NUTRITIONAL AND BIOLOGICAL EVALUATION OF TRANSGENIC POTATO LINES RESISTANT TO POTATO TUBER MOTH

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Potato (*Solanum tuberosum* L.) is the second economically important vegetable crop after tomato in Egypt. The nutritive value of potatoes, inducting vitamin C, is high, as they are particularly useful as a source of energy and proteins.

The main biotic constrains for potato are fungal, bacterial, viral diseases and potato tuber moth *Phthorimaea operculella* Zeller (PTM) infestation. The insect infestation decreases the yield by 20 to 40% according to the pressure of the insect and the growing season. The insect attacks potato plants in two ways, mining the foliage and also feeding on tubers, so it is considered as an important pest both in field and during storage.

Conventional breeding of potato takes much time and effort. The hybridization in potato even with the closest relatives usually requires several generations of backcrossing and selection to restore the yield and quality of the new cultivar. A major advantage of genetic modification of potato is that it can allow the transfer of specific genes that confer resistance, as opposed to the entire genomes of these wild varieties that have otherwise undesirable qualities. Potato and tomato crops were the first food crops to be genetically engineered (Ross, 1986).

Recently, a new strategy based on integration of the *cry* genes into the plant genome has emerged. This would give the transgenic plant self-protection and will have many advantages over topical application of biopestecides. Several *cry* genes have been introduced into plants, beginning with tobacco (Vaeck *et al*., 1987), and now including many major crops (Fujimoto *et al*., 1993; Koziel *et al*., 1993; Nayak *et al*., 1997). In potato, *Agrobacterium*-mediated transformation system is well established and widely used (Tavazza *et al*., 1988; Newell *et al*., 1991).

The field and storage studies to evaluate Bt-cry5 (rename as *Cry1Ic1)* potato lines for resistance to potato tuberworm in Egypt under natural infestations and their agronomic performance in both Egypt and Michigan was conducted (Douches *et al.,* 2004). A total of 27 Bt-

transgenic potato lines from six different *Bt* constructs were evaluated over a 5-yr period. Field tests to assess potato tuberworm resistance in Egypt have differentiated between the Bt-transgenic lines and the non-transgenic lines in the years 1997, 2000 and 2002. The Bt-cry5-Spunta lines (Spunta-G2, Spunta-G3, and Spunta-6A3) were the most resistant lines in field with 99-100% of tubers free of damage. In the 2001 storage study, these lines were also over 90% free of tuberworm damage after 3 months. In agronomic field trials in Michigan from 1997 to 2002, the Bt-transgenic lines, in most instances, performed similar to the non-transgenic line in the agronomic trials; however, in Egypt (1998-1999), the yields were less than those in Michigan. Results of expression of the Bt-cry5 gene in the potato tuber and foliage will provide the seed producer and grower a tool to which reduce potato tuber worm damage to the tuber crop in the field and storage, (Douches *et al* ., 2004). Insect bioassays on transgenic and non-transgenic potato tubers were studied by Mohammed *et al.* (2000)

Before commercialization, the produced transgenic crop must undergo estimation of many food safety parameters which are included in a standard term called substantial equivalence or SE. It is an internationally recognized standard that measures whether a biotech 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. (OECD, 2000; FAO/WHO, 2000).

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 essential vitamins, minerals, fatty acids, starch, carbohydrates and amino acids. If a new product is determined to be substantially equivalent, and has been assessed to be as safe as its traditional counterpart, there-

fore be regulated similarly. A determination that a biotech food or crop is not substantially equivalent does not mean that the product is unsafe. However, a product that is determined 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 (Marianna, 2000).

The aim of this work is to estimate the substantial equivalence of the genetically modified potato lines with its non modified counterpart using different nutritional composition parameters and a short-term feeding trial.

MATERIALS AND METHODS

Plant material

Spunta G2 (Sp G2), Spunta G3 (Sp G3), Spunta 6A-3 (Sp-6A-3) transgenic lines and Spunta control potato cultivar samples cultivated in a small field trial at AGERI were received by Regional Center for Food and Feed (RCFF) (Egypt) to be used in the feeding experiment.

The pSPUD5 construct (Fig. 1) harboring *Bacillus thuringienis* (Bt) (*Cry 1Ic1*) gene and the Neomycin Phosphtransferase (*NPTII*) gene was introduced into Spunta variety at David Dauches Lab. Department of Crop and Soil Science, Michigan State University, Michigan, USA and were kindly given to conduct the evaluation.

Rat experimental design

Biological assay was carried out using weighing age albino rats of 70-75 g, by cagging individually in metabolic cages comprising an upper living area for feeding and a lower device for collecting of urine and faeces. The temperature was ranged from 20 to 24°C and the relative humidity was between 45-50%. Four diets were prepared according to Eggum (1973) for four different experimental animal groups. The rats were fed on 150 mg nitrogen and 10 g dry matter/rat/day. General signs and behavior were observed daily. Urine and faeces were collected for the determination of T.D., B.V. and N.P.U.

Chemical composition evaluation

Protein, ash, fat, fiber, starch, total carbohydrates, fatty acids composition (according to AOAC 2000), iron, zinc, sodium, potassium, manganese (according to Chapman and Pratt 1961; Cottenic *et al.,* 1982), amino acids profile (according to Official Journal of the European Communities, 1998) and Vitamins B1, B2 and C (according to NMKL, 1996-methods no 189.2 and 113.2) were all measured or istemated on the examined samples.

Statistical analysis

All results of tested samples are reported as mean value and were compared with the control sample results using statistical analysis using *t*-test. Analysis of variance of the recorded data

was performed according to the method described by (Gomez, 1984).

Alergenisity test

Alergenisity 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 May, 2005 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 the Codex (2003) as the primary sequence search criteria for use in flagging proteins that might be of some concern of cross-reactivity for genetically modified plants (Codex Alinorm 03/34, 2003). 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.

RESULTS AND DISCUSSION

Genetic engineering is one of the many advances made to traditional breeding practices 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 hazards substances.

In the current work, the safety of transgenic *Bt*-Potato plants-that was intentionally engineered against one of the most devastating insects in potato, Potato Tuber Moth (PTM) that attacks potato plants in the field and infect potato tubers in storage was evaluated.

The usage of *Bt* spores, that contain the Bio-insecticidal toxic protein, have been widely used for more than 30 years in commercial agriculture globally particularly in organic communities world wide, and safety of *Bt*-toxins to humans, animals and the environment have been demonstrated. Furthermore, previous work in different crops engineered to express *Bt* genes along with *NPTII* (together or separately) have thoroughly demonstrated that proteins produced by both *Bt* and *NPTII* genes have no potential toxicity or allergenicity to mammalians and have

history of safe human consumption (Flavell *et al*., 1992; Nap *et al*., 1992; Fuchs *et al*., 1993a; Fuchs *et al*., 1993b; Lavrik *et al*., 1995; Betz *et al*., 2000; EFSA, 2006) were both *Bt* and *NPTII* proteins are rapidly digested in the acidic gastric fluids and do not contain any amino acids sequences that is closely related to those of known allergens or toxins (Betz *et al.,* 2000)

Verification of modified and non modified samples

RT-PCR run for the detection of 35S promoter revealed that the transgenic harbor. The results of the control were negative (data not shown). It is clear from the mentioned figures that, Spunta G2, G3 and 6A-3 had the promoter which was indicated by cutting the threshold line after 32 cycles for G2 and G3 and after 31 cycles for 6A-3 sample. The promoter gene was not detected in the control sample.

Effect of the GM potatoes on the digestibility performance and the behavior of experimental rats

No rats in any group died or had any abnormal signs throughout the period of the experiment. There were no marked differences in the true digestibility (T.D.), biological value (B.V.) and net protein utilization (N.P.U.) (Table 1) of the examined samples (SpG2, SpG3 and Sp6A-3), although the N.P.U. values were very low because of the poor quality of potato protein which is used as the sole source of protein.

Proximate analysis of examined Potato samples

Results of proximate analysis parameters including: Ash, fat, fiber, protein, starch and total carbohydrates are illustrated in (Table 2) 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 Habiba *et al.* (2000) and Hashimoto *et al.* (1999) who concluded the same results.

Amino acids composition

The content of 8 essential and 7 non essential amino acids were determined in modified and non modified samples. Results presented in (Table 3) showed that there were no significant differences (P >0.05) between all obtained values. This result is in agreement with Hashimoto *et al.* (1999) who mentioned the same conclusion.

Vitamins, Fatty acids and minerals content

Vitamins B1, B2 and C were determined and the obtained data in table 4 illustrate 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 4 also demonstrates the mineral content of the tested and the control samples which showed no significant differences (P >0.05). Also fatty acids contents in (Table 5) showed no significant differences

compared to the control sample ($P > 0.05$). These results are in agreement with Hashimoto *et al.* (1999) who concluded the same results.

Safety assessment studies is 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 (WHO, 1995), the United Nation Food and Agriculture Organization (FAO, 1996) and the Organization for Economic Cooperation and Development (OECD, 1996 & 1997).

According to this principle, if a new food or food derived from genetically modified crop is found substantial equivalent to its conventional counter part, it is considered as safe as the conventional one. Government 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. Therefore, in this study, the safety of the transgenic potatoes was evaluated by a feeding study in rats and measurement of the different digestibility parameters.

Throughout this work, there were no different observations between rats fed on transgenic and non transgenic potatoes and also there were no differences in the compositional analysis between the transgenic and the non transgenic counter parts.

In conclusion, this short term assessment of the novel potato by rat feeding experiment and the nutritional composition evaluation indicated that, all transgenic potatoes are not different.

In order to construct the experimental systems that make it possible to extrapolate transgenic potatoes to human, long term animal feeding study together with examining the other parameters like histopathological evaluation, protein stability and allergenicity tests must be assessed.

The results showed that, the genetically modified potato was substantially equivalent to the non modified potatoes in its biological effects on experimental rats and in its compositional analysis.

Alergenisity analysis

The query sequence was entered in a typical FASTA format. The amino acid sequence for the *Cry1IcI* did not show any significant similarity more that over 35% identities over any possible 80 amino

acids window. The results that we have dose not support the idea of that the *Cry1IcI* may cause any allergic reaction. All previous work (Flavell *et al*., 1992; Nap *et al*., 1992; Fuchs *et al*., 1993a; Fuchs *et al*., 1993b; Lavrik *et al*., 1995; Betz *et al*., 2000; EFSA, 2006), also alergenisity analysis for Bt showed that Bt and NPTII genes have no potential toxicity or alergenisity to mammalians and have history of safe human consumption.

SUMMARY

The present study was designed to estimate the substantial equivalence of genetically modified (GM) potato Spunta lines (SpG2, SpG3 and Sp6A-3) with the *cry1Ic1* gene compared to conventional non-transgenic potato Spunta cultivar (control group) through rat feeding experiments and through the determination of nutritional composition such as: protein, ash, fiber, fat, starch, total carbohydrates, amino acids, fatty acids, micro and macro-elements and vitamins B1, B2 and C. Short-term feeding study was conducted for nine days using experimental rats to estimate the digestibility parameters: True Digestibility, (T.D.), Biological Value (B.V.) and Net Protein Utilization (N.P.U.) as well as the behavior of rats under investigation. Rats in each group (SpG2, SpG3 and Sp6A-3 and control groups) grew normally without marked differences in appearance, behavior or in mortality rate. No marked differences were observed in the T.D., B.V. and N.P.U. in all groups. All tested chemical parameters showed no significant differences between the GM samples and the control. From these results, it can be concluded that the GM potato Spunta lines (SpG2, SpG3 and Sp6A-3) are equivalent to the non-GM potato Spunta cultivar. The results for allergenicity testing did not support the idea that the *cry1IcI* gene may cause any allergic reaction to the tested animals.

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Table (1): Digestibility parameters in examined rats.

Sample	T.D.%	B.V.%	N.P.U.%
Sp G2	70.2	60.73	42.63
Sp G3	70.6	62.8	44.34
Sp 6A-3	70.7	63.4	44.82
Spunta (Control)	71.6	64.4	46.11

Table (2): Proximate analysis of examined transgenic and non-transgenic potato lines.

Test	Sample type			
	Non GM potato sample	Sp G2	Sp G3	Sp 6A-3
Ash (%)	6.57	6.17	6.41	6.30
Fat (%)	0.27	0.25	0.34	0.25
Fiber (%)	2.70	2.83	2.93	2.86
Protein (%)	11.70	12.10	12.2	11.70
Total carbohydrates (%)	60.73	61.35	60.50	61.74
Starch (%)	69.51	69.33	69.45	69.61

Data are expressed as means of two replicate determinations.

Table (3): Amino acids profile of transgenic and non-transgenic potato lines.

Test	Sample type			
	Non GM potato sample	Sp G2	Sp G3	Sp 6A-3
Essential amino acids (g per 100 g protein)				
Isoleucin	3.56	3.71	4.07	3.79
Leucin	6.41	6.55	7.27	6.62
Lysin	5.64	5.99	6.43	5.69
Methionin & Cystin	2.98	3.04	3.10	3.07
Phenylalanin	4.19	4.13	4.59	4.24
Therionin	3.23	3.47	4.13	3.74
Valin	3.82	3.94	4.45	3.78
Non essential amino acids (g per 100 g protein)				
Alanin	4.72	4.68	5.10	4.45
Arginin	3.29	3.39	3.29	2.88
Aspartic acid	9.69	9.90	10.13	8.72
Glycin	3.43	3.56	3.96	3.56
Histidin	1.29	1.57	1.71	1.42
Prolin	3.11	2.99	3.79	3.06
Serin	2.70	2.98	3.76	3.43

Data are expressed as means of two replicate determinations.

Table (4): Vitamins and Minerals content of transgenic and non-transgenic potato lines.

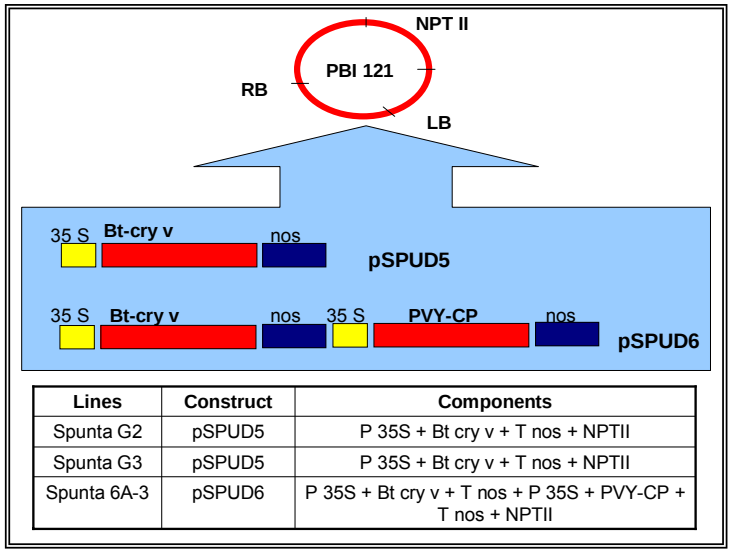
Test	Sample type			
	Non GM potato sample	Sp G2	Sp G3	Sp 6A-3
Vitamins (g/Kg)				
B1	0.00114	0.00131	0.00136	0.00123
B2	0.00024	0.00022	0.00023	0.00025
C	0.07180	0.08650	0.08080	0.07170
Minerals (mg/kg as dry weight)				
Iron	222.00	220.00	218.00	220.00
Zinc	25.86	27.34	28.28	28.24
Sodium	1994.00	2022.00	2001.00	1943.00
Potassium	29430.00	33490.00	30500.00	33500.00
Mangenese	8.11	9.90	9.93	8.66

Data are expressed as means of two replicate determinations.

Table (5): Fatty acids composition of transgenic and non-transgenic potato lines.

Test	Sample type			
	Non GM potato	Sp G2	Sp G3	Sp 6A-3
Linoleic acid (C18:2ω6) (%)	30.4	30.0	30.7	30.3
Linolinic acid (C18:3ω3) (%)	10.3	9.8	10.3	10.5
Oliec acid (C18:1ω9) (%)	50.6	49.2	50.5	49.7
Palmitic acid (C16:0) (%)	22.1	19.3	19.3	21.7
Palmitoleic acid (C16:1ω7) (%)	1.1	1.4	1.8	1.1
Stearic acid (C18:0) (%)	5.2	3.8	4.4	5.3

Data are expressed as means of two replicate determinations.



Lines	Construct	Components
Spunta G2	pSPUD5	P 35S + Bt cry v + T nos + NPTII
Spunta G3	pSPUD5	P 35S + Bt cry v + T nos + NPTII
Spunta 6A-3	pSPUD6	P 35S + Bt cry v + T nos + P 35S + PVY-CP + T nos + NPTII

Fig. (1): Bt-cryv cassette under control of CaMV 35S promoter.