pollinating^[22 and 23], self-pollination^[24] and cloned plants^[25]; (3) insertion mutagenesis, whereby the foreign gene may disrupt the gene it is inserted into or close to^[26] and (4) pleiotropy, whereby the foreign gene may have multiple effects on apparently unrelated genes^[27 and 28]. Variation has been correlated with the stress imposed by the presently utilized transformation procedure, depending on plant differentiation from *in vitro* cell culture. The extent of somaclonal variation, calculated as frequency of DNA changes, has been correlated with the extent and severity of the *in vitro* stress^[25,27].

PI values in transgenic rice populations previously produced by different approaches: PEG electroporation^[29], particle bombardment and cell electroporation^[20,27] showed that none of the transformation procedures is free from somaclonal variation. However, it is clear that transgenic plants produced by *A. tumefaciens* treatment carry, on average, fewer genomic changes than transgenic plants produced via other methods^[27]. Results, obtained by both RAPD and AFLP analysis showed a very low level of genomic variability between the transgenic and control plants indicating a high similarity between both of hem. AFLP and

Table 1: Total number of bands and polymorphism as revealed by RAPD and AFLP analysis.

Primer # & name	Total No. of bands	Polymorphism
1 OPA-03	77	No
2 OPA-08	88	No
3 OPA-11	55	No
4 OPB-05	76	No
5 OPB-07	88	No
6 OPB-14	53	Poly
7 OPB-16	86	Poly
8 OPC-05	75	Poly
9 OPC-18	88	No
10 OPC-20	104	Poly
11 OPO-16	66	No
RAPD Total	856	
1.1 E-ACC / M-CTG	696	Poly
6.6 E-AGG / M-CTT	891	No
7.7 E-AGG / M-CTG	595	Poly
8.8 E-ACG / M-CTA	671	No
10.10 E-AGC / M-CTA	752	poly
AFLP Total	3605	

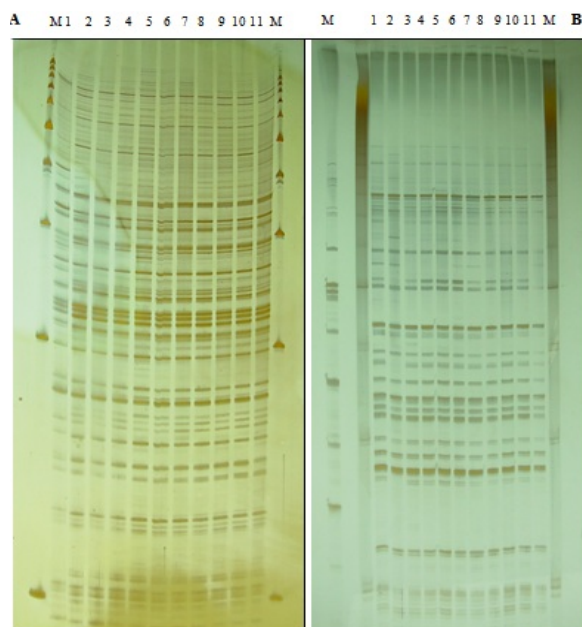


Fig. 1: AFLP profiles of the control and transgenic potato plants. Numbers 1 and 11 refer to Controls. Numbers 2-10 refer to the different transgenic potato plants. (A) Primer combination (1.1); (B) primer combination (7.7); (M) molecular weight standard (Biolab).

Table 2: Genetic similarity matrix calculated according to Jaccard's coefficient based on the RAPD and AFLP data.

RAPD	1C-S	PVY 23	11 C-D	PVY 31	PVY15	PVY 24	PVY 25	PVY 5	PVY 4	PVY 2
PVY 23	97.5									
11 C-D	97.5	100								
PVY 31	97.5	97.5	97.5							
PVY15	96.3	98.8	98.8	98.7						
PVY 24	96.3	98.8	98.8	98.7	100					
PVY 25	96.3	98.8	98.8	98.7	100	100				
PVY 5	96.2	96.3	96.3	98.7	97.5	97.5	97.5			
PVY 4	94.9	95.0	95.0	97.4	96.2	96.2	96.2	96.2		
PVY 2	93.7	93.8	93.8	96.2	94.9	94.9	94.9	97.4	96.1	
PVY 1	91.3	93.8	93.8	93.7	94.9	94.9	94.9	94.9	96.1	97.4
AFLP	1C-S	PVY 2	PVY 23	PVY 24	PVY 15	PVY 5	PVY 31	PVY 25	PVY 4	PVY 1
PVY 2	98.2									
PVY 23	97.0	96.4								
PVY 24	96.7	96.7	99.7							
PVY 15	96.4	95.3	98.2	98.5						
PVY 5	96.4	95.8	98.2	97.9	98.2					
PVY 31	96.4	97.0	99.4	99.1	97.6	98.8				
PVY 25	95.8	96.4	98.8	98.5	97.0	98.2	99.4			
PVY 4	95.5	96.7	98.5	98.8	98.5	97.9	99.1	98.5		
PVY 1	95.5	94.9	95.5	95.8	97.3	96.1	94.9	95.5	95.8	
11 C-D	94.9	94.3	97.9	97.6	96.1	96.1	97.3	97.9	96.4	95.8

Table 3: Levels of polymorphism and comparison of informativeness for RAPD and AFLP markers.

Parameter	RAPDs	AFLPs
Number of assay units	11 primers	5 combinations
Total No. of bands	856	3605
No. of polymorphic bands	53	151
Percentage of polymorphism (PI)	6.19	4.19
Effective No. of alleles per locus	16.15	23.9
Assay efficiency index	77.8	721
Average # of bands per assay	77.82	721
PIC value	0.969	0.954

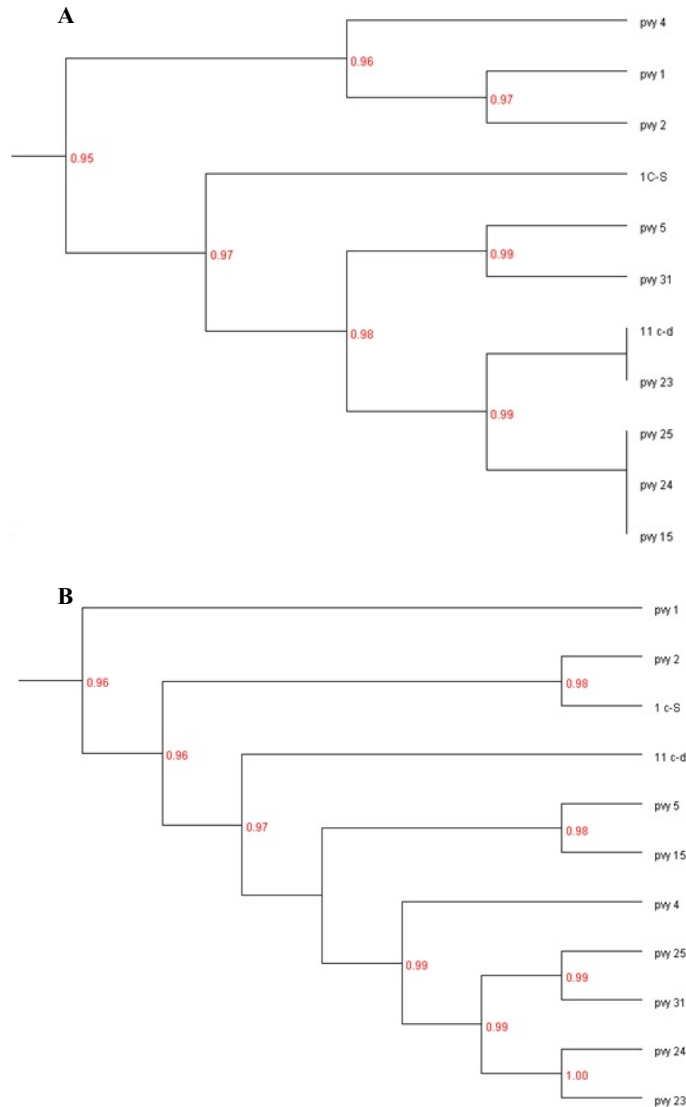


Fig. 2: Dendrogram, based on the Jaccard similarity index, showing the genetic distance between samples, determined by RAPD analysis (A) or AFLP analysis (B).

RAPD markers are good tools to investigate the occurrence and frequency of genomic polymorphism in plants [25,27,30]. The limitation is that these tools essentially analyze random or repetitive genome sequences and do not give direct information on the DNA region where exogenous genes are integrated.

Chemical Composition Evaluation: Genetic engineering is one of the many advances made to traditional breeding practice in plants, to enhance food quality and increase productivity. It is important to state that although the process involving recombinant

DNA technology is not inherently hazardous; some workers in the field of food safety may argue that the products of this technology may have the potential of being risky especially if the interest genes resulted in the production of unintentional hazardous substances.

In the current work, the safety of four of the nine transgenic PVY potato plants which showed the highest field resistance to PVY and controls were determined by examining key groups of reference components such as amino acid profiles, protein, vitamins and minerals, fatty acids, fiber, ash and moisture to give an indication of the safety of these transgenic plants in

comparison with the controls. Also, allergenicity testing was performed against the FARRP allergen protein database as described in materials and methods. Researchers have shown that NPTII proteins are rapidly digested in the acidic gastric fluids and do not contain any amino acids sequences that are closely related to those of known allergens or toxins ^[31].

Verification of Modified and non Modified Samples: RT-PCR was performed to detect the presence and the copy number of the 35S promoter ^[6].

Proximate Analysis of Examined Potato Samples: Results of proximate analysis parameters including: moisture, ash, fat, fiber and protein are illustrated in Table (4) which revealed that, there were no statistical significant differences ($P > 0.05$) between the obtained results of tested sample and the results of the control sample. These results are in agreement with ^[32,33] who concluded the same results.

Amino Acids Composition: The content of 16 essential and non essential amino acids was determined in modified and non modified samples. Results presented in Table (5) showed that there were no significant differences ($P > 0.05$) between all obtained values. This result is in agreement with ^[33] who concluded the same.

Vitamins and Minerals: Vitamins B1, B2 and C were determined and the obtained data in Table (6) illustrates again that there were no significant difference ($P > 0.05$) between the results obtained for the tested samples compared to that of the control sample. Table (6) also demonstrates the mineral content of the tested and the control samples which showed no significant differences ($P > 0.05$).

Fatty Acids Composition: Also fatty acids contents in (Table 7) showed no significant differences compared to the control sample ($P > 0.05$). These results are in agreement with ^[33] who concluded the same results.

Safety assessment studies are based on the application of the principal of "Substantial equivalence" which has been adopted by leading international food and feed regulatory bodies including the World Health Organization ^[34], the United Nation Food and Agriculture Organization ^[35] and the Organization for Economic Cooperation and Development ^[36,37].

According to this principle, if a new food or food derived from genetically modified crop is found substantial equivalent to its conventional counterpart, it is considered as safe as the conventional one ^[38]. Governmental authorities like Japan, United States, United Kingdom, European Union and many other countries have adopted substantial equivalence as an integral part of the bases of safety assessment of the food derived from crops developed through biotechnology and have approved numerous products based on this approach.

According to that, the safety assessment of the first generation of the transgenic potato crop had been carried out based on this concept "Substantial equivalence". Although compositional analysis and allergenicity test together with the protein stability test are reliable in reaching this concept, consumers and some scientists require more detailed evaluation with special reference to experimental animals feeding trials. The risk assessment of GM plants and derived food and feed follows a comparative approach, i.e. the derived food and feed are compared with their non-GM near isogenic counterparts in order to identify differences which subsequently are assessed with respect to their potential impact on the environment,

Table 4: Proximate analysis of examined transgenic Potato samples.

Test	Non GM Potato sample	PVY 4	PVY 5	PVY 23	PVY 31
Ash (%)	5.22	5.61	5.32	5.40	5.45
Fat (%)	0.33	0.36	0.32	0.31	0.27
Fiber (%)	4.69	3.83	4.31	3.41	3.93
Protein (%)	17.90	17.65	17.45	16.40	17.80
Moisture (%)	2.4	3.2	3.3	2.9	2.4
Energy (cal/g)	3460	3420	3530	3490	3550

Table 5: Amino acids profile of Desiree transgenic Potato samples.

Sample type					
Test	Non GM Potato sample	PVY 4	PVY 5	PVY23	PVY31
Aspartic acid	4.92	5.32	4.95	4.52	4.89
Therionin	0.35	0.34	0.30	0.37	0.23
Serin	0.42	0.40	0.39	0.42	0.24
Glutamic acid	2.88	2.64	2.81	2.49	2.13
Prolin	0.74	0.65	0.59	0.64	0.43
Glycin	0.30	0.29	0.25	0.32	0.22
Alanin	0.41	0.40	0.38	0.42	0.32
Cystin	0.16	0.16	0.13	0.20	0.28
Valin	0.56	0.57	0.53	0.54	0.52
Methionin	0.08	0.09	0.08	0.09	0.08
Isoleucin	0.38	0.40	0.34	0.35	0.31
Leucin	0.47	0.44	0.40	0.53	0.35
Phenylalanin	0.38	0.35	0.31	0.44	0.28
Histidin	0.33	0.31	0.30	0.34	0.22
Lysin	0.58	0.57	0.55	0.61	0.49
Arginin	1.11	1.15	1.18	1.15	0.86

Table 6: Vitamins and Minerals content of transgenic Potato samples.

Sample type					
Test	Non GM Potato sample	PVY 4	PVY 5	PVY23	PVY31
Vitamins (g per Kg)					
B1	0.00124	0.00121	0.00127	0.00119	0.00129
B2	0.00024	0.00021	0.00027	0.00023	0.00020
C	0.0718	0.0800	0.0781	0.0772	0.0794
Minerals (mg/kg as dry weight)					
Iron	129.2	120.7	137.6	130.2	121.0
Zinc	27.92	27.34	26.28	27.24	25.86
Sodium	130.1	126.6	125.4	130.2	128.5
Potassium	6211	6097	5997	6017	6544
Mangense	5.23	4.64	4.74	5.87	4.70

Table 7: Fatty acids composition of transgenic Potato samples

Test	Non GM Potato sample	PVY4	PVY5	PVY23	PVY31
Linoleic acid (C18:2 ω 6) (%)	30.4	28.8	30	29.7	28.4
Linolenic acid (C18:3 ω 3) (%)	10.3	12.6	9.6	10	10.6
Oleic acid (C18:1 ω 9) (%)	52.6	62	58.2	53.8	55
Palmitic acid (C16:0) (%)	25.1	24.8	26.8	27.3	29.7
Palmitoleic acid (C16:1 ω 7) (%)	0	0	0	0	0
Stearic acid (C18:0) (%)	5.2	5.6	6.6	5.1	5.1

safety for humans and animals, and nutritional quality (Concept of Substantial Equivalence or Comparative Safety Assessment). This approach has been developed and accepted by international organizations like the EC, the FAO/WHO, Codex Alimentarius and OECD ^[39].

In conclusion, the nutritional composition evaluation indicated that, all transgenic potatoes are not different from controls; and the genetically modified potato was substantially equivalent to that of the non modified potatoes in its compositional analysis and so did the molecular marker studies, therefore it can be concluded that transgenic material when properly assessed can be equivalent to controls.

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