Ann. appl. Biol (1989), **114**, 579-586
Printed in Great Britain

The lack of enzymic browning in the wild potato species *Solanum hjertingii* compared with commercial *Solanum tuberosum* varieties

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(Accepted 10 February 1989)

Summary

Enzymic browning of tuber tissue was evaluated quantitatively for 50 genotypes, representing seven accessions, of *Solanum hjertingii*, a wild potato species from north-east Mexico, together with five commercial varieties of *Solanum tuberosum* with a known range of enzymic browning.

Ninety-four percent of *S. hjertingii* clones examined showed less browning than the commercial varieties, with 66% exhibiting half the 'potential browning' (a measure of the total enzymic browning resulting from disruption of cellular compartmentalisation in that system) of cv. Maris Piper, a low enzymic browning cultivar. Of these clones, 18 showed no visual discoloration when sliced. Results clearly indicate that the lack of enzymic browning in *S. hjertingii* is a 'true' character.

The efficiency of one subjective and two objective methods for obtaining different measures of enzymic browning was assessed. Potential browning of a rehydrated freeze-dried powder was adopted as the most efficient technique for screening enzymic browning.

Introduction

Enzymic browning is common in many plant tissues following a breakdown in cellular integrity or cellular injury in response to pests and diseases (e.g. slugs, fungal and bacterial pathogens), environmental stresses (e.g. freezing and drought), mechanical damage (bruising and internal cracking) and processing procedures (cutting and peeling). In potatoes, discoloration of tuber flesh is of major concern to growers and processors, due to increased labour costs for sorting blemished and damaged tubers, and the cost of preventing browning during processing.

Tuber discoloration caused by enzymic browning is well documented (Muneta, 1977; Walker, 1977; Rhodes & Wooltorton, 1978) and is caused by the oxidation of pre-formed phenolics, principally the amino-acid tyrosine and the o-diphenol chlorogenic acid, by the copper-containing enzyme complex polyphenol oxidase (PPO), in the presence of molecular oxygen. Tyrosine oxidation initiates the formation of the black polymeric pigment melanin (Mason,

1955), whilst lighter brown o-quinones are formed following the oxidation of chlorogenic acid (Pierpoint, 1970).

There is considerable variation in the level of enzymic browning shown by potato varieties included in the National Institute for Agricultural Botany recommended list (1986), with further variations superimposed onto varietal differences by climate (Mapson, Swain & Tomalin, 1963) and to a lesser extent, by edaphic factors (Burton, 1966; Mondy, Bond Gedde-Dahl & Owens Molbey, 1967; Birecki, Bizien & Henderson, 1971). Because of these inherent variations, processors have to resort to chemical means to prevent enzymic browning even when using recommended 'non-browning' commercial varieties.

The chemical control of enzymic browning involves the inhibition of PPO by adjusting the pH (Amla & Francis, 1961), adding chelating agents of the PPO copper prosthetic group (Matthew & Parpia, 1971), or by adding reducing agents either to decolorise the o-quinones formed or to reduce them back to o-hydroxyphenols (Ponting, 1960). However, the commonest and most effective commercially economic means of prevention is the use of bisulphites or sulphydryl compounds (Lerner, 1953). Concern is growing however on the use of such additives, and their use has been banned on fresh vegetables (not at present including potatoes) in the United States by the Food and Drugs Administration, after linking sulphite application to the death of 13 people (Anon., 1986).

Through traditional plant breeding it may be possible to select from within existing varieties for reduced enzymic browning, but due to the narrow genetic base of cultivated potatoes such characters are not readily available and it has become increasingly important to look to the wild tuber-bearing *Solanum* gene pool for specific characters for which breeding value has been determined (Ford-Lloyd & Jackson, 1986).

In the present context, several wild potato species from the taxonomic Series Longipedicellata, principally *Solanum hjertingii* Hawkes from north-east Mexico, have been reported to possess a non-browning character; however, previous assessments were purely subjective, using either tuber slices (Woodwards & Jackson, 1985) or gratings (Firbas, 1961). The need remains however, firstly to verify the occurrence of non-browning in *S. hjertingii* as a 'true' character, and secondly to quantify the character in relation to currently available commercial varieties.

In this paper we report the results of a study into the enzymic browning of seven accessions of *S. hjertingii* compared with five currently available commercial varieties of *S. tuberosum*, using both subjective and objective techniques to assess tuber discoloration.

Materials and Methods

True potato seed from seven accessions (i.e. a sample of genotypes from a single source) of *S. hjertingii* was obtained from the Commonwealth Potato Collection (CPC). Fifty clones of *S. hjertingii* and five commercial *S. tuberosum* varieties (Table 1) were grown at the Institute of Food Research (Norwich, UK), in a glasshouse (15 – 20°C) in 10 litre (30 cm diameter) pots using a peat based compost (3:2:1 peat:loam:coarse sand), supplemented with a basal fertiliser. Individual clones were harvested, cleaned, weighed and stored at 6°C.

Tuber slices 4 mm thick were cut transversely across the long axis of the tuber. Slices for fresh and frozen reflectance measurements and visual assessment were taken from the centre of the tuber, whilst the slices adjacent to these were used to produce a freeze-dried powder. Ten slices, one from each of 10 tubers were taken from each genotype, five slices for fresh reflectance measurement and visual assessment and five for freezing. Fresh slices were incubated immediately whilst slices for freezing were placed into a commercial freezer at – 18°C for 6 h, thawed and subsequently incubated. All slices were incubated at 30°C for 3 h in 9 cm

Table 1. *Accessions of* Solanum hjertingii *and commercial* Solanum tuberosum *varieties grown under glasshouse conditions, at I.F.R.N. 1986*

Solanum hjertingii Accession	Date of seed production	Code (genotype codes)
CPC 2624 × sibs	1984	a (a1, a2, a6, a7)
CPC 3208 × sibs	1975	b (b1, b2, b3, b4, b5, b6, b7, b8, b9)
CPC 3210 × sibs	1981	d (d1, d2, d3, d4, d5, d6)
CPC 3210 × sibs	1982	e (e1, e2, e3, e4, e5, e6)
CPC 3979 × sibs	1975/1980	f (f1, f2, f3, f4, f5, f6, f7, f8, f9, f10, f11)
CPC 3979 × sibs	1981	g (g1, g2, g3, g4, g5, g6, g7, g9)
CPC 5697 × sibs	1975/1980	h (h1, h2, h3, h4, h5, h6)
Solanum tuberosum commercial varieties		
cv. Golden Wonder		GW
cv. Maris Piper		MP
cv. Pentland Dell		PD
cv. Pentland Javelin		PJ
cv. Désirée		D

Petri dishes lined with moistened filter paper. Slices for freeze-drying (20 slices per genotype) were cut and immediately immersed into liquid nitrogen, freeze-dried and brought back to equilibrium at 20°C, finely ground and stored at −70°C until required.

Enzymic browning was measured in three ways. Firstly, subjective assessments of fresh and frozen slices were made on a discoloration scale of 1-6, modified from Woodwards & Jackson (1985), with 1 indicating no discoloration and 6 representing total discoloration of the slice. Secondly, a Hunter Labscan colour difference spectrophotometer was used to measure the L, a_L and b_L values of the fresh and frozen tuber slices before and after incubation. The linear white/black element of the sample on a scale 0-100, was represented by L, with a_L and b_L indicating the red/yellowness and blue/greenness of the sample respectively referenced to a control white tile. From these values the total colour difference (ΔE) was determined $\Delta E = (\Delta L^2 + \Delta a_L^2 + \Delta b_L^2)$. Thirdly, a spectrophotometric analysis of the freeze-dried potato macerate of each genotype was undertaken to measure potential browning (a measure of the total enzymic browning resulting from the breakdown of cellular compartmentalisation in that particular system and the release of endogenous substrates and water-soluble enzymes in tuber tissue). From a bulked powder of 20 tuber slices, duplicate 0.5 g extracts (fresh weight), were wetted with 2.5 ml of double distilled water, sonicated for 30 s at 30 KHz, and incubated at 30°C for 3 h on a tilting bed mechanism. A supernatant was obtained by centrifugation at 6000 *g* for 3 min in an Eppendorf microcentrifuge and the absorbance measured at 500 nm.

Results

The results of the tuber evaluation for enzymic browning clearly indicate that *S. hjertingii* characteristically exhibits lower levels than the best *S. tuberosum* variety, cv. Maris Piper, irrespective of the technique used to measure browning.

In terms of potential browning of freeze-dried macerates, all *S. hjertingii* accessions showed

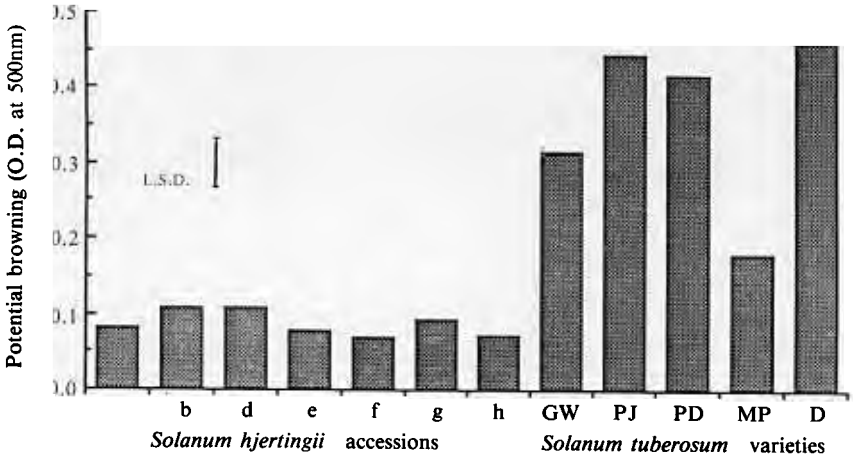

Fig. 1. Comparison of the potential browning of freeze-dried potato macerates of *Solanum hjertingii* accessions and commercial *Solanum tuberosum* varieties. See Table 1 for details of accessions and cultivars.

Table 2. *Visual assessment of* Solanum hjertingii *accessions and commercial* Solanum tuberosum *varieties using frozen potato slices*

Solanum hjertingii Accession	Code	Mean score		C.V.*	Score range
CPC 2624 × sibs 1984	a	2.0	(n = 20)	40.8%	(1-3)
CPC 3208 × sibs 1985	b	3.3	(n = 45)	30.0%	(2-6)
CPC 3210 × sibs 1981	d	3.6	(n = 30)	53.5%	(1-6)
CPC 3210 × sibs 1982	e	3.2	(n = 30)	30.0%	(2-4)
CPC 3979 × sibs 1975/80	f	2.5	(n = 55)	46.0%	(1-4)
CPC 3979 × sibs 1981	g	1.9	(n = 40)	34.0%	(1-3)
CPC 5697 × sibs 1975/80	h	2.3	(n = 30)	80.0%	(1-6)
Mean for all accessions		2.6	S.E.D. = 0.18 d.f. = 249		
Solanum tuberosum commercial varieties					
cv. Golden Wonder		6.0	(n = 5)	0%	(6)
cv. Pentland Javelin		5.6	(n = 5)	9.7%	(5-6)
cv. Pentland Dell		5.0	(n = 5)	0%	(5)
cv. Maris Piper		3.6	(n = 5)	15.2%	(3-4)
cv. Désirée		5.6	(n = 5)	9.7%	(5-6)

*Coefficient of variation

Data for *S. hjertingii* represents means of several genotypes for a given accession, whilst the data for the commercial varieties are for single genotype and consequently present a biased low C.V. value.

significantly lower browning values ($P = 0.05$) than the commercial varieties, with the exception of cv. Maris Piper (Fig. 1). In fact, the values for the other four varieties, Golden Wonder, Pentland Javelin, Pentland Dell and Désirée, were three to five times higher than *S. hjertingii* accessions (b and d) which showed the greatest browning. On the basis of potential browning of freeze-dried macerates, 18 of the *S. hjertingii* genotypes representing all seven accessions screened, were characterised by negligible tissue browning (visual scores 1-2)

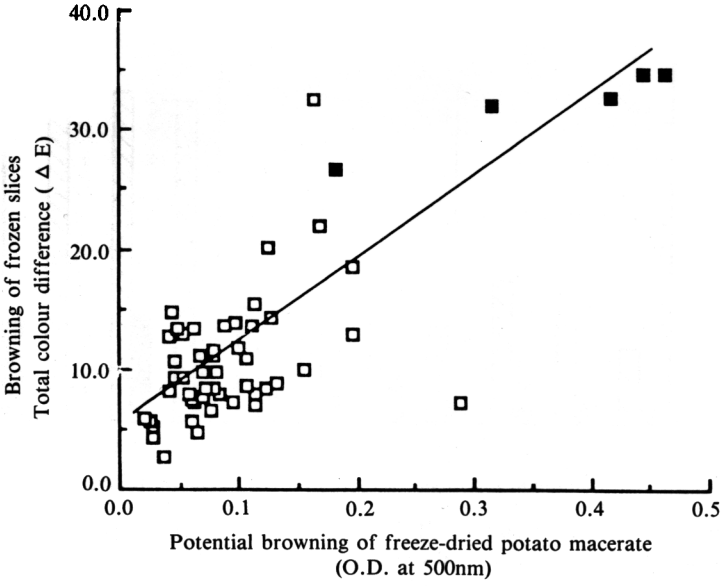

Fig. 2. Correlation between frozen slice discoloration, measured by reflectance and the potential browning of freeze-dried potato macerates, for *S. hjertingii* genotypes □ and commercial *S. tuberosum* varieties ■ . The regression line equation for the relationship is $y = 5.715 + 68.124x$. ($r = 0.95$).

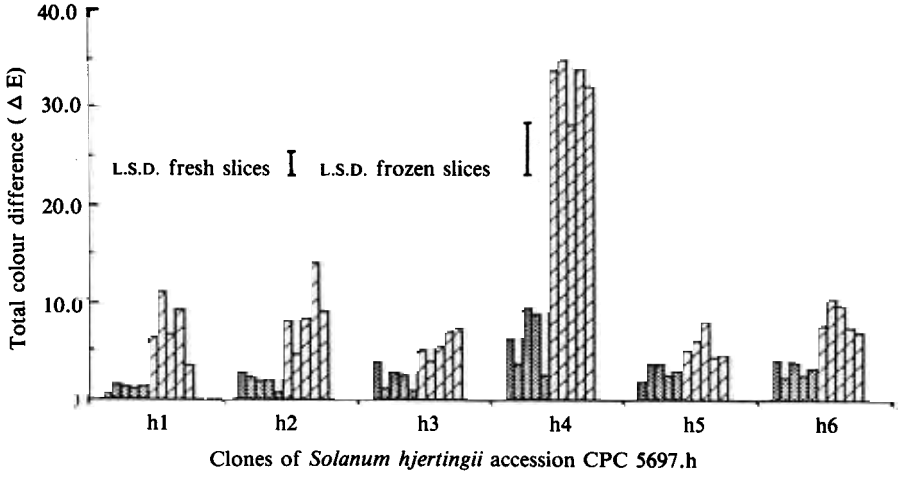

Fig. 3. Within accession and genotype variation in enzymic browning ΔE (total colour difference), of fresh ▩ and frozen ▨ slices of *Solanum hjertingii* accession CPC 5697.h.

equivalent to an optical density of < 0.07. While potential browning gives an objective measure for each accession or variety, visual assessments of frozen tuber slices (Table 2) could discriminate between cv. Maris Piper with a mean score of 3.6, and the *S. hjertingii* accessions taken together, with a mean browning score of 2.6 (S.E.D. = 0.18, D.F. = 249). Visual assessments of fresh tuber slices were undertaken but results were inconclusive.

There was a close correlation ($r = 0.95$) between the two objective techniques which were used to screen material, namely reflectance measurement of frozen slices and potential browning of freeze-dried potato macerate. In Fig. 2 the variation within *S. hjertingii* and marked differences between *S. hjertingii* genotypes and *S. tuberosum* varieties are also evident.

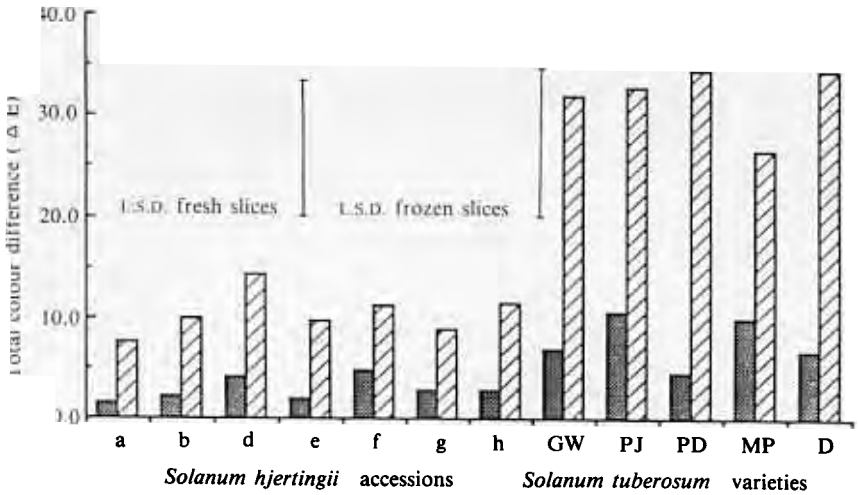

Fig. 4. Comparison of enzymic browning ΔE (total colour difference), of fresh ■ and frozen ▨ tuber slices of *Solanum hjertingii* accessions and commercial *Solanum tuberosum* varieties.

Variation in enzymic browning within accessions (i.e. between genotypes) and within a genotype (i.e. between slices from the same genotype) is clearly showed by accession CPC 5697.h (Fig. 3). The degree of browning was greater in frozen slices than in fresh as illustrated earlier, but the ranking of each genotype was not the same for fresh and frozen slices. Although significant differences ($P = 0.05$) are seen within genotypes in both fresh and frozen slices, it is however, the large difference between genotype CPC 5697.h4 and the other genotypes which is of particular interest. Such a high ΔE value for CPC 5697.h4 can be considered atypical for this accession, as can those of CPC 3208.b3, CPC 3210.d1 and CPC 3210.d4 in their particular accessions, which are probably responsible for the high coefficients of variations shown in the visual assessment scores (Table 2).

The difference in mean browning measurement for *S. hjertingii* accessions and *S. tuberosum* between fresh and frozen slices is illustrated in Fig. 4. There were significant differences ($P = 0.05$) between the *S. hjertingii* accessions and all *S. tuberosum* varieties using frozen slices and in the fresh slices with the exception of cv. Pentland Dell. The differences in browning were not as marked in fresh slices. Furthermore there were no significant differences between the *S. hjertingii* accessions, nor between the varieties for either fresh or frozen slices. Of the varieties, cv. Maris Piper had the lowest ΔE value in frozen slices, as with the freeze-dried macerate (Fig. 1), but was not significantly different from the *S. hjertingii* accession CPC 3210.d.

Discussion

Work by Firbas (1961) and Woodwards & Jackson (1985) has suggested that *S. hjertingii* and other wild species of potato from the Series Longipedicellata possess a non-browning character. The work in this paper has quantified the occurrence of non-browning as a 'true character' of *S. hjertingii* and not a random event. Other species in the Series Longipedicellata were shown by Woodwards & Jackson (1985) to possess varying degrees of enzymic browning, and of these *S. polytrichon* and *S. papita* showed least browning, although in the same taxonomic series *S. fendleri* exhibited high levels of tissue browning.

Two forms of tuber discoloration have been assessed in this paper. The first of these was

enzymic browning of fresh tuber slices in response to cutting damage, where discoloration is limited to the cells in the vicinity of the cut surface and which is restricted by the rate of oxygen diffusion at the surface. Total discoloration is made up of two components, namely the potential of the tissue to brown and the number of damaged cells per unit area. The second form of discoloration measured was potential browning, which is the severest measure of browning in potatoes, although not normally reproduced in the field.

The use of the Hunter colour difference spectrophotometer is a very sensitive screening technique, but the use of tuber slices still presents the problem of oxygen diffusion, although the browning of frozen slices correlated well with potential browning values from potato macerates, where diffusion of oxygen into the tissue is not limited. Thawed slices for reflectance measurement are difficult to handle in any quantity, but the distinct advantage of measuring reflectance of frozen slices is that intra-genotype variation can easily be assessed.

The significance of the lack of enzymic browning for potato improvement will depend to a considerable extent upon the ease with which the character can be manipulated. Its widespread occurrence in *S. hjertingii* adds support to the genetic hypothesis put forward by Woodwards & Jackson (1985) that non-browning is a dominant trait. Current work is underway to elucidate its genetic basis. It is clear that direct introduction into commercial varieties through traditional crossing programmes will prove difficult because of the strong crossability barriers between *S. tuberosum* and *S. hjertingii* at the tetraploid level (Woodwards & Jackson, 1985). The determination of the biochemical basis of non-browning and its genetic control is an important preliminary stage in the incorporation of the character into existing commercial varieties through genetic engineering.

In preliminary work McIlroy (1976) suggested that a possible reason for the lack of browning in *S. hjertingii* was reduced PPO (diphenolase) activity and not reduced substrate availability. Using a crude potato sap Woodwards (1982) showed that *S. hjertingii* was polymorphic for PPO (diphenolase) activity with 17 isozyme bands identified on polyacrylamide gels, with a maximum of seven bands in any one genotype. However, no examination was carried out on PPO (monophenolase) activity. Recent work (Gubb *et al.*, in prep.) on selected *S. hjertingii* genotypes discussed in this paper has shown that both substrate availability (particularly tyrosine), and PPO (monophenolase) activity vary markedly between genotypes even in the same accession.

Acknowledgement

The principal author would like to acknowledge the receipt of a SERC CASE award for this work.

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(Received 17 October 1988)