

Review

The potential of unintended effects in potato glycoalkaloids

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Unintended changes have long been considered as byproducts associated with genetic improvement of crop plants. The issue has been hotly debated during recent years following the identification of some unwanted characters in genetically engineered crop plants. In this context, the subject of unintended effects of plant transformation on known toxic compounds has been an area of immense interest. Compositional changes in these toxins may have a profound impact on human and animal health. Potato glycoalkaloids are known toxic compounds to humans and animals. These days, food safety evaluation tests of transgenic potato varieties are conducted on routine basis to keep the glycoalkaloid levels below a threshold level. Some transgenic potato varieties have been found with altered glycoalkaloid levels, which have created doubts on the process of transformation and tissue culture conditions. In this review, we summarize recent work on unintended effects in crop plants with special emphasis on compositional changes in potato glycoalkaloids as a result of genetic transformation.

Key words: Unintended effects, transgenic potato, glycoalkaloids, food safety, environmental stress.

INTRODUCTION

Genetic improvement of crop plants is a routine process that has been in practice since time immemorial. The advent of modern biotechnology however revolutionized this process and as a result, new and improved varieties of crop plants were developed. The modern molecular techniques have been successfully used to transform crop plants for several useful traits, including better shelf life, nutritional content, flavor, color, texture and tolerance to environmental stresses. Along with successful manipulation of the plants with the above mentioned characters, concerns have been raised that genetic engineering may provide a source for the introduction of unintended changes in transgenic plants, causing them to contain undesirable metabolites or changes in their composition (Kok and Kuiper, 2003).

Recent literature reveals a number of transgenic plants with accumulation of undesired metabolites or at least

changes in their composition. The potential sources of these unintended effects are considered to be present in the transformation process and tissue culture conditions. Due to these concerns, the new genetically engineered varieties of crop plants have therefore triggered systematic research on assessment of unintended effects of key nutrients and metabolites (Filipecki and Malepszy, 2006). The need for such assessment gets further impetus, if genetic transformation of a particular crop plant tends to bring changes in the levels of key nutrients and anti-nutritional factors, which have relevance to human and animal health.

Several international organizations have formulated regulations for risk assessment studies of transgenic crop plants to monitor any unintended changes. Some of these include, the Organization for Economic Cooperation and Development (OECD), the Food and Agricultural Organization of the United Nations (FAO), the World Health Organization (WHO), and the US Food and Drug Administration (FDA) (Rogan et al., 2000). The evaluation tests are based on the strategy of substantial equivalence, developed by OECD and further elaborated

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by FAO/FDA. According to this concept, the new genetically engineered plants are compared for effects with wild counterparts, which have a known history of safe use (FAO, 2000).

Potato, one of the most important members of family Solanaceae, is a rich source of carbohydrates, vitamins, minerals and proteins. In many parts of the world, particularly developing countries, potato is consumed as a sole source of food. One of the major problems facing the developing world is the need to feed an increasing population, for which potato production must be increased by many folds.

Improvement of potato through conventional or gene manipulation techniques is often associated with unwanted or unintended changes that appear in the progenies. These changes are sometimes of paramount importance to take into consideration. For example any alteration in the glycoalkaloid levels, is of major concern to the scientific and food safety community. Glycoalkaloids are naturally occurring toxins, which can be found in many plants of the Solanaceae family including potato (Matthews et al., 2005). In recent years, following introduction of foreign genes in potato, food safety evaluation tests have been routinely conducted to test whether or not the transformation events have any unintended effects on the total glycoalkaloid levels.

Although no transgenic potato variety tested so far, has been found with glycoalkaloids exceeded the threshold safety levels (20 mg/100 g⁻¹ fresh weight), there are concerns that the transgene can interact with several variable environmental factors, and may bring a dramatic change in the glycoalkaloid levels. Also there is limited amount of scientific data available regarding the synergistic effect of individual glycoalkaloids, which may probably, has the potential to enhance the toxic effects of total glycoalkaloids by many folds. In this review, we summarize recent progress in understanding the sources of unintended effects with particular emphasis on changes in glycoalkaloids in both conventional and transgenic potato varieties and the possible implications of these changes on pathogen resistance and food safety.

UNINTENDED EFFECTS IN CROP PLANTS

Unintended effects represent a statistically significant difference in the phenotype, response, or composition of the modified plant compared with the parent from which it is derived (Cellini et al., 2004). Both conventional and modern gene transformation practices are confronted with undesired changes in the resultant modified plants. There are a number of examples in which the conventional methods brought undesired effects in the resultant plants. For example two potato varieties developed through conventional breeding were withdrawn from commercial release due to high levels of tubers glycoalkaloids (Zitnak and Johnson, 1970; Hellenas et al., 1995). A conventionally bred pest resistant celery variety was

found with high levels of psoralens that caused light sensitive rashes and burns in pickers (Ames and Gold, 1990). Similarly, a spring barley variety, Chariot, selected for high malting quality showed reduced quality in terms of high levels of grain splitting, an undesired character (R. Ellis, SCRI *pers comm.*; *comments on the UK recommended lists for cereals, 1999 to 2002*).

In contrast to conventional breeding methods, the advent of modern gene manipulation techniques has made it possible to engineer crop plants for individual genes of known functions. Although genetic engineering of crop plants for individual genes is considered to be a targeted approach, individual components of the transformation process provide basis for unintended effects in the progenies. There are a number of published examples of genetically modified plants with undesired traits. Some of these include altered levels of toxins and nutrients under certain environmental conditions, susceptibility to pathogens, altered insect resistance, altered interactions with soil microorganisms and plant reproductive characteristics (Latham et al., 2006).

Some transgenic plants were studied in details for unintended effects. Genetically modified commercial Bt maize varieties showed increased stem lignin content relative to their non-Bt isogenic parents (Saxena and Stotzky, 2001). A commercial herbicide tolerant soybean variety showed stem splitting and yield reduction (up to 40%) under high soil temperature (45°C), and a 20% higher lignin content at normal temperature (25°C). Similarly the commercial round up ready herbicide tolerant cotton variety showed increased boll drop after spraying (Pline et al., 2003). Unintended effects are not always undesired or unwanted; some may be useful for humans and animals. In Bt corn, a substantial reduction was observed in mycotoxins, which adversely affect human and animal health (Munkvold et al., 1999).

SOURCES OF UNINTENDED EFFECTS

Transformation associated mutations

In the literature, various factors of the transformation process have been described as potential sources of unintended effects. In transgenic plants, the transformation process, mediated either by agrobacterium or particle bombardment induce mutations in the host chromosomal DNA, leading to abnormal phenotypes. Mutations associated with transgene integration and other elements of the transfer (T-DNA) have been extensively studied in transgenic plants (Cellini et al., 2004; Filipecki and Malepszy, 2006; Yin et al., 2004). Studies on transgenic *Arabidopsis* and Aspen revealed deletions and rearrangement of the host chromosomal DNA at the transgene integration sites (Forsbach et al., 2003; Kumar and Fladung, 2002; Kaya et al., 2000; Filleur et al., 2001; Tax and Vernon, 2001). The inserted transgene varies in its activity and expression in the transgenic lines

depending upon its copy number (Pawlowski and Somers, 1996; Hobbs et al., 1990; Schubert et al., 2004).

Apart from mutations associated with the insertion effect and copy number of the transgene and associated mutations, the transformation process induces genome wide mutations, which provide a source of unintended changes in the resultant transgenic plants. Several DNA polymorphism analysis studies have provided clues of genome wide mutations in transgenic plants (Wang et al., 1996; Labra et al., 2001; Arencibia et al., 1999). Some authors have ascribed these genome wide mutations to tissue culture conditions, agrobacterium infection and the use of antibiotics (Larkin and Scowcroft, 1981; Somers and Makarevitch, 2004). The genome wide distribution of T-DNA, particularly in gene rich regions is considered to be a contributor to enhanced functional inactivation of the host genes.

Several studies revealed that agrobacterium T-DNA preferentially integrates in transcriptionally active regions of the host chromosomes (Herman et al., 1990; Azpiroz-Leehan and Feldmann, 1997; Koncz et al., 1992). In transgenic *Arabidopsis*, rice and barley, a higher T-DNA density was found in the gene rich regions (Alonso et al., 2003; Sha et al., 2004; Garrido et al., 2004). Recently, Kim et al. (2007) conducted genome wide analysis of T-DNA integration sites in *Arabidopsis* genome, generated under non-selective conditions. Unlike previous findings, this study described a high frequency of T-DNA insertions in the heterochromatic regions, including centromeres, telomeres and recombinant deoxyribonucleic acid (rDNA) repeats. The authors argue that such arrangement of T-DNA insertion regions are disfavored under selective conditions as the case in previous studies.

***In vitro* conditions/somaclonal variation**

Another potential source of unintended effects in transgenic plants is somaclonal variation that arises during tissue culture conditions. The molecular mechanism underlying somaclonal variation is not fully understood, however it is considered to be a major contributor to variable phenotypes in regenerated plants. It has long been considered a source of useful unintended effects *in vitro* cultured plants. Some of these characters include morphological traits, biotic and abiotic stress tolerance and production of secondary metabolites (Veilleux and Johnson, 1998). A number of molecular techniques have been used to detect sequence variation between source material and the resultant somaclones in various crop plants. These molecular techniques include random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and representational difference analysis (RDA) (Labra et al., 2000; Linacero et al., 2000; Oha et al., 2007). Somaclonal variation has been determined in a wide range of plant

species including cotton, banana, date palm, garlic, tomato, soybean, rice, asparagus, potato and oil palm (Jin et al., 2008; Bairu et al., 2006; Saker et al., 2000; Al-zahim et al., 1999; Soniya et al., 2001; Gesteira et al., 2002; Yang et al., 1999; Raimondi et al., 2001; Bordallo et al., 2004; Rival et al., 1998).

In the literature many factors have been mentioned to be responsible for somaclonal variation. The most prominent are genotype, type of explant, cultivation period and cultural conditions (Evans and Sharp, 1988). Desiccation, wounding, improper nutrient supply and osmotic stress are other factors, which are induced during *in vitro* culturing (Filipecki and Malepszy, 2006). Explants are subjected to these stress factors along with growth regulators and antibiotics. The combination of these stress factors brings various genetic and epigenetic changes, leading to unwanted characters in the resultant progenies. Genetic changes include ploidy changes, chromosome rearrangements, somatic recombination, gene addition/deletion, point mutations and insertions of transposons, while, epigenetic changes are comprised of DNA methylation and histone modifications.

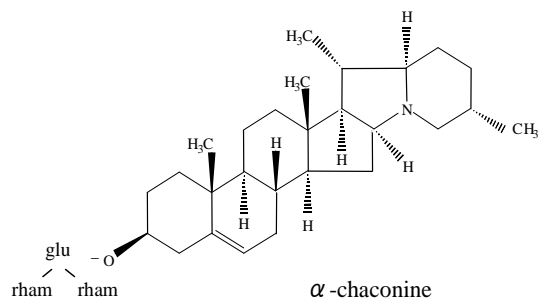
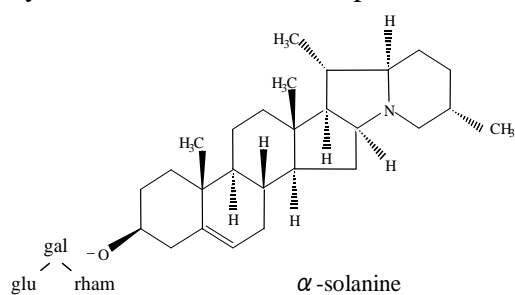
In the presence of hormones and antibiotics, individual cells carrying rDNA molecules are induced to regenerate into whole plants expressing the transgene. Because of the necessity of *in vitro* conditions for plant transformation, it is normally difficult to distinguish between somaclonal variation and variation due to transformation process. Recently Labra et al. (2004) used a floral dip technique in *Arabidopsis* plants to distinguish between somaclonal variation and variation due to transformation. Floral dip technique does not need any tissue culture regeneration step and through AFLP or RAMP, the genome wide differences can be easily compared with plants, which are passed through a tissue culture regeneration step.

POTATO GLYCOALKALOIDS

Members of the family *Solanaceae* synthesize a variety of secondary metabolites, including alkaloids. Potato, an important member of this family contains several types of alkaloids. The most important group of alkaloids in commercial potato varieties is the glycoalkaloids. These are sugar molecules (usually a trisaccharide) linked to the steroidal alkaloid solanidine (Matthews et al., 2005). The two major types are α -solanine and α -chaconine, which constitute 95% of the total potato glycoalkaloids. The other glycoalkaloids found in potato are β - and γ -solanines and chaconines, α - and β -solamarines and aglycons demissidine and 5- β -solanidan-3- α -ol, and leptines, commersonine, demissine and tomatine in wild potatoes (Lachman et al., 2001) (Figure 1). The total glycoalkaloid content of potato tubers varies widely and values between 2 and 410 mg/100 g of fresh weight (FW) have been found (Lisinska and Leszczynski, 1989).

In most cases, the glycoalkaloid content in whole tubers

Glycoalkaloids in cultivated potatoes



Glycoalkaloids in wild potatoes

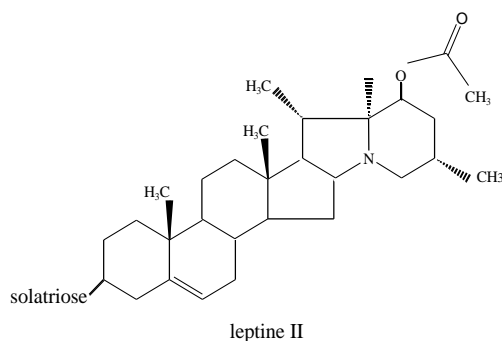
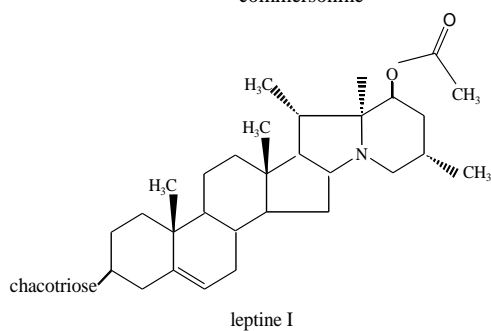
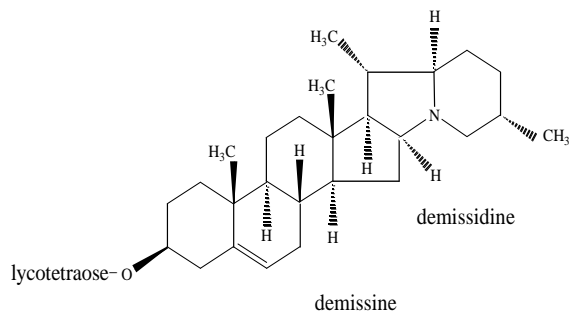
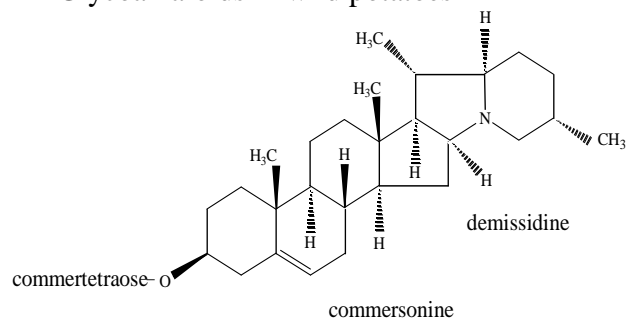


Figure 1. Glycoalkaloids in cultivated and wild potatoes. The two common forms of glycoalkaloids that is, solanine and chaconine are abundantly present in cultivated potatoes, while the other forms of glycoalkaloids are concentrated in wild potatoes.

ranges from 10 to 150 mg/ 100 g FW (Gelder et al., 1988). Unpredictable variations in the glycoalkaloid content can arise from differences in variety, locality, season, cultural practices, and stress factors. Glycoalkaloids are thought to have a role in plant defense against diseases and pathogen infestation. However, at higher levels, glycoalkaloids may have toxic effects on humans and animals. Glycoalkaloids affect the normal functioning of the nervous system by inhibiting the enzyme acetylcholinesterase, which regulates acetylcholine, a chemical responsible for conducting nerve impulses (Roddick et al., 2001).

Severe glycoalkaloid poisoning causes symptoms ranging from gastrointestinal disorders through confusion, hallucination, and partial paralysis to convulsions, coma, and death (Smith et al., 1996). These days, the widely accepted safety limit for the level of total glycoalkaloids in

tubers is 200 mg/kg of FW (Smith et al., 1996). Apart from their toxic effects, glycoalkaloids also have beneficial effects. These include lowering of cholesterol in hamsters, protection of mice against *Salmonella typhimurium* infection, prevention of human colon and liver cancer cells, enhancement of general anesthetics against cholinesterase and potentiation of a malaria vaccine (Friedman, 2004).

GLYCOALKALOID BIOSYNTHESIS IN POTATO

The glycoalkaloid biosynthetic pathway in potato is still not fully understood; however it is thought to be via the mevalonate/isoprenoid pathway (Krits et al., 2007). The key enzymes of this pathway, leading from Acetyl-Co-A to solanidine and then to solanine and chaconine are shown in Figure 2. Downward in the pathway, solanidine, the

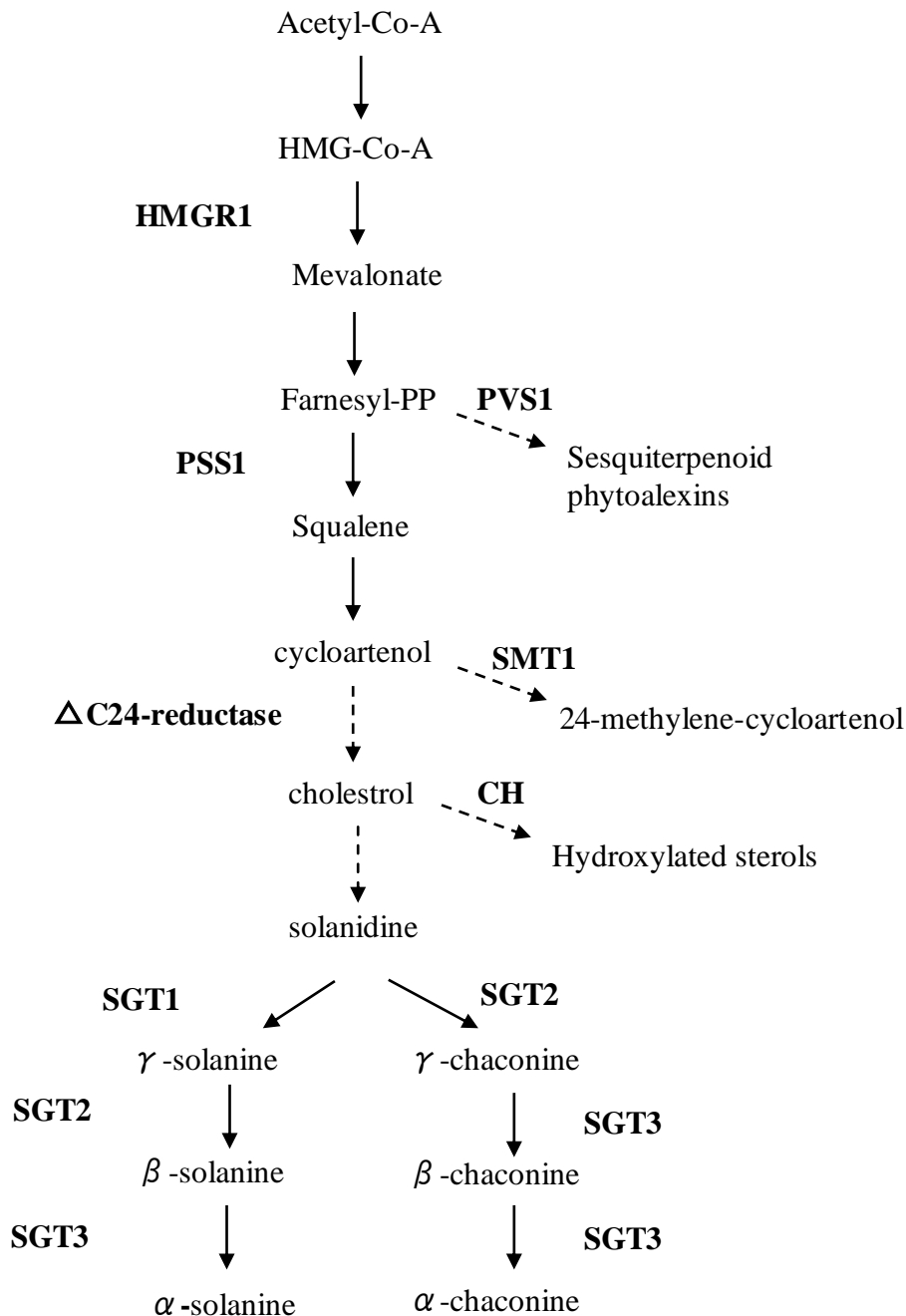


Figure 2. Glycoalkaloid biosynthetic pathway in potato. The glycoalkaloid biosynthetic pathway starts from Acetyl-Co-A. The enzymes catalyzing various known steps are HMGR1 (3-Hydroxy-3-methylglutaryl coenzyme A reductase), PVS1 (vetispiradiene sesquiterpene cyclase), PSS1 (Squalene synthase), SMT1 (Sterol C24-methyltransferase type1), CH (Cholesterol hydroxylase), SGT1 (Solanidine galactosyltransferase), SGT2 (Solanidine glucosyltransferase), SGT3 (Glycoesterol rhamnosyltransferase) (modified from Krits et al., 2007).

precursor of solanine and chaconine has been proposed to be synthesized from the key precursor in plant sterol synthesis, cycloartenol, in a biosynthetic route including cholesterol (Bergenstråhle et al., 1996; Friedman and McDonald, 1997; Mc Cue et al., 2007). However, the

enzymes catalyzing solanidine biosynthesis from cycloartenole have yet to be discovered. The possible link between glycoalkaloids and sterol biosynthesis has been the subject of research in the recent past. An important clue came out from the work on transgenic potato for

Soybean type 1 sterol methyltransferase (SMT1) (Arnqvist et al., 2003).

Over-expression of (SMT1) in potato plants led to an increased sterol levels and a reduced cholesterol as well as glycoalkaloid levels. This was presumably due to an increased channeling of cycloartenol into alkylated sterols, thus reducing the non alkylated form of sterol (cholesterol). The decrease in glycoalkaloid levels as a result of the reduction in cholesterol suggests the precursor role of cholesterol for glycoalkaloids biosynthesis (Arnqvist et al., 2003). Further studies in transgenic potato and *Arabidopsis* revealed that downregulation of both cholesterol and glycoalkaloids can be achieved, which supports the role of cholesterol as a metabolic precursor in glycoalkaloid biosynthetic pathway (Arnqvist, 2007). The precursor role of cholesterol in the glycoalkaloid metabolic pathway was further verified in a recent study by Mandimika et al. (2007a). The effect of α -chaconine on gene expression of the human colon carcinoma cell line Caco-2 intestinal epithelial cell line was observed.

The most important finding of this study was up-regulation of the expression of several genes involved in cholesterol biosynthesis. Cholesterol, an abundant component of the plasma in eukaryotic cells, plays an important role in maintaining membrane integrity and fluidity. Glycoalkaloids in toxic levels disrupt membrane integrity by formation of destabilizing complexes between the lipophilic moiety of glycoalkaloids and cholesterol present in the membranes (Keukens et al., 1995). The induction of cholesterol biosynthetic pathway in transgenic potato might be induced through feedback regulation due to depletion of cellular cholesterol by α -chaconine (Mandimika et al., 2007a). In addition, other genes working in the glycolakaloid pathway were studied. One of such genes is StDWF1 that encodes a sterol Δ 24-reductase. The role of this gene was studied in transgenic potato lines (Nurun, 2011). The author of this study reported that down regulation of StDWF1 in transgenic potato resulted in lowered levels of both cholesterol and glycoalkaloids, demonstrating an important role of StDWF1 in the glycoalkaloid biosynthetic pathway.

COMPOSITIONAL CHANGES IN POTATO GLYCOALKALOIDS

Potato has wide food versatility and a full complement of nutrients due to which it is consumed in many parts of the world (Woolfe, 1987). Because of the auto-tetraploid genome, low genetic variation and asexual mode of propagation, potato has been a target of genetic improvement. The strategies, which are currently being undertaken for genetic improvement of cultivated genotypes, include interspecific hybridization and genetic transformation (Esposito et al., 2002). In the former strategy, wild *Solanum* species are used as a source of

useful genes to broaden the gene pool of existing cultivars. The later strategy is based on isolation of individual genes from parental source and their manipulation into plant cells. So far both these strategies have been successfully used to transfer several useful traits in potato.

Some of these traits include disease resistance (Hoy, 1999), improved tuber quality (Edwards and Gatehouse, 1999), and allelic diversity (Carputo et al., 2000). With the transfer of useful traits, some unwanted or undesired effects, associated with tuber quality, yield and chemical composition of newly produced genotypes have also been observed. One of the unintended effects of potato genetic manipulation concerned with human health is the potential compositional changes in glycoalkaloids. Potato glycoalkaloids are considered to be undesirable for human consumption at concentration >200 mg/1000 g of total tuber weight (Friedman, 1997). Based on its toxic nature, any compositional change in potato glycoalkaloids, brought about either by traditional or transgenic approaches is seen as a potential health hazard.

CHANGES DUE TO CONVENTIONAL APPROACHES

In some cases, the new varieties developed by traditional plant breeding appeared to have higher levels of glycoalkaloids. Examples of potato varieties developed by traditional plant breeding that showed increased glycoalkaloid content include Lenape variety, a *Solanum tuberosum* \times *Solanum chacoense* cross (Sturckow and Low, 1961) for pest resistance. Due to high alkaloid content, this variety was not released for general planting (Zitnack and Johnson, 1970). Another conventionally bred potato variety (Magnum Bonum), popular in Sweden, was withdrawn from the market for similar reasons (Hellenas et al., 1995). In a traditional crossing of *Solanum brevidens* and *Solanum tuberosum*, the progeny was found to contain demissine, a toxic steroidal alkaloid. Apparently, a hydrogenase found in *S. brevidens* that produces tomatidine from teinamine, produced demissine from solanidine, a compound found in *S. tuberosum* but not in *S. brevidens* (Laurila et al., 1996).

CHANGES DUE TO TRANSGENIC APPROACHES

In case of genetic engineering of plant species, undesired effects arise due to transformation system employed and interactions between the transgene and the plant genome (Bregitzer et al., 1998). Several examples demonstrated the existence of unintended effects of genetic modification events on potato glycoalkaloid levels (Table 1). The expression of a nutritionally valuable protein (*Soybean glycinin*) in potato increased the content of glycoalkaloids (Hashimoto et al., 1999a, b). On the other side, expression of a yeast invertase gene in potato resulted in altered carbohydrate metabolism and as a result, a

Table 1. Unintended effects of potato transformation on glycoalkaloids.

Trait	Unintended effect	Potato variety	Reference
Expression of soybean glycinin	Glycoalkaloid content increased	-	Hashimoto et al. (1999a, b)
Expression of yeast invertase	Glycoalkaloid content decreased	-	Engel et al. (1998)
Expression of S-adenosyl-methionine decarboxylase	Glycoalkaloid content decreased	-	Pedros et al. (1999)
Insect and virus resistance	No significant changes	Russet burbank	Rogan et al. (2000)
Modified glycoprotein processing protein	Glycoalkaloid content decreased	-	Tylor et al. (2000)
Soybean type 1 sterol methyltransferase (GmSMT1)	Glycoalkaloid content decreased	-	Arnqvist et al. (2000)
Ech42 gene encoding for an endochitinase	No significant changes	-	Fabrizio et al. (2002)
Insect resistance	Leaf glycoalkaloid content decreased	Desiree	Birch et al. (2002)
Potato virus Y resistance	Glycoalkaloid content increased in peel	Desiree	Bianco et al. (2003)
ADP- ribosylation factor (ARF)	Glycoalkaloid content decreased	Desiree	Zuk et al. (2003)
Flavonoid biosynthesis	Glycoalkaloid content changed	-	Stobiecki et al. (2003)
Cry V	No significant changes	Spunta	El Sanhoty et al. (2004)
Sterol alkaloid glycosyltransferase (Sgt1).	Inhibition of solanine. TGA remained constant	Lenape/Desiree	McCue et al. (2005)
Inulin type fructane biosynthesis	No significant changes	Desiree	Catchpole et al. (2005)
Antisense invertase and maize ribosome-inactivating proteins (RIPs)	Significant changes in Glycoalkaloid content	Hermes	Matthews et al. (2005)
Blight resistance	Glycoalkaloid content increased	Desiree	Vaananen et al. (2005)
Modification in carbohydrate metabolism	No significant changes	Record/Desiree	Shepherd et al. (2006)
Resistance to potato virus Y (PVYn)	Glycoalkaloid content decreased	Irga	Sadowska et al. (2007)

-, Information is not available.

reduction in the glycoalkaloid content was observed (Engel et al., 1998).

According to Cellini et al. (2004), the lower glycoalkaloid content in the aforementioned example is associated with

differences in plant maturity between the transgenic lines and controls, which is a confirmation of the possible links between metabolic and developmental processes. Transgenic potato transformed for the gene encoding S-adenosyl-methionine decarboxylase showed increased vitamin C content as well as a significant reduction in the glycoalkaloid levels (Pedros et al., 1999). Similar reduction in the glycoalkaloid content was reported in transgenic potato for the gene encoding a modified glycoprotein processing protein (Taylor et al., 2000).

The transgenic potato lines for S-adenosyl-methionine decarboxylase and glycoprotein processing protein were further studied by Shepherd et al. (2006). They observed a significant decrease in the glycoalkaloid content in the transgenic lines including those with empty vector and tissue culture derived controls. As reduction in the glycoalkaloid content was also reported for tissue culture derived control lines, the authors are of the view that somaclonal variation might also contributed to these unintended changes and the mechanism governing tissue culture induced compositional changes in plant metabolites works independently of the process of transformation and gene insertion. Potato is generally considered to be highly prone to somaclonal variation; however the impact of somaclonal variation on compositional changes in secondary metabolites is not fully understood and needs extensive study. Some experiments revealed that both transformation process and tissue culture conditions may collectively bring unintended effects in terms of glycoalkaloid changes. Birch et al. (2002) investigated the effect of genetic transformation for pest resistance on foliar solanidine based potato glycoalkaloids. The lines were transformed for three insecticidal proteins that is, snow drop lectin, jackbean lectin, and cowpea trypsin inhibitor. The transgenic lines produced lower level of leaf glycoalkaloids relative to either tissue cultured controls or standard controls.

A possible explanation for this is that transformation and tissue culture both have some effects on one or more physiological processes. On the molecular level it is difficult to explain factors causing these unintended effects, which could be due to target gene insertion, marker gene insertion, chromosomal re-arrangements, altered gene expression and tissue culture conditions (Birch et al., 2002). There is a possibility that the target gene insertion may disrupt an endogenous gene responsible for a key nutrient or anti nutrient. However, this kind of disruption would be expected to lead to measurable changes that would be detected by nutritional analyses or through phenotypic and agronomic changes observed during clone selection (Rogan et al., 2000). In case of potato, the chances of this possibility are greatly reduced given that potato is a tetraploid with multiple copies of each gene (OECD, 1997). For disruption of a pathway, a transgene would have to be inserted precisely in all four copies of the same gene on different

chromosomes, which is extremely a matter of chance (Rogan et al., 2000).

As glycoalkaloids provide protection against phyto-pathogens including feeding insects, the foliar glycoalkaloids analysis in any potato transformation experiment is of crucial importance. Any inadvertent lowering of foliar glycoalkaloids in transgenic potato plants can cause an undesired increase in susceptibility to those pests which are sensitive to threshold concentrations of glycoalkaloids for insect deterrence or toxicity, potentially reducing the benefits of expressing anti-insect transgenes in potato (Birch et al., 2002). Transgenic potato lines, resistant to potato virus Y were evaluated for any compositional changes in the glycoalkaloid content (Bianco et al., 2003). Total glycoalkaloid content was nearly doubled in peel samples of resistant relative to control lines, and these levels were lower than the limit recommended for food safety, that is, 20 to 60 mg of TGA per 100 g fresh weight. It was established that tubers produced by virus-resistant clones were substantially equivalent in glycoalkaloid content to those produced by conventional potato varieties.

However, this research work raises a very important question about the possible interaction between leaf glycoalkaloids and the induced PVY resistance. It is still to be explored whether the changes occurred in the TGA levels in the PVY transgenic potato lines were either due to transformation process and tissue culture conditions or due to some correlation between the leaf glycoalkaloids and the PVY resistance. Similar studies were conducted by Sadowska et al. (2007). They developed a number of potato transgenic clones for PVY resistance and conducted compositional analysis of various nutrients including glycoalkaloids over several years of cultivation.

In this study, the glycoalkaloid content in green and normal tubers of both transgenic clones and controls was determined. In most of the transgenic clones, the glycoalkaloid content was reduced compared to controls. However, in transgenic clones, the glycoalkaloid content was higher in green tubers relative to normal tubers of either the transgenic or control lines. In transgenic clones, the glycoalkaloid content was found to be correlated with tuber size and maturity. Again it is premature to say, whether the observed changes in the glycoalkaloids have occurred due to the modified trait and/or tissue culture conditions.

ENVIRONMENTAL STRESS AND ITS IMPACT ON POTATO GLYCOALKALOIDS

Glycoalkaloid levels vary considerably between genotypes and among crops of the same cultivar (cv) produced under various growing conditions (Bintcliffe et al., 1982; Sinden et al., 1984). This finding is indicative of the fact that along genotype, environmental variation has a drastic effect on potato glycoalkaloids. The previous

literature reveals that both biotic and abiotic stresses tend to affect potato glycoalkaloid levels. It has long been known that exposure of tubers to light can cause a rapid increase in total glycoalkaloid concentration (Percival et al., 1993). Various stress factors during growth, harvest and handling (weather or inadequate storage conditions) can produce similar effects. Unusually cold and wet conditions during tuber development and growth have often been assumed as a cause of high glycoalkaloid levels (Sinden and Webb, 1974).

Hot and dry conditions during plant growth have also been suggested to be responsible for elevated glycoalkaloid concentrations (Levy et al., 1993). Papathanasiou et al. (1999) demonstrated the effect of cool temperature and combined stresses (water logging, warm temperature and drought stress) on glycoalkaloids of early maturing cultivars. They observed significant increases in glycoalkaloid levels as a result of the combined stresses. However, the cultivars behaved differently to these combined stresses in terms of glycoalkaloid increases. Recently Bejarano et al. (2000) investigated the effect of drought stress on glycoalkaloid levels. They used some drought tolerant varieties along with control (Desiree) and exposed them to drought stress.

The effect of drought stress on glycoalkaloids was analyzed. A sharp increase in glycoalkaloid content was observed in the control, while the tolerant varieties showed very less increase. These results suggested the widely recognized association between adverse environmental conditions and high glycoalkaloid concentrations in potato tubers. However, research work on the elucidation of molecular mechanisms that explain the nature of this association is rare. Another important factor that lacks proper research attention is the behavior of the transgene to environmental fluctuations and the resultant effect on glycoalkaloids. A couple of experiments have been conducted that try to explain the transgene-environment interaction and its effects on potato glycoalkaloids.

Transgenic potato lines with anti-sense invertase gene and maize ribosome-inactivating proteins (RIPs) were exposed to stress conditions (Blight and gangrene) in order to evaluate changes in glycoalkaloids between transgenic and non transgenic plants (Matthews et al., 2005). Significant differences were observed in the levels of glycoalkaloids between transgenic and control plants and between infected and non-infected material. The introduction of yeast anti-sense invertase gene previously showed a reduction in the levels of steroidal glycoalkaloids in a number of potato cultivars (Hey et al., 1995). Anti-invertase gene lowers the levels of reducing sugars, thus helps diminishing browning during cooking, while maize derived ribosome-inactivating protein (RIP) gives higher resistance to the potato cyst nematode.

The anti-sense invertase potato lines showed reduced glycoalkaloid content compared to controls, suggesting a

correlation between severity of the plant susceptibility to stress (Blight and gangrene) and the glycoalkaloid levels. However, the maize RIP lines showed no consistent trend of variation in glycoalkaloid content relative to controls. Matthews et al. (2005) further explained that genetic manipulation of carbohydrate metabolism and pathogen resistance often lead to changes in the profile of plant defense compounds present in the organs of potato plants including tubers. They assumed that the mechanism behind glycoalkaloid changes might include direct effects due to changes in the hexose pool and/or indirect effects due to changes in the susceptibility of the plants to infection and infestation.

IMPLICATIONS OF GLYCOALKALOID CHANGES ON PATHOGEN RESISTANCE

The analysis of glycoalkaloids in genetically modified crop plants holds immense importance. Genetic modification of potato for a particular trait may bring changes in the leaf glycoalkaloids and this in turn may alter susceptibility of the plant to known or possibly unknown potato pathogens.

Apart from their known toxic effects on humans and animals, one beneficial aspect of glycoalkaloids is that they play an important role in plant defenses against major potato pathogens. These include *Erwinia* soft rot, (Austin et al., 1988), *Fusarium* species (Percival et al., 1998) and some other fungi (Fewell and Roddick, 1997). Among insects, glycoalkaloids give protection against Colorado potato beetle, (Deahl et al., 1991), potato leafhopper, *Empoasca fabae*, (Sanford et al., 1992) and wireworm, larvae of *Agriotes obscurus* L., (Jonasson and Olsson, 1994). From previous research findings, it seems apparently that for known potato pathogens, there may be a threshold level of foliage glycoalkaloid, at which the pathogen can feed and reproduce.

Studies with artificial diets on two potato feeding aphid species, *M. euphorbiae* and *M. persicae* have demonstrated that glycoalkaloids at low to medium levels (10 to 40 mg/100 ml in artificial diets) can stimulate feeding and reproduction (Birch et al., 2002). Increase in the glycoalkaloid levels can have toxic effects on the pathogen, but this assumption has never been verified. On the other side, it is assumed that reduction in the foliar glycoalkaloids may increase susceptibility of the transgenic potato plants to infectious pathogens. Based on this assumption, it is possible that the glycoalkaloid content may have some correlation with the degree of biotic stress, and with increasing susceptibility to stress, glycoalkaloids may tend to increase and vice versa.

Regarding potato fungal pathogens, it is assumed that glycoalkaloids may be involved in the high level of field resistance of the foliage to late blight. However this assumption has never been verified in the improved varieties for pathogen resistance developed through conventional plant breeding. One such study conducted

on the genetic improvement of potato for late blight and soft rot resistance in six potato progenies, showed no positive correlation of the incorporated trait with that of variable glycoalkaloid levels (Didier et al., 2003). The authors used six partial progenies from crosses between *Solanum tuberosum* and accessions of *Solanum andigena*, *Solanum berthaultii*, *Solanum phureja*, and *Solanum vernei* to investigate the possible correlation between resistance to *Phytophthora infestans* and/or to *Erwinia carotovora* subsp. *atroseptica* and the concentration of glycoalkaloids in tubers. They concluded that neither race-specific nor partial resistance to late blight and soft rot in the accessions used as progenitors of resistance depended on high α -solanine or α -chaconine concentrations.

According to Fewell and Roddick (1993, 1997), one of the possible reasons for this lack of correlation is that glycoalkaloids are usually less toxic to potato pathogens than to potato non-pathogens. In some cases, this lower toxicity is related to the ability of the fungus to degrade glycoalkaloids (Didier et al., 2003), which is not the case for all potato pathogens. Furthermore, the effect of elevated levels of glycoalkaloids on non potato pathogens needs further investigation. In this context, the potential interaction between glycoalkaloids and non potato pathogens in the soil atmosphere surrounding the plant is important. Based on these facts, it seems that any increase in potato foliage glycoalkaloids, brought about either by conventional breeding or gene transformation have no or negligible effects on plant responses to pathogen infection. While on the other side, lowering of foliage glycoalkaloids may have the potential to increase susceptibility of the plant to a particular pathogen, but this needs further research.

IMPLICATIONS OF GLYCOALKALOID CHANGES ON FOOD SAFETY

On the food safety side, changes in the glycoalkaloid content, whether small or large are of crucial importance for humans and animals health. The current food safety guidelines recommend the limits of glycoalkaloids for new potato varieties to be 20 mg/100 g fresh weight of tubers in order to minimize over consumption of high glycoalkaloid content. Although no variety developed through genetic engineering so far, has been reported to contain glycoalkaloids exceeded the recommended limits, there are concerns that several known and unknown factors may increase glycoalkaloids above the safe limits. These factors include effects of the transgene insertion coupled with somaclonal variation and transgene interaction with environmental fluctuations.

We have already discussed somehow the compositional changes in glycoalkaloids as a result of the above mentioned factors. The compositional analysis of glycoalkaloids in potato varieties developed through gene

transformation (Table 1) reveals that the glycoalkaloid changes are within safe limits and pose no real threat to humans and animals. However, there are certain factors which may influence the individual or combined effects of α -solanine and α -chaconine. For example, more recently discovered synergism between α -solanine and α -chaconine can induce both beneficial and toxic effects. This kind of synergism between α -solanine and α -chaconine and its resultant toxic effects have been discussed by several authors (Friedman et al., 2005; Smith et al., 2001; Rayburn et al., 1995).

The most important research on the synergistic effect of α -solanine and α -chaconine was conducted by Smith et al. (2001). They demonstrated the individual and combined effect of α -solanine and α -chaconine on feeding activity of snail (*Helix aspersa* L.). They observed more antifeeding effect of α -solanine and α -chaconine in combination rather than their individual application. In more general terms, the occurrence of such synergism may have even more toxic effects on humans and animals than generally perceived. According to Friedman (2006), due to this synergism, it is difficult to predict the toxicity of a mixture of two glycoalkaloids using results of individual compounds or of mixtures of differing ratios present in different potato varieties. The author further adds that mixtures of glycoalkaloids can vary in their toxic effects depending upon their ratios of α -solanine and α -chaconine. This was further confirmed in a recent study that was conducted to determine the individual and combined effects of α -solanine and α -chaconine on gene expression in intestinal epithelial cells (Caco-2 cells) (Mandimika et al., 2007b).

The Caco-2 cells were exposed to either pure α -chaconine or α -solanine or glycoalkaloid mixtures of varying α -chaconine/ α -solanine ratios for 6 h. Following these applications, changes were found in the expression of genes, catalyzing key pathways of cholesterol biosynthesis, growth signaling, lipid and amino acid metabolism, cell cycle, and cell death/apoptosis. Microarray analysis revealed variable expression of these genes depending upon the solanine/chaconine ratios. In mixtures of glycoalkaloids, the effect of α -chaconine is more toxic. Its toxicological potency has been evaluated to be about 10-fold higher than that of α -solanine, which means that α -chaconine may have more effect in cases of potato poisoning. This has led to an even more careful analysis of the total potato glycoalkaloids with accurate α -solanine and α -chaconine ratio that can induce toxicity irrespective of total glycoalkaloids remain below the recommended levels. It may therefore be better to use potato varieties with a low α -chaconine/ α -solanine ratio to enhance food safety.

CONCLUSION

Genetic modification of potato and associated unintended

effects on glycoalkaloids has been investigated in a number of studies. In some modified varieties, though significant increases were observed, the glycoalkaloid content remained below the recommended food safety limits. If we compare the unintended changes in glycoalkaloids of genetically modified potato varieties to those of conventionally bred varieties, it seems clear that conventional approaches pose more serious threat in terms of unexpected changes in glycoalkaloids. In the light of previous findings, it is evident that some potato varieties developed through conventional breeding were found to contain very high levels of glycoalkaloid content. This fact is well established now that conventionally bred varieties accumulate more unwanted characteristics, which are then eliminated through backcrossing but unintended effects on key toxins or allergens are not routinely evaluated at the molecular levels. In case of transgenic varieties, food safety assessment tests are routinely conducted for assessing changes in potential toxins and allergens. That is one reason, why even small changes in these metabolites in the transgenic varieties create doubts on the transformation process employed.

According to published data, it is evident that genetic transformation of potato may pose fewer risks in terms of unintended effects on glycoalkaloids compared to conventional methods. However, there are several questions, which must be answered in order to fully understand the toxic nature of glycoalkaloids. For example the full elucidation of the mechanism of synergism between α -solanine and α -chaconine and interaction between the transgene and environmental fluctuations that may bring dramatic changes in glycoalkaloid levels. As glycoalkaloids are highly amenable to any change in the genetic architecture of the plant, it is possible that during biotic or abiotic stress condition, the new genetically modified varieties accumulate variable amounts of glycoalkaloids. However, there has been limited research on the effect of environmental stress conditions on glycoalkaloids of genetically modified varieties, which needs to be expanded. Based on these facts, it is premature to say on concrete basis that transgenic application may have no unintended effects on potato glycoalkaloids. Therefore, glycoalkaloid analysis in transgenically modified potato varieties should be continued to ensure that the glycoalkaloid content remains below the safety limits and pose no real threat to humans and animals.

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