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Steroidal glycoalkaloid content of potato, tomato and their somatic hybrids

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Summary. Analyses of leaves and 'tubers' from somatic hybrids of potato and tomato ('pomato' with plastids of potato, 'topato' with plastids of tomato) produced by fusion of protoplasts from liquid cultures of dihaploid potato and mesophyll of tomato revealed the presence of the two major potato glycoalkaloids (α-solanine and α-chaconine) as well as the tomato glycoalkaloid (α-tomatine). The total alkaloid content of leaves was greater than that of 'tubers' and similar to levels in the foliage of parent plants. However, glycoalkaloids were more abundant in hybrid 'tubers' than in normal potato tubers by a factor of 5–15. In hybrid foliage, approximately 98% of the alkaloid present was of potato origin whereas in 'tubers' the reverse was the case, with tomatine comprising 60–70% of the total alkaloid. The similarities in alkaloid content and ratios between the pomato and the topato lines indicate that plastomes do not influence the biosynthesis and distribution of these alkaloids. The results indicate that major secondary metabolites may prove useful for assessing the hybrid nature of such plants.

Key words: *Solanum tuberosum* – *Lycopersicon esculentum* – Protoplast fusion – Pomato – Topato – Alkaloids

Introduction

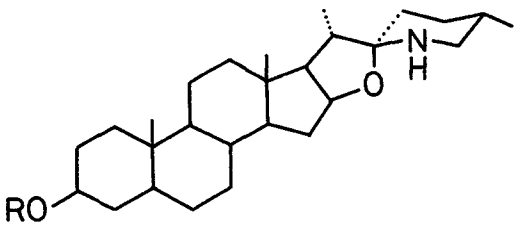
Since the development of reproducible in vitro methods for fusing higher plant protoplasts about a decade ago, significant advances have been made in the somatic hybridisation of plants through regeneration from cultured cells (Melchers 1982). Not only do such methods overcome the problems and limitation of sexual reproduction, but they are now considered to be sufficiently developed to be of use in plant breeding (Melchers 1980a).

In early work, somatic hybrids could only be produced from species (usually of the same genus) which were capable of producing hybrids sexually (Melchers and Labib 1974; Power et al. 1976; Smith et al. 1976; Dudits et al 1977). More recently, success has been achieved not only with sexually incompatible intrageneric species, e.g. *Datura innoxia* and *D. discolor* or *D. stramonium* (Schieder 1978), *Petunia parodii* and *P. parviflora* (Power et al. 1980), but also with plants considered taxonomically more distant e.g. *Solanum tuberosum* and *Lycopersicon esculentum* (Melchers 1978; Melchers et al. 1978) and *Arabidopsis thaliana* and *Brassica campestris* (Gleba and Hoffmann 1979). Studies on the somatic hybridization of potato and tomato have so far produced at least nine morphologically distinct hybrid lines, some with plastids of potatoes (pomatoes), some with plastids of tomatoes (topatoes). These plants do not grow as quickly as parent plants (some grow only on organic media or as scions on stocks of tomato) and often show morphological abnormalities such as abnormal, infertile flowers and small parthenocarpic fruits. Instead of round tubers the hybrids produce elongated 'tubers' resembling thickened stolons (Melchers 1980b). The appearance of plants and tubers is shown in Melchers et al. 1978; Poulsen et al. 1980; Schiller et al. 1982; Melchers 1983. Karyological data have not proved particularly reliable in establishing the hybrid nature of regenerated plants as the shape of the chromosomes too similar in the fusion partners (Melchers 1980b), but hybrid identification has been achieved by a variety of other methods. For example, Melchers and Labib (1974) utilised complementation of two non-allelic genes for chlorophyll deficiency in normal chlorophyll production. Power et al (1976) employed differential growth habit and drug resistance. Isoenzyme patterns have been studied in soybean/*Nicotiana* hybrids by Wetter (1977) and analyses of ribulose bisphosphate carboxylase by electrofocussing conducted on potato/tomato hybrids by Melchers et al. (1978) and Poulsen et al. (1980). It was found that the large subunit of RuBP carboxylase (which is coded for by chloroplast DNA) was of either potato or tomato origin. Restriction endonuclease analyses of plastid DNA (Schiller et al. 1982) has subsequently confirmed the results of RuBP carboxylase analyses. Two other approaches used successfully in potato/tomato hybrid analyses are chilling resist-

ance, as measured by photosynthetic electron transfer activity (Smillie et al. 1979) and production of volatile metabolites by callus tissue (Ninnemann and Jüttner 1981).

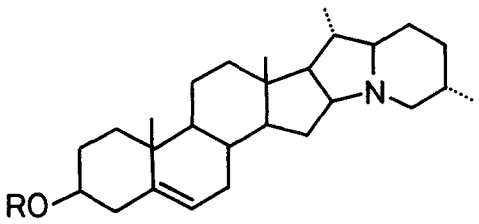
The use of intermediates or end products of primary metabolism for determining hybridization is not without its problems. At this level of organisation, organisms from widely differing taxonomic groups tend to be remarkably similar and consistent differences may be found only at the macromolecular (DNA/protein) level which necessitates highly sophisticated analytical techniques. Similarly, physiological activity, although arising from a particular genotype, is not only an expression mainly of primary metabolism but often plastic in nature and modifiable according to the particular environment. In contrast, higher plants show immense activity and diversity in the area of secondary metabolism (Bell and Charlwood 1980), the products of which tend to be less prone to environmental modification. Also, being more restricted in their taxonomic distribution, secondary metabolites may be more useful indicators of the biochemical uniqueness or individuality of a species, genus, family, etc. Thus, in seeking biochemical markers of genome hybridisation, perhaps more consideration should be given to utilising major secondary metabolites, the identification and assay of which may also technically less demanding than those required for primary macromolecules.

Based on this assumption, we have analysed potato/tomato somatic hybrids for steroidal glycoalkaloids,



α-Tomatine, R = xyl - 2 glu - gal -

Tomatidine, R = H



α-Solanine, R = rham - glu - gal -

α-Chaconine, R = 2 rham - glu -

Solanidine, R = H

Fig. 1. Structures of steroidal alkaloids of tomato and potato

compounds which are produced by most plants belonging to the genera *Solanum* and *Lycopersicon*. A large number of different compounds exist which are not consistently species specific, but in the case of potato and tomato, the alkaloid complement is different (Schreiber 1968; Ripperger and Schreiber 1981). The tomato plant produces a single glycoalkaloid, α-tomatine (Fig. 1) which is a tetraoside of a spirosolane-type aglycone tomatidine whereas in potato, two major glycoalkaloids are present, α-solanine and α-chaconine (Fig. 1), both of which are triosides of a solanidane-type aglycone solanidine differing only in the structure of their sugar moiety. Although similar chemically, the tomato and potato alkaloids are sufficiently distinct to be separated relatively easily by chromatographic techniques. All three alkaloids are also similar with respect to their distribution within the plant, being present in all organs (with the possible exception of mature fruits from which they may disappear) but predominant in aerial parts, particularly leaves and flowers (Roddick 1974; Jadhav et al. 1981).

Material and methods

Plant material

Potato protoplasts were obtained from suspension cultures of *Solanum tuberosum* L. prepared from the dihaploid stock HH 258. (2n = 24). Tomato mesophyll protoplasts were derived from light green leaves of *Lycopersicon esculentum* var 'cerasiforme' (Dunal) Alef. mutant yellow-green 6 (Rick) (2n = 24). Procedures for the preparation and fusion of protoplasts and for regeneration from hybrid callus tissue have been described earlier by Melchers (1978) and Melchers et al. (1978).

The hybrid tissues analysed here were referred to as 2a/2a/36d, S_2, S_4 and S_5 and 2a/1y/7c, S_{13}, S_{14} and S_{26}. The first notation '2a' refers to the petri dish containing (several) hybrid calli whilst 2a/36d and 1y/7c refer to a particular callus after further transfers. 'S'-notations refer to individual shoots regenerated from the same callus. In this study, plants from shoots 2a/2a/36d S_2, S_4 and S_5 were morphologically similar but harvested separately whereas 2a/1y/7c S_{13}, S_{14} and S_{26} were virtually identical and were bulked for analyses. Thickened stolons or elongated tubers produced by these hybrids were also analysed. The tuber tissue S_2 and S_5 from 2a/2a/36d were combined whereas S_{15}, S_{25} and S_{26} from 2a/1y/7c were individually analysed. For comparison, leaf tissue from intact plants of potato (HH 258 dihaploid) and tomato (subspecies *cerasiforme* mutant yellow-green) cultivated under the same greenhouse conditions, and tubers from the intact potato plants were also analysed for alkaloids.

Extraction and purification of alkaloids

Leaf tissue was freeze-dried prior to extraction and ground to a coarse powder in a mortar and pestle. Tubers were extracted fresh and were homogenised in a blender. In each case, the initial extractant was 94% (v/v) methanol containing 2% (v/v) acetic acid, using 10–15 ml/g of tissue. After extracting overnight, the slurry was Buchner filtered and re-extracted as before but with 64% (v/v) methanol. Extracts were combined and

reduced to dryness by rotary evaporation at 45 °C. The residue was taken up in three 10 ml washings of 2% (v/v) acetic acid. In the case of leaf tissue, the combined extract was partitioned three times against equal volumes of diethyl ether to remove pigments. Any traces of ether were removed in an air stream. The pH of the aqueous acidified extract was adjusted to 10.0 using concentrated ammonia and the extract placed in a water-bath at 80 °C for 30 min and then in a fridge overnight at 4 °C. The extract was then centrifuged at 27,000 *g* for 30 min at 4 °C, the supernatant discarded and the pellet washed with 1% (v/v) ammonia and re-centrifuged as before. After drying in a $CaCl_2$ desiccator, the precipitate was dissolved in three 50 ml volumes of methanol at 45 °C. These were filtered, bulked, evaporated to dryness under vacuum at 35 °C and flask contents taken up in a small volume of methanol and made to 10 ml. A 1 ml aliquot of this extract was applied as a band to a TLC plate (silica gel G, 0.5 mm) which was developed in 95% (v/v) methanol. Alkaloid bands were visualised using modified Dragendorff's reagent and located by reference to authentic compounds (Sigma Chemical Company, Poole, England) and then eluted from the gel with ethanol. The purified tomatine was dissolved in 5 ml of 96% (v/v) ethanol whereas the combined solanine and chaconine extract was re-chromatographed on silica gel in 95% (v/v) ethanol. The separated alkaloids were eluted as before, the ethanol evaporated off under vacuum at 35 °C and the alkaloids dissolved in 5 ml of 7% (w/v) phosphoric acid.

Assay of alkaloids

Tomatine was assayed either by a radioligand method (Heftmann and Schwimmer 1973) involving binding of the alkaloid to ^{14}C-labelled cholesterol, precipitation of the complex and measurement of the radioactivity remaining in solution or, where quantities were smaller, by a spectrophotometric method (Roddick and Butcher 1972) based on chromogen formation with conc. sulphuric acid and measurement of absorbance at 325 nm. Solanine and chaconine were assayed by a spectrophotometric method based on that of Bergers (1980). This involved reacting 0.4 ml of alkaloid solution in 7% (w/v) phosphoric acid with 4 ml of 70% (w/v) sulphuric acid and measuring absorbance at 405 nm after 10 min. Calibration graphs were prepared for all alkaloids using authentic standards.

Results

Analyses of hybrid leaf tissue by TLC with 95% methanol as solvent revealed two major Dragendorff-positive spots with Rf's of 0.28 and 0.60 which corresponded to α-solanine and/or α-chaconine (no separation in this solvent) and α-tomatine respectively. In contrast, parental tissue gave major spots of Rf either 0.28 or 0.60 (Fig. 2). In this solvent, some minor, non-parental Dragendorff-positive spots were observed but not identified. Further TLC using 95% ethanol as solvent resolved the lower spot into two components of Rf 0.20 and 0.31, which corresponded to solanine and chaconine, respectively (Fig. 2). Separation of these three 'alkaloids' and co-chromatography with suspected authentic alkaloids gave single spots in each case. The chromogen characteristics of the extracted compounds, produced by spraying TLC plates with 50% (v/v) sulphuric acid and heating to 120 °C, were also consistent with those of authentic alkaloids. Aliquots of 'alkaloids' were hydrolysed by refluxing in 20 ml of 1.0 N hydrochloric acid for 6 h after which flask contents were evaporated to dryness under vacuum at 45 °C and then taken up first into diethyl ether and then into distilled water. TLC of ether extracts on silica gel with a chloroform : methanol (94 : 6) solvent revealed Dragendorff-positive compounds with Rf's corresponding to tomatidine (0.48) and solanidine (0.18). TLC of the aqueous extract of the hydrolysate on kieselguhr using methyl acetate : isopropanol : water (90 : 5 : 5) as solvent and ceric ammonium sulphate as locating reagent gave spots corresponding to xylose (Rf 0.82), glucose (Rf 0.62) and galactose (Rf 0.50) from 'tomatine' and to glucose, galactose and rhamnose (Rf 0.91) from 'solanine' and 'chaconine'.

The identities of extracted alkaloids were confirmed by fast atom bombardment mass spectrometry (FAB-MS) using a Kratos MS9/50 TC mass spectrometer set at 1,500 resolution. Samples were added to the copper probe tip in methanol and evaporated down prior to the addition of 2 µl glycerol and 1 µl 1.0 M acetic acid. The probe tip was bombarded with a fast atom beam of xenon produced by an Ion Teck 11 NF atom gun operated at 9 kV (nominal).

Results of quantitative alkaloid analyses of hybrid and parental leaf tissues are shown in Table 1. The hybrids 2a/1y/7c (pomato) and 2a/2a/36d (topato) differed in the total concentration of alkaloid present. In the former, levels resembled that in the tomato parent whereas the latter was more akin to the potato parent. This difference was not due to the tomatine content, which was virtually identical in all the hybrid leaf tissue examined, but to the lower solanine and chaconine levels (approximately half) in 2a/1y/7c. On the whole, hybrid solanine and chaconine levels were of the same order as those in the parent potato tissue but hybrid

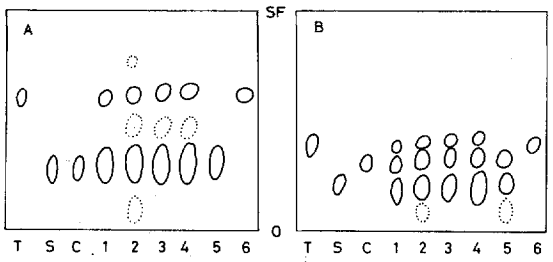


Fig. 2. Thin-layer chromatograms in 95% methanol (**A**) and 95% ethanol (**B**) of alkaloid extracts of leaves of hybrid and parent potato and tomato plants. T = tomatine; S = solanine; C = chaconine; 1 = 2a/1y/7c ($S_{13} + S_{14} + S_{26}$); 2 = 2a/2a/36d (S_2); 3 = 2a/2a/36d (S_4); 4 = 2a/2a/36d (S_5); 5 = potato; 6 = tomato; O = origin; SF = solvent front. Compounds were visualised with modified Dragendorff's reagent

Table 1. Glycoalkaloid content of leaf tissues of potato/tomato hybrids and of parent plants

Material	Alkaloid concentration (mg/g dry wt) ± SD				Chaconine	% of total alkaloid		
	Tomatine	Solanine	Chaconine	Total	Solanine ratio	Tomatine	Solanine	Chaconine
Tomato	7.55±2.06	–	–	7.55	–	100	–	–
Potato	–	2.04±1.20	9.50±3.02	11.54	4.66	0	17.7	82.3
2a/1y/7c ($S_{13}+S_{14}+S_{26}$)	0.21±0.06	0.72±0.25	5.39±1.93	6.32	7.49	3.3	11.4	85.3
2a/2a/36d (S_2)	0.21±0.07	1.50±0.40	11.46±0.40	13.17	7.64	1.6	11.4	87.0
2a/2a/36d (S_4)	0.16±0.03	1.97±0.80	12.59±2.22	14.72	6.39	1.1	13.4	85.5
2a/2a/36d (S_5)	0.23±0.06	1.70±0.40	10.93±3.39	12.86	6.43	1.8	13.2	85.0

Data are means of at least three and up to five replicate extractions

Table 2. Glycoalkaloid content of 'tubers' of potato/tomato hybrids and of the potato parent

Material	Alkaloid concentration (mg/g dry wt)				Chaconine	% of total alkaloid		
	Tomatine	Solanine	Chaconine	Total	Solanine ratio	Tomatine	Solanine	Chaconine
Potato	–	0.06	0.06	0.12	1.00	–	50.0	50.0
2a/1y/7c (S_{15})[a]	0.40	0.08	0.18	0.66	2.25	60.6	12.1	27.3
2a/1y/7c (S_{25})[a]	0.79	0.22	0.12	1.13	0.55	69.9	19.5	10.6
2a/1y/7c (S_{26})[a]	1.40	0.34	0.23	1.97	0.68	71.1	17.3	11.6
2a/2a/36d (S_2+S_5)	0.45	0.09	0.13	0.67	1.44	67.2	13.4	19.4

[a] Tissues showed some fungal infection and greening

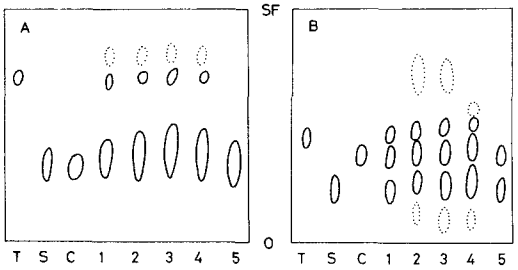


Fig. 3. Thin-layer chromatograms in 95% methanol (**A**) and 95% ethanol (**B**) of alkaloid extracts of tubers of potato/tomato hybrids and of normal potato plants. T=tomatine; S=solanine; C=chaconine; 1=2a/1y/7c (S_{15}); 2=2a/1y/7c (S_{25}); 3=2a/1y/7c (S_{26}); 4=2a/2a/36d (S_2+S_5); 5=potato; O=origin; SF=solvent front. Compounds were visualised with modified Dragendorff's reagent

tomatine was only about 2.5% of that in the parent tomato leaf. However, despite differences between the two hybrids in the absolute amount of alkaloid, relative levels as indicated by the chaconine/solanine ratio and the % composition were remarkably similar. No significant differences in either absolute or relative amount of alkaloid were apparent in the different lines (S_2, S_4 and S_5) from the hybrid 2a/2a/36d.

Although tubers were extracted fresh, some of the material became slightly infected/dehydrated in transit and results were therefore calculated on a dry weight basis. This also makes them directly more comparable with leaf data (as shown in Table 1). Qualitative TLC analyses similar to that done on leaves showed that all the hybrid tubers elaborated alkaloids of both potato and tomato origin with some showing additional minor Dragendorff-positive compounds of unknown identity (Fig. 3). Consistent with the situation that normally obtains in the intact plant, total alkaloid levels in hybrid tubers were much lower than in hybrid leaf tissue but higher than in parental potato tubers by a factor of between 5–15 (Table 2). In all the hybrid tubers analysed, tomatine was the major alkaloid present comprising 60–70% of the total. The percent solanine was very much as in leaf tissue but the percent chaconine was much lower. The relative amounts of tomatine and chaconine were almost reversed in tubers compared with in leaves. It was not possible to draw any conclusions regarding differences in tuber alkaloid either between different hybrids or between different lines of the same hybrid.

Discussion

The hybrid nature of plants regenerated from calli 2a/2a/36d and 2a/1y/7c is also expressed in their steroidal

glycoalkaloid content, alkaloids of both potato and tomato parents being present in their aerial and subterranean organs. This finding is in keeping with earlier work which established hybridity by RuBP carboxylase electrofocusing analyses (Melchers et al. 1978), peptide mapping (Poulsen et al. 1980), plastid DNA analyses (Schiller et al. 1982), and also with conventional breeding studies in which F_1 hybrids of *Solanum* species (which elaborated different glycoalkaloids) contained the glycoalkaloids of both parents (Prokoshev et al. 1952; McCollum and Sinden 1979). The present study demonstrates that certain secondary metabolites may also prove useful as biochemical markers in such studies.

Although the overall distribution of alkaloids in hybrid plants was essentially as in normal plants (more alkaloid in aerial than subterranean organs), different parental contributions were apparent in the alkaloid complement of leaves and tubers. For example, in both hybrids leaf alkaloids were predominantly of the potato type whereas in tubers the tomato alkaloid was more abundant. It is well known that in potato, high alkaloid shoots develop from low alkaloid tubers. In our hybrid plants this pattern persists for the potato alkaloids but is reversed with respect to the tomato alkaloid. Since transport of potato and tomato glycoalkaloids between shoot, root and tubers apparently does not occur (Roddick 1982), the observed alkaloid patterns must result from different biosynthetic/accumulation capacities of these organs possibly reflecting different mechanisms controlling expression of genes for glycoalkaloids.

The intracellular site of synthesis of potato and tomato alkaloids is not certain but circumstantial evidence points to the microbodies rather than to plastids or mitochondria (Roddick 1976, 1977). It seems likely, therefore, that synthesis of these compounds is coded for by nuclear genes. The small subunit of RuBP carboxylase is encoded in nuclear genes and Poulsen et al. (1980) found peptides of both potato and tomato origin in this subunit in hybrid plants. In contrast, the large subunit which is coded for by chloroplast DNA, was derived exclusively from potato in 2a/1y/7c and from tomato in 2a/2a/36d. Because of the absence of close similarities in glycoalkaloid content between pomatoes and potatoes and between topatoes and tomatoes, any influence of the plastome on the biosynthesis and distribution of these compounds is definitely excluded.

Ninnemann und Jüttner (1981) reported that genomes which coded for volatile metabolites specific to potato or to tomato were both expressed not only in hybrid plants but also in hybrid callus tissue. Of interest also is the observation (Ninnemann and Jüttner 1981) that the number and amount of volatile secondary compounds were significantly greater in hybrid callus than in parental tissue. A quantitative enhancement in glycoalkaloid level (largely arising from the additional accumulation of tomatine) was particularly noticeable in hybrid tuber tissue which contained at least five (and up to 15) times as much alkaloid as tubers from parent potato plants. Two of the hybrid lines (2a/1y/7c, S_{25} and S_{26}) had tuber alkaloid level in excess of 20 mg/100 g fresh weight which is considered the upper acceptable level for domestic consumption. However, these data (and also those from 2a/1y/7c, S_{15} tubers) should be viewed with some caution as this material showed signs of fungal infection and greening from light exposure. Both these factors are known to cause elevation of solanine and chaconine levels in potato tubers (Locci and Kuć 1967; Jadhav and Salunkhe 1975) but whether they did so in hybrid tubers, and whether they also influenced the concentration of tomatine, remains unknown. It may be significant that tubers of the hybrid 2a/2a/36d were not infected or green and yielded data which were broadly comparable with those from 2a/1y/7c tubers. On the other hand, glycoalkaloid levels in leaves of hybrids were not markedly higher compared with parent plants but were, in fact, equivalent to levels in one or other of the parents. These results could have implications in plant breeding as conventional programmes normally aim to reduce (or maintain low) tuber glycoalkaloids because of their toxicity and effect on taste, but to increase foliage glycoalkaloids because of their possible antifungal and insect-antifeedant properties (Roddick 1985). Thus, if tuber-bearing somatic hybrids of potato and tomato were ultimately to be of commercial value, it would be desirable to reverse the pattern of alkaloid changes seen here in leaves and tubers.

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