African Journal of Biotechnology Vol. 10(34), pp. 6401-6410, 11 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2604
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Comparative analysis of transformed potato microtubers and its non-transformed counterpart using some biochemical analysis along with inter simple sequence repeat (ISSR) marker

Mahmoud M. Saker[1], Amal A. Mohamed[2]* and Amina A. Aly[3]

[1]Plant Biotechnology Department, National Research Centre, Dokki, Cairo, Egypt.
[2]Plant Biochemistry Department, National Research Centre, Dokki, Cairo, Egypt.
[3]Natural Products Department, National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt.

Accepted 8 April, 2011

The rapid progress of transgenic biotechnology has significantly promoted the development and production of genetically modified (GM) crops. The aim of this study was to compare some compositional analysis and genetic variation of transformed potato microtubers (*Solanum tuberosum* L. Desiree) line (which harbor potato virus Y coat protein gene) with its conventional line in order to understand the metabolic and genomic changes associated with the transformation process. The constituents measured were: amino acids profile, minerals content, reducing sugar, vitamin C, total phenolic, total flavonoid, β-carotene and antioxidant activity. Also, PCR based assay using inter simple sequence repeat (ISSR) was employed to characterize the genetic variation between both lines. The data demonstrated that, there were no significant differences between the transformed and non-transformed line in total amount of amino acids, some minerals, total phenolic, total flavonoid and β-carotene contents. Obtained results of the ISSR profile was efficiently discriminated in both selected lines at DNA level. Generally, it was concluded that the transformed microtubers line was confirmed to be similar to that of the non-transformed microtubers line.

Key words: Amino acids, genetic modification, phenolic content, reducing sugar, *Solanum tuberosum*.

INTRODUCTION

Tremendous progress in plant molecular biology has opened ample opportunities to improve crop plants in a way that is not feasible a few years ago. A transgenic plant contains a gene or genes that have been artificially inserted. The inserted gene sequence, known as transgene, may come from an unrelated plant, or from a completely different species. One of the purposes of inserting a combination of genes in a plant is to make it as useful and productive as possible (FAO, 1996). Recombinant gene technology, the most well-known modern biotechnology, provides several advantages for agricultural productivity such as improving yield quality, pest or disease resistance, tolerance to environmental stresses and reduction in agricultural chemical usage (Herbers and Sonnewald, 1998). Transgenic plants are produced in such a way that they express foreign proteins with industrial or pharmaceutical value. On the other hand, the novel traits in genetically modified organisms (GMOs) may also contain new proteins that either directly or indirectly threaten health and could cause allergies or be toxic (Taylor and Hefle, 2001).

Potato (*Solanum tuberosum* L.) is the fourth most economically important food crop after wheat, rice and maize in the world. Potato is an excellent source of carbohydrate and produces more dry matter and high quality protein than the major cereal crops (Al-Saikhan, 2000). Furthermore, there is preliminary evidence to suggest that potatoes contain significant levels of

*Corresponding author. E-mail: amin_amal@yahoo.com.

important antioxidants, including phenolic compounds, flavonoid and carotenoids (Brown, 2005). Potato has a high nutritive value and is a rich source of vitamins A, B and C besides several minerals such as calcium, phosphorus and iron (Dale et al., 2003; Andre et al., 2007). Because of the importance of the potato around the world, great efforts have been made to develop new potato breeds that are resistant to different pathogens. One of these pathogens is potato virus Y (PVY), a member of *Potyviruses* (Hollings and Brunt, 1981). *Potyviruses* are one of the most important virus groups of cultivated potato all over the world and can reduce yield up to 80% (Hooker, 1981). Modification of potato using genetic engineering holds enormous potential to alleviate this problem. An efficient safe transformation system of transgenic potato plants harboring potato virus Y coat protein gene (CP-PVY) conferring resistance against potyvirus Y has been developed (Saker, 2003). Additionally, potato microtubers grown in an aseptic manner on defined nutrient media in a controlled environment are an attractive model for biochemical and physiological studies of conventional and transformed potatoes (Coleman et al., 2001). New foods or food components derived from genetic engineering should be compared with its conventional counterpart to establish the extent of equivalence. Substantial equivalence (SE) is an internationally recognized standard that measures whether a genetically modified crop share similar health and nutritional characteristics with its conventional counterpart (Schauzu, 2000). The differences between the new food and its conventional counterpart are evaluated to determine their relevance in human diet health and safety. Methods of the identification of genetically modified (GM) foods can be divided into three categories: (i) chemical composition and nutritional value; (ii) protein and enzymatic activities based methods and (iii) nucleotide-based amplification methods, such as polymerase chain reaction (PCR). Every detection method has its own specificity and limitations (FAO/WHO, 2000). For example, to evaluate the safety and quality of some GM potato lines harboring insecticidal crystal gene (CryV), the compositional analysis, anti-nutrients compounds and toxicological effects were detected (El-Sanhoty et al., 2004). The authors reported that there were no significant differences in all the chemical composition between GM potatoes lines and its conventional one. As well, molecular techniques have proven to be a critically useful tool to characterize transgenic plants (Wassenegger, 2001).

Molecular markers such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) were used by El-Khishin et al. (2009) to investigate the frequency of genomic polymerphism between transformed potato lines and its non-GM counterpart. Microsatellites, also called inter simple sequence repeats (ISSR), are an abundant and dispersed class of repetitive DNA throughout most plant genomes (Morgante and Olivieri, 1993). In potato, ISSR markers linked to genes for resistance to potato virus Y (PVY) were used to identify the corresponding resistance loci on chromosomes XII (Flis et al., 2005). Therefore, this study was carried out to evaluate the transformed potato microtubers line harboring virus Y coat protein (CP-PVY) gene with its conventional line using some biochemical analysis and ISSR marker.

MATERIALS AND METHODS

Transgenic potato microtubers (*Solanum tuberosum* L. cv. Desiree) line harboring the coat protein gene of potato virus Y (CP-PVY) without insertion of antibiotic resistance selectable marker genes and its conventional line were used. These lines were developed in a previous work done by Saker (2003) at the Center of Excellence for Advanced Science (CEAS), National Research Centre (NRC), Cairo, Egypt. The developed shoots of the transformed and non-transformed lines were sectioned into nodal cutting and cultured onto MS medium (Murashige and Skoog, 1962) supplemented with 0.3 mg/l gibberellic acid (GA_3) and 0.1 mg/l 6-benzylaminopurine (BA). Cultures were incubated at 22°C in a growth room under 16 h/day photoperiod (2000 lux). Both clones were mass micropropagated and microtubers were produced from the transformed clones in liquid MS medium containing 5 mg/l BA and 8% sucrose in darkness without agitation (Chandra et al., 1988). Microtubers were harvested after 60 days growth on tuberization media and were used for different analysis.

Amino acids composition

The amino acids profile was determined using the method described by Cohen et al. (1989). Each sample was dried to constant weight, hydrolyzed, evaporated and loaded into the reverse phase HPLC (Shimadzu, Kyoto, Japan). The identification of amino acids was carried out by comparing their retention times with those of the standards from Sigma (Brückner and Westhauser, 1994).

Determination of minerals content

Minerals content (Mg, K, Ca, Na, N, Fe and Zn) were estimated after wet digestion as described in AOAC (2000). Sodium, calcium and potassium contents were detected by using flame emission spectrophotometry (Genway, PFP7 model). Magnesium, iron and zinc were quantitatively determined using atomic absorption spectrophotometer (Perkin Elmer-100) with appropriate hollow cathode lamps. Nitrogen contents were determined by Micro-Kjeldahl method whereas phosphorus was measured by using molybidate method (Raboy et al., 2001). Results were expressed as % for macronutrient and as ppm for micronutrient.

Determination of vitamin C (ascorbic acid)

Extraction of ascorbic acid was carried out with meta-phosphoric acid (3%) at room temperature for 30 min using a shaker. Extracts were analyzed according to the standard HPLC method (Cho et al., 2000). HPLC analysis was conducted with a Hewlett-Packard 1050 series pumping system and a Waters 486 UV-detector. Reversed

phase separations were carried out using a 250 x 4.6 mm i.d. and 5 µm Hichrom C18 column. Concentrations of vitamin C were calculated from the integrated areas of samples and the corresponding standard.

Preparation of methanolic extract

The extracts were prepared using the method of Matkowshi and Piotrowska (2006). Briefly, 0.5 g of the dried powder from each line was refluxed with 5 ml 80% methanol in a water bath at 45°C for 3 h. The extracts were filtered and dried under vacuum at 45°C using a rotary evaporator and the extraction was repeated twice. The resulting residue was re-dissolved in methanol (80%) and used for the determination of reducing sugar, phenolic, flavonoid, β-carotene and antioxidant activity.

Determination of reducing sugar

Reducing sugar was determined by using the method of Miller (1972). Briefly, 3.0 ml of the methanolic extract was added to 3.0 ml of DNSA (3,5-dinitro-salicylic acid) reagent. Absorbance was recorded using spectrophotometer (Thermo Unicam UV300) at 515 nm. The amount of reducing sugar was calculated using standard curve prepared from glucose. The quantity of reducing sugar was expressed as mg of glucose equivalent per gram of dried samples.

Determination of total phenolic content

Total phenolic content of the extracts was determined by using the Folin-Ciocalteu reagent according to Julkunen-Tiitto (1985). Methanolic extract (0.5 ml), 0.5 ml of Folin-Ciocalteu reagent, 10 ml of 7.5% sodium carbonate and deionized water were added to a final volume of 25 ml. After 1 h, the absorbance of the sample was measured at 725 nm against a blank by spectrophotometer. Gallic acid was used as the standard for preparing the calibration curve. The results were expressed as mg of gallic acid equivalent per gram of the dried samples.

Determination of total flavonoid content

Colorimetric aluminum chloride method was used for flavonoid determination (Nabavi et al., 2008). The methanolic extract (250 µl) was mixed with 1.25 ml of distilled H_2O and 75 µl of 5% $NaNO_2$ solution. After 5 min, 150 µl of 10% $AlCl_3$ solution was added and filtered. Then 500 µl of 1.0 M NaOH and 275 µl of distilled H_2O were added to the mixture. The absorbance of the sample was measured at 510 nm against a blank by spectrophotometer. The results were expressed as mg of quercetin equivalent per gram of dried samples.

β-Carotene content

β-Carotene was determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone : hexane mixture (4:6) for 1 min and filtered. The absorbance of the filtrate was measured at 453, 505 and 663 nm. β-Carotene content was calculated according to the following equation:

β-Carotene (mg/100 ml) = 0.216 (A_{663}s) - 0.304 (A_{505}) + 0.452 (A_{453}).

Where, A is the absorbance and β-carotene was finally expressed as µg g^{-1} d.w.

Determination of antioxidant activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was determined by the method described by Yen and Duh (1994). The radical scavenging activity of the synthetic antioxidant compounds such as butyl-hydroxytoluene (BHT) and tert-butyl-hydroxyanisole (BHA) were also determined as a positive control. The plant extract (0.1 ml) was added to 3 ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated using the following equation:

$DPPH^{\cdot}$ scavenging effect (%) = [(A0 – A1/ A0)] x 100

Where, A0 is the absorbance of the control reaction (containing all reagents except the test compound) and A1 is the absorbance in the presence of the tested extracts

Genomic DNA isolation for ISSR analysis

Total genomic DNA was isolated and purified from frozen microtubers material using the DNeasy Plant Mini Kit (QIAGEN, Chatsworth, CA). The concentration of DNA was determined at a wavelength of 260/280 nm using a spectrophotometer and the quality was verified by electrophoresis on 1.4% agarose gel.

Primers and ISSR-PCR assays

15 primers (Operon Technologies, Alameda, California, USA) were used for the ISSR analysis; five ISSR primers that produced clear and reproducible fragments were selected for the amplification of the DNA samples as shown in Table 1.

Amplification reactions were carried out in a Perkin Elmer Thermalcycler 9600, the PCR reaction mixture (25 µl) was composed of 20 ng of total genomic DNA (2 µl), 10 µM of primer (2.5 µl), 2.5 µl of reaction buffer, 0.35 µl of Taq DNA polymerase (2u/µl) and 10 mM of each dNTP. Each reaction mixture was overlaid with 25 µl of mineral oil to avoid evaporation during PCR cycling. The PCR was programmed for an initial denaturation at 94°C for 5 min, 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 36°C and 2 min extension at 72°C, followed by a final extension for 7 min at 72°C. PCR amplification products were electrophoresed on 1.4% agarose gels in 1x TBE buffer by loading 25 µl of the reaction mixture into prepared wells. Gels were run for 2 to 3 h at 60 V, and were stained with ethidium bromide (10 µgml^{-1}) according to Sambrook et al. (1989). The molecular weights of the amplification products were calculated using 100-bp DNA ladder standards (Gibco BRL/Life Technology; Rockville, Maryland). ISSR banding patterns were visualized using a UV transilluminator and photo documentation was performed under UV light using a photo imaging system.

ISSR data analysis

Amplicons were scored as 1 (presence) or 0 (absence). Only strong bands were scored for analysis, a pair wise similarity matrix was generated using Jaccard's similarity coefficient and using the unweighted pair group method with t he arithmetic averaging

Table 1. List of primers name (ID) and their nucleotide sequences used in the PCR analysis.

Primer number	Primer name (ID)	Repeats	Primer sequence 5'———3'
1	17898A	$(CA)_6AC$	5`-CACACACACACAAC-3`
2	HB-08	$(GA)_6GG$	5`-GAGAGAGAGAGAGG-3`
3	HB-09	$(GT)_6GG$	5`-GTGTGTGTGTGTGG-3`
4	HB-12	$(CAC)_3GC$	5`-CACCACCACGC-3`
5	HB-15	$(GTG)_3GC$	5`-GTGGTGGTGGC-3`

algorithm (UPGMA), cluster analysis was performed to develop a dendrogram. These computations were carried out using NTSYS-PC Version 2.02 h (Rohlf, 1998).

Statistical analysis

All data were reported as mean ± standard errors (SE) for the three independent samples (n = 3). Analysis of variance and significant differences among means were tested by one-way ANOVA using the COSTAT computer package (Snedecor and Cochran, 1980). The least significant difference (LSD) at $p \leq 0.05$ level was calculated.

RESULTS AND DISCUSSION

Biochemical analysis

One of the goals of plant genetic engineering is to create crops that are tailored to provide better nutrition for humans and their domestic animals. There are about 20 different amino acids that constitute the protein in food substances; these include both the essential and non-essential amino acids. In this study, only 16 of these acids were detected due to the conversion of glutamine and asparagines to glutamic and aspartic acids, respectively, and complete destruction of tryptophan during acid hydrolysis (Hassan and Umar, 2008). The results of amino acids profile as shown in Table 2 indicated that both potato microtuber lines, contain almost all the essential amino acids needed. Aspartic acid was the most abundant amino acid in both lines. Also, the data obtained from the transformed line showed that transformation process by CP-PVY gene led to an increase in the levels of certain amino acids (isoleucine, phenylalanine, threonine and alanine). Improvement of nutritive value of potato plants, in particular amino acids composition, has been a major long-term goal of plant breeding programs. There were no differences in the total amount of amino acids (∑AA) between the transformed line (440.14 mg $100g^{-1}$ N) and non-transformed line (439.67 mg $100g^{-1}$ N). Efforts have been made to enhance nutritive value in transgenic plants, in this concern. Molvig et al. (1997) reported that, over-expression of sunflower seed albumin gene in lupins plant resulted in dramatic increase only in methionine, along with a significant decrease in cysteine content. The results of this work are in a harmony with the results obtained by other authors (Juśkiewicz et al., 2004; Zduńczyk et al., 2005) who indicated that transgenic potato lines (with genetically improved resistant to potato virus PVY) had the same chemical composition (protein and amino acids profile) when compared to non-transgenic one.

Recently, extensive genetic manipulation to improve the mineral nutrition of plants for both macronutrients such as calcium and nitrogen, and micronutrients such as zinc and selenium has been promoted. With regards to the minerals content of microtubers lines, the values in Table 3 revealed that the transformation process by CP-PVY gene led to a significant increase of some minerals (phosphorus, nitrogen, iron and zinc) in the transformed line when compared with the non-transformed one. Potato tubers contain several minerals that are important in diet, including phosphorous, calcium, zinc, nitrogen and iron (Yilmaz et al., 2005). The results are in accordance with the results previously reported by Li et al. (2008) who revealed significant differences in some mineral content between GM rice and its non-transgenic counterpart. On the other hand, other researchers (Hashimoto et al., 1999; El-Sanhoty et al., 2004) indicated that there were no significant differences in the mineral content ($P \leq 0.05$) between GM and non-GM potato lines harboring CryV. The efforts to enhance the synthesis and bioavailability of other minerals through biotechnological approaches are active, including iron and zinc (Zimmermann and Hurrell, 2002).

The results in Table 4 show that, the level of reducing sugar, vitamin C, phenolic, flavonoid and β-carotene contents were comparable in both microtubers evaluated lines. The content of reducing sugar in the transformed line was significantly higher (6.52 mg g^{-1} dw) than the content found in the non-transformed line (5.09 mg g^{-1} d.w.). Vitamin C content ranged from 22.76 mg 100 g^{-1} dw (transformed line) to 18.63 mg 100 g^{-1} dw (non-transformed one); these differences were statistically significant. The total phenolic content of both lines ranged from 7.76 to 8.33 mg g^{-1} dw, whereas, total flavonoid ranged from 5.43 to 5.26 mg g^{-1} dw. There were no significant differences ($P \leq 0.05$) between both lines in the contents of total phenolic, total flavonoid and β-carotene. In this study, the result of reducing sugar

Table 2. Amino acids composition of the transformed and non-transformed potato microtubers lines after 60 days growth on tuberization media.

Amino acid (mg 100 g^{-1} N)	Potato microtubers line	
	Transformed	Non-transformed
Isoleucine (Ile)	24.21±0.09	22.20±0.03
Leucine (Leu)	26.74±0.10	25.91±0.14
Lysine (Lys)	30.61±0.05	32.81±0.15
Methionine (Met)	14.61±0.09	14.81±0.17
Phenylalanine (Phe)	27.41±0.01	23.28±0.04
Threonine (Thr)	32.14±0.12	29.84±0.06
Valine (Val)	26.11±0.06	24.66±0.03
[a]∑EAA	181.83	173.51
Alanine (Ala)	35.71±0.14	29.74±0.01
Arginine (Arg)	27.16±0.08	28.02±0.15
Aspartic (Asp)	88.21±0.15	90.41±0.02
Cysteine (Cys)	5.16±0.17	7.99±0.03
Glutamic (Glu)	32.9±0.16	30.9±0.04
Glycine (Gly)	25.11±0.06	28.2±0.01
Histidine (His)	10.51±0.13	13.8±0.14
Tyrosine (Tyr)	11.64±0.01	12.97±0.04
Serine (Ser)	21.91±0.09	24.13±0.01
[b]∑NEAA	258.31	266.16
[c]∑AA	440.14	439.67

[a]∑EAA, Total essential amino acids; [b]∑NEAA, total non-essential amino acids; [c]∑AA, total amount of amino acids; ±SE, standard errors.

Table 3. Content of macronutrient and micronutrient of transformed and non- transformed potato microtubers lines after 60 days growth on tuberization media.

Mineral		Potato microtubers line	
		Transformed	Non-transformed
Macronutrient (%)	Magnesium (Mg)	0.23^{a}±0.09	0.26^{b}±0.11
	Potassium (K)	3.90^{a} ±0.12	4.07^{b}±0.02
	Calcium (Ca)	0.07^{a}±0.17	0.08^{a}±0.15
	Sodium (Na)	28.20^{a}±0.21	27.71^{a}±0.13
	Phosphorus (P)	0.45^{a}±0.17	0.35^{b}±0.08
	Nitrogen (N)	2.18^{a}±0.09	1.18^{b}±0.15
Micronutrient (ppm)	Iron (Fe)	28.70^{a}±0.18	25.37^{b}±0.16
	Zinc (Zn)	23.85^{a}±0.14	22.91^{b}±0.11

Each value is expressed as mean ± SE. Data with different superscript letters are significantly different ($P \leq 0.05$).

showed significant differences between both lines. The amount and kind of sugar in a particular cultivar depended upon the growing environment, tubers maturity stage at harvest, cultural practices (irrigation, fertilization, etc.) and storage time (Weaver et al., 1978). As the reducing sugar affect flavor and color of potato products, the acceptable sugar content in tubers should not exceed 0.33% of the fresh mass (Roe and Faulks, 1991; Duplessis et al., 1996). Recently, gene encoding glycolysis enzymes were introduced into potato through metabolic engineering in order to decrease the reducing sugar level (Navratil et al., 2007). Vitamin C is one of the most important nutritional quality factors in many horticultural crops and responsible for many biological activities in the human body. In this work, the level of vitamin C content showed significant differences ($P \leq 0.05$) between transformed and non-transformed line. In this concern, Hemavathi et al. (2010) revealed that potato

Table 4. Reducing sugar, vitamin C, phenolic, flavonoid and β-carotene contents in the transformed and non-transformed potato microtubers lines after 60 days growth on tuberization media.

Potato microtubers lines	Reducing sugar (mg g^{-1}d.w.)	Vitamin C (mg $100g^{-1}$d.w.)	Phenolic (mg g^{-1}·d.w)	Flavonoid (mg g^{-1}d.w.)	ß-carotene (µg g^{-1}d.w.)
Transformed	6.52^{a}±0.02	22.76^{a}±0.02	7.76^{a}±0.03	5.43^{a}±0.06	3.41^{a}±0.04
Non-transformed	5.09^{b}±0.04	18.63^{b}±0.14	8.33^{a}±0.05	5.26^{a}±0.07	2.63^{a}±0.02
LSD (P < 0 .05)	0.119	3.51	NS	NS	NS

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different (P ≤ 0.05). NS: not significant.

plants overexpressing the L-gulono-c-lactone oxidase gene showed increased accumulation of vitamin C level and conferred stress tolerance to the transformed plants. Phenolic contents are known to be important, naturally occurring antioxidants, which function as scavengers of damaging free-radical molecules inside the cell. These compounds are involved in a number of biosynthetic processes, including the maintenance of cell wall integrity and chemical defense against pathogen attack and can make a significant contribution to the nutritional value of potatoes (Lachman and Hamouz, 2005). Genetic factors and growing conditions may play an important role in the formation of secondary metabolites; including phenolic and flavonoid content (Islam et al., 2003). The results of phenolic and flavonoid contents are in agreement with the published literature data and ranges of the historical conventional control values determined in previous studies (Prescha et al., 2002; El-Sanhoty et al., 2004). The authors indicated that there were no significant differences in some secondary metabolites in GM potato lines as compared to the control line. Carotenoids are highly beneficial for human nutrition and health because they provide essential nutrients and important antioxidants in our diets. Although significant progress has been made in developing food crops rich in carotenoids by altering the expression of carotenoid biosynthetic genes, in many cases it has proved to be difficult to reach the desired levels of carotenoid enrichment. Potato is qualified as an excellent source of vitamin A (in the form of beta-carotene). Genes encoding the enzymes of the carotenoids biosynthetic pathway have been cloned in potato tubers and a wide range of β-carotene, ranging from 5 to 78 µg/g dw among transgenic potato lines have been observed (Ducreux et al., 2005).

Potato extracts exhibit antioxidant activity and the antioxidant properties of potato plants have been associated with phytochemicals such as α-tocopherol, vitamin C, β-carotene and phenolic compounds (Rumbaoa et al., 2009). The ability of phenolic compounds to quench reactive oxygen species by hydrogen donation was measured through the DPPH radical scavenging activity (Singh et al., 2008). In this work, both methanolic extracts and synthetic antioxidants (BHT and BHA) compounds exhibited dose-dependent anti-DPPH radical scavenging activities (Figure 1). Free radical scavenging activity increased with increasing sample concentration, for example; at the concentration of 150 µg ml^{-1}, the scavenging effect of methanolic extracts and the reference compounds (BHT and BHA) on the DPPH radical decreased in the following order BHA > BHT > transformed > non-transformed and the values were 75.91, 73.49, 45.04 and 39.75%, respectively. These results indicated that methanolic extract of the transformed clone exhibited scavenging of free radicals more than the non-transformed extracts. One of the richest sources of antioxidants in the human diet is potato tubers (Lachman et al. 2000). The main potato antioxidants are polyphenols, ascorbic acid, carotenoids, tocopherols, α-lipoic acid and selenium. Polyphenolic compounds, especially, flavonoid are effective antioxidants (Bors and Saran, 1987) due to their capability to scavenge free radicals of fatty acids and oxygen. Transformation process, genotype and growth conditions, such as water availability, light quality and temperature, affect the synthesis and accumulation of phenolic compounds in some parts of the plant; and consequently, antioxidant activity (Lukaszewicz et al., 2002; Reyes and Cisneros-Zevallos 2003). It could be stressed that, the flavonoids-enriched plants showed improved antioxidant capacity; however, there is a complex relationship between antioxidant capacity and flavonoids content, suggesting the great participation of other compounds in the antioxidant potential of the plants. These other compounds are still to be recognized.

ISSR amplification

Many molecular marker techniques are available today. PCR-based approaches are in demand because of their simplicity and requirement for only small quantities of DNA sample. ISSR are arbitrary multiloci markers produced by PCR amplification with a microsatellite primer. In the study, five random primers (17898A, HB-08, HB-09, HB-12 and HB-15) exhibited polymorphism with the transformed and non-transformed potato lines (Figure 2). Five ISSR primers detected a total of 50 amplification fragments at an average of 10 bands per primer, varying from 6 (17898A) to 13 (HB-12) fragments per primer and ranged from 115 to 1277 bp in size as shown in Table 5.

All the tested primers revealed polymorphisms among

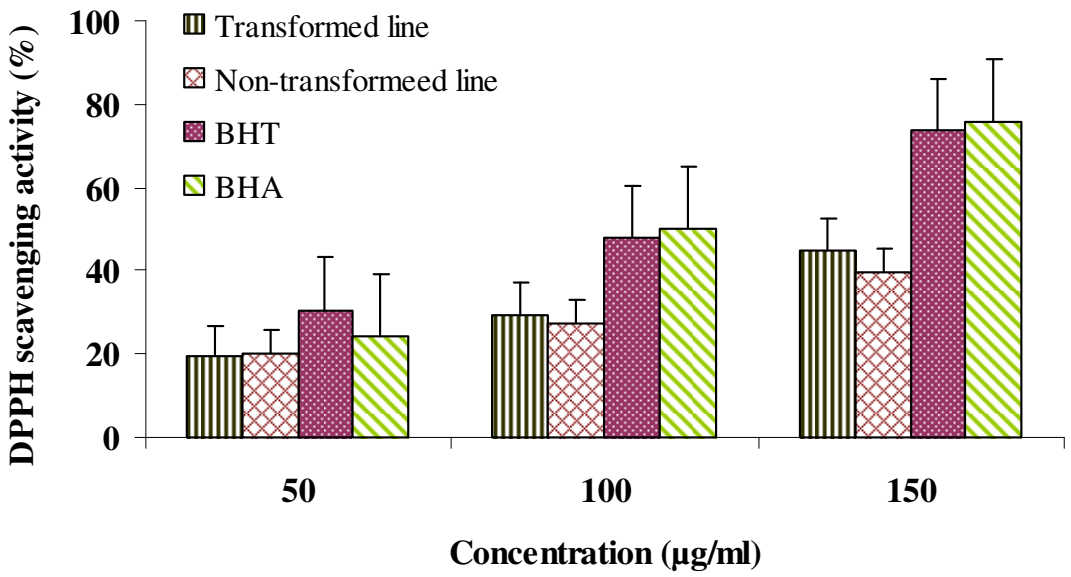

Figure 1. DPPH radical scavenging activity of methanolic potato microtubers lines extracts compared with synthetic antioxidants (BHT and BHA). Bars on the columns represent the standard errors.

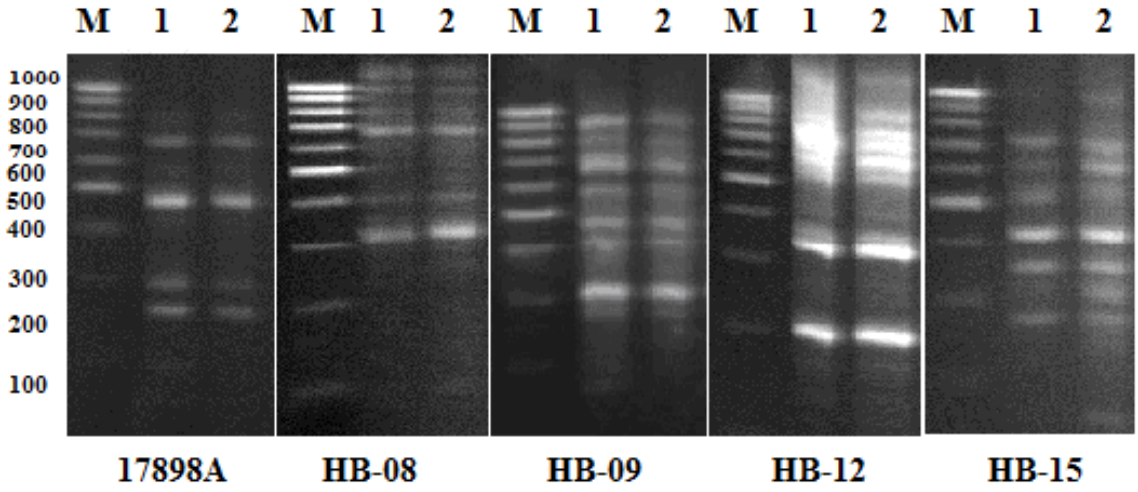

Figure 2. Agarose gel electrophoresis of ISSR amplifications of the two potato microtubers lines with primers 17898A, HB-08, HB-09, HB-12 and HB-15. Lane M, DNA marker (100 to 1000 bp); lanes 1 and 2 refer to transformed and non-transformed lines, respectively.

the transformed and non-transformed lines ranging from 11.11% for primer HB-09 to 41.67% for primer HB-15, respectively. Also, monomorphic bands ranged from 4 (17898A) to 10 (HB-12). The overall percentage of polymorphism for the five primers across the transformed and non-transformed lines was 29.84. The dendrogram of genetic distances between the transformed and non-transformed lines based on band polymorphisms generated by ISSR is shown in Figure 3. The cluster analysis of ISSR markers separated both lines into two distinct clusters. The similarity coefficient values between both transformed and non-transformed lines based on band polymorphisms generated by ISSR are presented in Table 6. The similarity value of 0.70 was found between the transformed and non-transformed lines. Few attempts have been made earlier to assess diversity between transformed and non-transformed potato lines (Flis et al., 2005). In this work, the DNA of two lines (transformed and non-transformed) were compared using five primers to study the genetic diversity between both lines. The results showed that, each primer generated distinct ISSR patterns that were different from the others. The appearance of specific amplified bands from each micro-tuber line was used as a good tool for polymorphism

Table 5. Total number of amplified fragments, polymorphic bands, monomorphic bands, percentage of polymorphic bands, mean of band frequency and amplified fragment size based on ISSR analysis using five primers.

ISSR primer	Total amplified fragment	Polymorphic band	Monomorphic band	*Percentage of polymorphic band	Mean of band frequency	Amplified fragment size
17898A	6	2	4	33.33	0.833	232-1277
HB-08	10	3	6	40.00	0.800	258-1100
HB-09	9	4	8	11.11	0.944	253-937
HB-12	13	3	10	23.08	0.885	137-1085
HB-15	12	5	7	41.67	0.792	115-978
Total	50	17	35	149.19	4.254	
Average per primer	10	3.4	7	29.84	0.850	

*Percentage of polymorphic bands = Number of polymorphic bands/ total Number of amplified fragment x100.

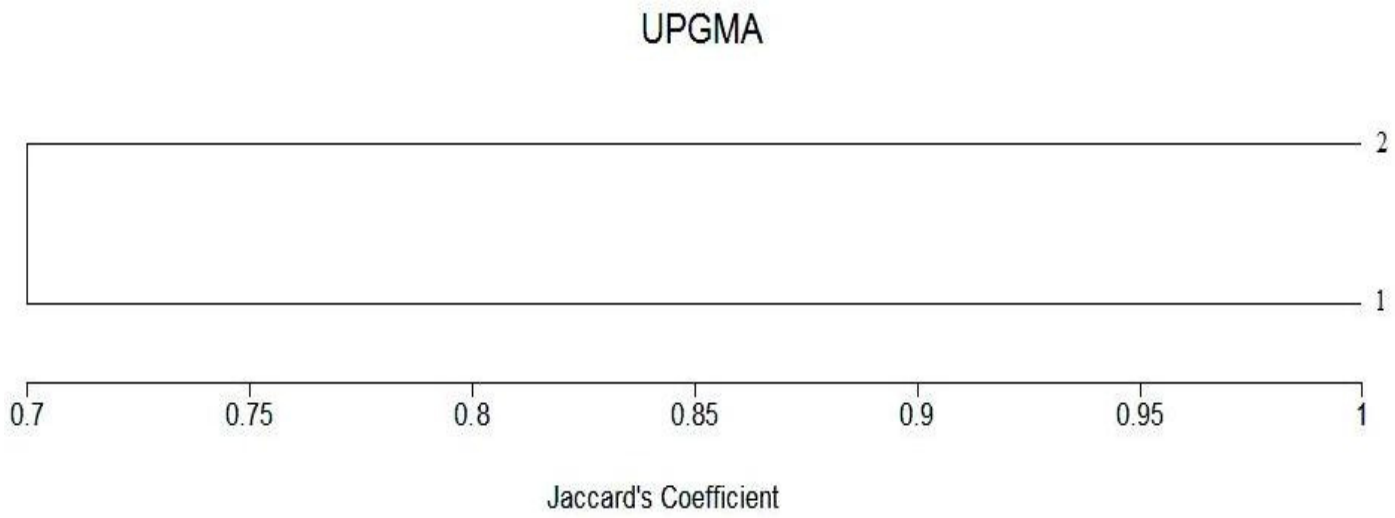

Figure 3. UPGMA cluster analysis for the transformed (1) and non-transformed (2) potato microtubers lines based on genetic distance of ISSR.

Table 6. Genetic similarity matrix of transformed (1) and non-transformed (2) potato microtubers lines calculated according to Jaccard`s coefficient based on the ISSR data.

	1	2
1	1.000	
2	0.700	1.000

detection, and defined by amplified band of characteristic size.

These results are in agreement with those of El-Khishin et al. (2009) who used RAPD and AFLP fingerprints methods to investigate the frequency of genomic polymorphism between transformed potato lines and its non-GM counterpart. It was found that a close genetic relationship was present between the transformed and non-transformed potato lines. This study provides evidence that ISSR polymorphisms could be distinguished between transformed and non-transformed lines. It was concluded that ISSR-PCR provides a quick, reliable and highly informative system for DNA fingerprinting that is amenable for routine applications (Prevost and Wilkinson, 1999). There is a concern that gene insertion and regeneration may cause mutations or somaclonal variation that could have safety implication and affect other genes (Ehrenfeld et al., 2004; Arif et al., 2009). Also, Bornet et al. (2002) indicated that ISSR marker could be successfully used to assess the genetic diversity between cultivated potatoes. It was also found that an ISSR study using a limited number of cultivars and very few primers clearly differentiated between all cultivars, thus ISSR was revealed to be a good tool for the genetic identification of potato and for future germplasm-management programs.

Conclusions

Since potato is an important vegetable crop in Egypt, the safety assessment of transgenic potato should be well conducted before its commercialization. As part of the safety assessment, much work has been carried out on compositional analysis of transgenic potato to determine whether the insertion of transgenes affects the food and feed quality (El-Sanhoty et al., 2004; El-Khishin et al., 2009). This study concludes that essentially nutritional equivalence was found between transgenic and non-transgenic potato lines. It was further added that, there were no significant differences between the transformed and non-transformed lines in the total amount of amino acids, some minerals, total phenolic, total flavonoid and β-carotene contents. These preliminarily results thus confirm that the nutritional quality of transgenic potato line was mostly substantially equivalent to that of the non-transgenic counterpart. No detrimental changes in the nutritional composition of the transgenic potato were observed as a result of the insertion of CP-PVY gene. The potential risks associated with GM foods should be assessed on a case-to-case basis, taking into account the characteristics of the GM food and possible threats of the receiving environments. Also, as prime conclusion, the results indicated that microtubers nutrient composition or concentration could be changed by the introduction of the CP-PVY gene. With respect to food nutrition, the increase of the degree of some of these nutrients in transgenic tuber is beneficial for human health, but the mechanism should be further analyzed.

REFERENCES

Al-Saikhan MS (2000). Antioxidants, proteins, and carotenoids in potato (*Solanum tuberosum*, L.). Ph.D. Dissertation, Texas A&M University, College Station.

Andre CM, Ghislain M, Bertin P, Oufir M, Herrera MD, Hoffmann L, Hausman JF, Larondelle Y, Evers D (2007). Andean potato cultivars (*Solanum tuberosum L.*) as a source of antioxidant and mineral micronutrients. J. Agric. Food Chem., 55: 366-378.

AOAC (2000). (Association of Official Analytical Chemists). Official Methods of Analysis. 17th ed., Arlington Virginia, USA.

Arif M, Thomas PE, Crosslin JM, Brown CR (2009). Agrobacterium-mediated transformation of potato cv. Desiree using potato leafroll virus replicase and potato virus Y coat protein genes and development of replicase mediated resistance against natural infection of potato leafroll virus. Pak. J. Bot., 41: 1477-1488.

Bornet B, Goraguer F, Joly G, Brancjard M (2002). Genetic diversity in European and Aregntinian cultivated potatoes (*Solanum tuberosum subsp. Tuberosum*) detected by inter-simple sequence repeats (ISSRs). Genome, 45: 481-484.

Bors W, Saran M (1987). Radical scavenging by flavonoid antioxidants. Free Rad. Res. Commun., 2: 289–294.

Brown CR (2005). Antioxidants in potato. Am. J. Potato Res., 82:163-172.

Brückner H, Westhauser T (1994). Chromatographic determination of D-amino acids as native constitutents of vegetables and fruits. Chromatographia, 39: 419-426

Chandra R, Dodds H, Tovar P (1988). *In vitro* tuberization in potato. Newsletter, 55: 10-20.

Cho CM, Ko JH, Cheong WJ (2000). Simultaneous determination of water-soluble vitamins excreted in human urine after eating an over dose of vitamin pills by a HPLC method coupled with a solid phase extraction. Talanta, 51: 799 -806.

Cohen SA, Meys M, Tarvin TL (1989). The Picotag Method. A Manual of Advanced Techniques for Amino Acid Analysis. (WM 02, Rev. 1), Waters Millipore, Millford, MA, USA, p. 25.

Coleman WK, Donnelly DJ, Coleman SE (2001). Potato microtubers as research tools: A review. Amer. J. Potato Res., 78:47-55.

Dale MF, Griffiths DW, Todd DT (2003). Effects of genotype, environment, and postharvest storage on the total ascorbate content of potato (*Solanum tuberosum)* tubers. J. Agric. Food Chem., 51: 244-248.

Ducreux LJ, Morris WL, Hedley PE, Shepherd T, Davies HV, Millam S, Taylor MA (2005). Metabolic engineering of high carotenoid potato tubers containing enhanced levels of betacarotene and lutein. J. Exp. Bot., 56: 81-89.

Duplessis PM, Marangoni AG, Yada RY (1996). A mechanism for low temperature induced sugar accumulation in stored potato tubers: the potential role of the alternative pathway and invertase. Am. Potato J., 73: 483-494.

Ehrenfeld N, Romano E, Serrano C, Arce-Johnson P (2004). Replicase mediated resistance against potato leafroll virus in potato Desiree plants. Biol. Res., 37: 71-82.

El-Khishin DA, Abdul Hamid A, El Moghazy G, Metry EA (2009). Assessment of genetically modified potato lines resistant to potato virus Y using compositional analysis and molecular markers. Res. J. Agric. Biol. Sci., 5: 261-271.

El-Sanhoty R, Abd El-Rahman AA, Bogl KW (2004). Quality and safety evaluation of genetically modified potatoes Spunta with CryV gene: Compositional analysis, determination of some toxins, anti-nutrients compounds and feeding study in rats. Nahrung, 48: 13-18.

FAO (1996). Biotechnology and food safety. Report of a Joint FAO/WHO consultation 30 September-4 October 1996. FAO Food and Nutrition. Paper 61, FAO, Rome.

FAO/WHO (2000). Safety aspects of genetically modified foods of plant origin. Report of a joint FAO/WHO expert consultation on foods derived from biotechnology, Geneva.

Flis B, Hennig J, Strzelczyk ZD, Gebhardt C, Marczewski W (2005). The *Ry-fsto* gene from *Solanum stoloniferum* for extreme resistant to potato virus Y maps to potato chromosome XII and is diagnosed by PCR marker GP122718 in PVY resistant potato cultivars. Mol. Breed., 15: 95-101.

Hassan LG, Umar KJ (2008). Nutritive value of Night shade (*Solanum americanum* L.) leaves. Electronic J. Food and Plants Chem., 3: 14-17.

Hashimoto W, Momma K, Katsube T, Ohkawa Y, Ishige T, Kito M, Utsumi S, Murata K (1999). Safety assessment of genetically engineered potatoes with designed soybean glycinin: compositional analyses of the potato tubers and digestibility of the newly expressed protein in transgenic potatoes. J. Sci. Food Agric., 79: 1607-1612.

Hemavathi A, Upadhyaya CP, Akula N, Kim HS, Jeon JH, Ho MO (2010). Enhanced ascorbic acid accumulation in transgenic potato confers tolerance to various abiotic stresses. Biotechnol. Lett., 32: 321-330.

Herbers K, Sonnewald U (1998). Altered gene expression brought by inter and intracellularly formed hexoses and its possible implications for plant–pathogen interactions. J. Plant Res., 111: 323-328.

Hollings M, Brunt AA (1981). *Potyviruses*. In Handbook of Plant Virus Infections and Comparative Diagnosis, (Ed), Kurstak, E., Amsterdam, Elsevier, pp. 731-780.

Hooker J. (1981). Compendium of Potato Diseases. APS Press, St. Paul, Minn.

Islam IK, Okuno S, Yoshimoto M, Yamakawa O (2003). Composition of phenolics and anthocyanins in a sweet potato cell suspension culture. Biochem. Eng. J., 14: 155-161.

Julkunen-Tiitto R (1985). Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. J. Agric. Food Chem., 33: 213-217.

Juśkiewicz J, Zduńczyk Z, Frejnagel S, Fornal J (2004). Caecal fermentation in rats fed diets containing tubers of transgenic potato.

J. Anim. Feed Sci., 13: 93-96.
Lachman J, Hamouz K, Orsák M, Pivec V (2000). Potato tubers as a significant source of antioxidants in human nutrition. Rostlinná Výroba, 46: 231-236.
Lachman L, Hamouz K (2005). Red and purple coloured potatoes as a significant antioxidant source in human nutrition – a review. Plant Soil Environ., 51: 477-482.
Li X, He XY, Luo YB, Xiao GY, Jiang XB, Huang KL (2008). Comparative analysis of nutritional composition between herbicide-tolerant rice with bar gene and its non-transgenic counterpart, J. Food Compos. Anal., 21: 535-539.
Lukaszewicz M, Matysiak-Kata I, Aksamit A, Oszmian´ski J, Szopa J (2002). 14-3-3 Protein regulation of the antioxidant capacity of transgenic potato tubers. Plant Sci., 163: 125-130.
Matkowshi A, Piotrowska M (2006). Antioxidant and free radical scavenging activities of some medicinal plants from the *Lamiaceae*. Fitoterapia, 77: 346-353.
Miller GL (1972). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem., 31: 426-428.
Molvig L, Tabe LM, Eggum BO, Moore AE, Craig S, Spencer D (1997). Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius* L.*)* expressing a sunflower seed albumin gene. Proc. Natl. Acad. Sci. USA, 94: 8393-8398.
Morgante M, Olivieri AM (1993). PCR-amplified microsatellites as markers in plant genetics. Plant J., 3: 175-182.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays for tobacco tissue cultures. Physiol. Plant, 15: 473- 497.
Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR (2008). Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica Mey*. Pharmacologyonline, 2: 560-567.
Nagata M, Yamashita I (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J. Jap. Soc. Food Sci. Technol., 39: 925-928.
Navratil O, Fischer L, Čmejlova J, Linhart M, Vacek J (2007). Decreased amount of reducing sugars in transgenic potato tubers and its influence on yield characteristics. Biol. Plant, 51: 56-60.
Prescha A, Biernat J, Szopa J (2002). Quantitative and qualitative analysis of lipids in genetically modified potato tubers with varying rats of 14-3-3 protein synthesis. Nahrung, 46: 179-183.
Prevost R, Wilkinson MJ (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appl. Genet., 98: 107-112.
Raboy V, Young KA, Dorsch JA, Cook A (2001). Genetics and breeding of seed phosphorus and phytic acid. J. Plant Physiol., 158: 489-497.
Reyes LF, Cisneros-Zevallos L (2003). Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum* L.*).* J. Agric. Food Chem., 51: 5296-5300.
Roe MA, Faulks RM (1991). Color development in a model system during frying: role of individual amino acids and sugars. J. Food Sci., 56: 1711-1713.
Rohlf FJ (1998). NTSYSpc: numerical taxonomy and multivariate analysis system. Version 2.02 h. Setauket, New York: Exeter Software.
Rumbaoa RGO, Cornago DF, Geronimo IM (2009). Phenolic content and antioxidant capacity of Philippin sweet potato (*Ipomoea batatas*) varieties. Food Chem. 113: 1133-1138.
Saker MM (2003). Production of biosafe transgenic potato plants with coat protein gene for potato virus Y. Arab J. Biotech., 6: 125-138.
Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A Laboratory Manual. 2nd ed. NY, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press. 1659 p.
Schauzu M (2000). The concept of substantial equivalence in safety assessment of foods derived from genetically modified organisms. AgBiotechNet. ABN 044.http://www.bfr.bund.de/cm/245/schauzu.pdf
Singh N, Kamath V, Narasiminanmurthy K, Rajin PS (2008). Protective effects of potato peel extract against carbon tetrachloride-induced liver injury in rats. Env. Toxicol. Pharmacol., 26: 241-246
Snedecor GW, Cochran GW (1980). Statistical Methods, 7th edition. The Iowa State, University Press, Ames, Iowa.
Taylor SL, Hefle SL (2001). Ingredient issues associated with allergenic foods. Curr. Allergy Clin. Immunol., 14: 12-18.
Wassenegger M (2001). Advantages and disadvantages of using PCR techniques to characterize transgenic plants. Mol. Biotechnol., 17: 73-82.
Weaver ML, Timm H, Nonaka M, Sayre RN, Reeve RM, McCready RM, Whitehand LC (1978). Potato composition: II. Tissue selection and its effects on total sugar, total reducing sugar, glucose, fructose, and sucrose contents. Am. Potato J., 55: 83-93.
Yen GC, Duh PD (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. J. Agric. Food Chem., 42: 629-632.
Yilmaz G, Tuzen M, Kandemir N, Mendil D, Sari H (2005). Trace metal levels in some modern cultivars and Turkish landraces of potato. Asian J. Chem., 17: 79-84.
Zduńczyk Z, Frejnagel S, Fornal J, Flis M, Palacios MC, Flis B, Zagórski-Ostoja W (2005). Biological response of rat fed diets with high tuber content of conventionally bred and transgenic potato resistant to necrotic strain of potato virus Y (PVYN). Part. I. Chemical composition of tubers and nutritional value of diets. Food Control, 16: 761-766.
Zimmermann MB, Hurrell RF (2002). Improving iron, zinc and vitamin A nutrition through plant biotechnology. Curr. Opin. Biotech., 13: 142-145.