Project Report

Understanding and improving potato flavour characteristics

Ref: R246

Final Report: June 2004 - November 2007

G Bryan, D Lloyd & J Bradshaw: *SCRI*

2008

Project Report 2008/3

Additional copies of this report and a list of other publications can be obtained from:

Publications
Potato Council Ltd
4300 Nash Court
John Smith Drive
Oxford Business Park South
Oxford
OX4 2RT

Tel: 01865 782222
Fax: 01865 782283
e-mail: publications@potato.org.uk

Most of our reports, and lists of publications, are also available at *www.potato.org.uk*

Contents

1. Summary

Aims of project

The main scientific objective of the project were to gain a fundamental understanding of the genetic and biochemical control of flavour characteristics in potato using differences between conventional Tuberosum potatoes and the more extreme Phureja potatoes such as cultivar Mayan Gold. Commercial objectives were to generate Phureja potatoes with consumer appeal, improved Tuberosum breeding material carrying desirable Phureja flavour and "mouth-feel" characteristics, and the development of molecular and biochemical markers that can be used for marker-assisted breeding (MAB).

Work undertaken during reporting period (June 2004 – Nov 2007)

In the first year of the project significant differences were found in volatile and sensory profiles of Phureja and Tuberosum potatoes. Four Phureja/Tuberosum segregating populations were tested for agronomic performance and for variation in the target sensory/volatile traits. From these a single population, FT.4, a backcross of an F1 hybrid to the Phureja parent Mayan Gold, was selected for detailed analysis in years two and three of the project. The FT.4 cross was analysed phenotypically (sensory, volatile, agronomic characters) and with molecular markers (AFLPs, SSRs). This population represented a significant challenge in terms of the experimental design for the analysis of sensory traits on a large number of clones (>120). A linkage map comprising ~230 markers was generated, with all 12 expected potato chromosomes being identified. This map was used to perform QTL analysis with the volatile and sensory data. QTL analysis was performed using a regression-based approach whereby trait mean values of plants with any particular segregating marker are compared with values for plants lacking the same marker. For markers unlinked to trait effects these mean values will not differ significantly. However, for cases where a marker is genetically linked to a gene giving a trait effect the mean trait values for the marker present vs absent classes will differ to some extent. A large number of QTLs (120) were identified, and many of these appear to co-localise on the chromosomes. However, further work will be required to determine whether these trait co-localisations are real or spurious, given the very approximate QTL positions. Some of the markers that explain larger components of trait variation or several co localizing traits may be utilised as markers in potato breeding, subject to validation on larger panels of material and conversion to marker types more suited to high throughput in a modern potato breeding programme.

Conclusions

Significant levels of heritable variation have been detected for sensory traits, as well as volatiles produced during cooking of potatoes.

These variations are, to some extent, explained by 'interspecific' differences between Phureja and Tuberosum cultivar groups, although there is considerable variation among Phureja breeding clones.

Linkage and QTL analysis suggest that the genetic factors underlying organoleptic traits are complex, but that there may be significant clustering of QTLs.

Significant correlations among certain 'positive' sensory traits suggest that the genetic analysis of flavour should focus on identifying candidate genes underlying QTLs for the larger genetic effects detected in this study.

The high levels of α-copaene in Phureja tubers seen in previous studies at SCRI as well as in this project suggest a possible role in flavour. Future studies should focus on this, as well as the role of other factors (e.g. 'umami') in sensory preference. Now that there is genetic information on the α-copaene trait, the extent to which this co-localises with obvious candidate genes (Sesquiterpene synthases etc), will inform an understanding of the genetics underlying this trait.

2. Experimental Section

Introduction

Organoleptic attributes of food products are those characters that are perceived by the five senses during their consumption. They involve but are not limited to: aroma and taste (often considered together as "flavour" since they may be difficult to identify separately), colour and texture. Following cooking of potatoes, regardless of the method used (boiling, baking, deep-frying), various transformations take place that result in a pleasant and characteristic 'potato flavour'. This relatively neutral and bland, yet characteristic, flavour may account for the world-wide acceptance and consumption of the potato – it can be prepared in many different ways, and has been accommodated into most national cuisines.

The vast majority of established potato cultivars belong to the species *Solanum tuberosum* ssp *tuberosum* (hereafter referred to as *S. tuberosum*) which, because of the restricted range of material used in its introduction to Europe and elsewhere, has a fairly narrow genetic base. Indeed, results of previous studies on European cultivated potato indicate that the range of variation for flavour and texture (mouth-feel) descriptors is often too small to allow detection of significant and/or repeatable genetic differences between cultivars. What is more, sensory traits are poorly understood at the genetic and biochemical level and so have proved remarkably difficult to manipulate. In general, regardless of the crop being studied, breeding for organoleptic quality is difficult due to its complexity and because of the lack of efficient selection criteria. To date, most research into improvement of potatoes has been directed towards resistance to the major biotic stresses and 'quality' traits. Selection for quality has focussed on the reduction of glycoalkaloids, eliminating after-cooking-blackening and improving fry colour.

In South America, at least seven types of cultivated potato are grown and some are considered particularly flavoursome and of good eating quality. Varieties of S. phureja (now classified as Group Phureja of *S. tuberosum*) are particularly prized for their delicate yellow flesh, flavour and speed of cooking. In sensory analysis *S. phureja* cultivars have been shown to possess a distinctive mouth-feel and higher flavour intensity than *S. tuberosum* (Dobson *et al*., 2004). They also contain higher levels of carotenoids and have a greater range of flesh and skin colour than commonly seen in our European potatoes (Griffiths *et al*., 2007). In consequence, *S. phureja* populations have been identified as a promising source of specialist 'niche' cultivars, and as a gene pool for introgession of novel traits into the higher yielding *S. tuberosum* (De,Maine *et al*., 1993). That is, the use of Phureja and Phureja-Tuberosum hybrids could greatly expand the range of variation for these traits giving us the opportunity to analyse them genetically and biochemically. The initial goal of this project is to assess the range of variation in a set of *S. phureja* clones and representative *S. tuberosum* cultivars for molecular, sensory and volatile characters. Prior to the project SCRI generated a set of four Phureja-Tuberosum hybrid populations, to be used for genetic analysis of organoleptic traits. A small number of individuals (~20 from each population) would be assessed for sensory and volatile attributes with a view to identifying the most variable progeny for all subsequent analysis. This part of the project was designed to identify genetic factors influencing organoleptic traits, which would pave the way for the development of molecular and chemical markers for these increasingly important characters.

Material and methods

Germplasm

Backcross Populations: in order to have hybrid material for a mapping population, crosses were set up between a diploid Tuberosum (2DH40(3)) and a Phureja (DB337(37)) plant – the Phureja parent is also known as Mayan Gold. Two individuals, [99.FT.1(1), 99.FT.1(5)] from the resulting F1 populations, were backcrossed to the initial parents to give four backcross populations, 02.FT.1 and 02.FT.4, derived from crosses back to the Phureja parent, and 02.FT.24 and 02.FT.26, derived from the backcross to the Tuberosum parent. A yield trial was set up in 2004 with 50 clones from each of the four backcross populations. The parents and the F1s were also included in this ware trial. On the basis of this trial, populations FT.4 and FT.26 were chosen for more extensive trialling. Ware trials of the selected backcross populations FT.4 and FT.26 were planted on 5th May 2005 and 26 April 2006 and harvested on the 27th September 2005 and 25 September 2006. Two replicates were planted following a randomised complete block design, and standard fertiliser, cultural practice and chemical control of aphids and late blight for ware growing in the east of Scotland was used.

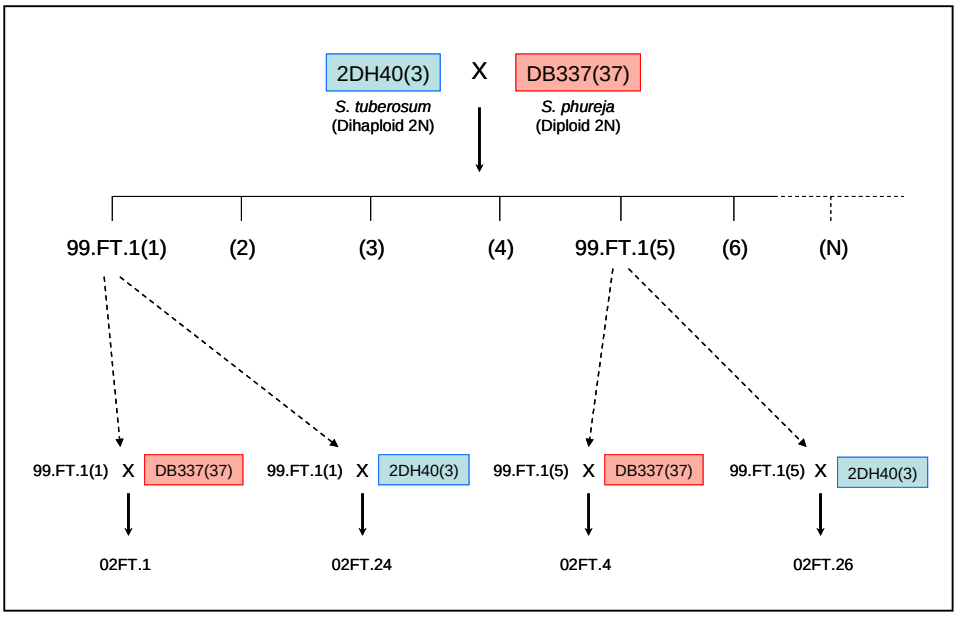

FIGURE 1. ORIGIN OF THE FOUR BACKCROSS POPULATIONS GENERATED AT SCRI FOR STUDYING FLAVOUR.

Agronomic Characteristics

At harvest, total yield (kg) was recorded for each of the clones from the FT.4 and FT.26 populations. A photographic record was made in 2005 of all clones that were to be used for sensory testing, volatile and molecular analysis: tubers were washed and photographed under consistent lighting conditions, alongside a colour card and scale bar. These images were used for image analysis in order to estimate skin and flesh colour for each clone and to make estimates of tuber size and shape.

Sensory Analysis

Sensory data on the FT.4 backcross were generated by Charis Innovative Food Services Limited in 2004, and then by Sensory Scotland. Tubers were stored at 2°C until used for sensory assessment. Prior to cooking, tubers were peeled and cut into approximately 30g cubes. A total of 500g of sample was cooked in 1 L of boiling distilled water to which 1% (w/v) cooking salt was added. Cooking times for each variety were determined by initial penetrometer tests and empirical studies.

Samples were drained of excess water and squeezed through a potato ricer. Samples were then transferred to pre-warmed bowls, covered with tin-foil and kept warm in an oven at 70°C prior to serving to the panel of sensory assessors (N=~16). Tubers were scored for 22 attributes which were grouped into six main categories. These were 1) appearance (white, yellow, grainy, shiny); 2) odour (intensity, creamy, earthy); 3) flavour (intensity, creamy, earthy, salt, metallic, sweet); 4) after-taste (intensity, metallic, bitter); 5) acceptability. All attributes were scored on a scale from 0 (poor rating for the character) to 100 (good rating for the character).

Volatile Analysis

Tubers of the FT.4 backcross population and controls were prepared for volatile analysis using conditions as similar as possible to those used for the sensory analysis. Tubers were peeled and cut into cubes, each weighing approximately 30 grams. These were prepared immediately prior to cooking to minimize oxidation of the cut tuber surface. Approximately 500 g of tuber cubes were cooked by placing them directly into 1 L of boiling water containing 1% (w/v) NaCl. The samples were cooked until the tines of a fork were able to slide in and out of the tuber cubes smoothly and easily. Cooked tuber cubes were drained of liquid and passed through a ricer (2 mm apertures) directly into a 2 L culture flask. 1 µl of β-Ionone was added to the mashed potato matrix as an internal standard. The culture flask was then sealed with a five necked flask cover stopped with borosilicate stoppers. All joints between glassware were further sealed with PTFE tape to minimise contamination from extrinsic volatiles.

Culture flasks were then placed in water baths at 50ºC and allowed to equilibrate for 30 minutes. Air was then drawn into the culture flask through a molecular sieve (to remove water) and activated carbon filter (to remove extrinsic volatiles), through the headspace and through a silicosteel sorbent tube containing Tenax TA adsorbent polymer, at a flow rate of 100 ml min^{-1}. Volatiles were entrained for a period of 30 minutes after which the sorbent tubes were then back-flushed with dry, carbon filtered N_2 for further 30 minutes to remove condensation. They were then sealed with Swagelock caps for storage prior to analysis.

Entrained volatiles were analysed by thermal desorption gas chromatography mass spectrometry (ATD-GC-MS). Entrained volatiles were desorbed from the sorbent tubes by heating at 200 ºC and concentrated on a cold trap at -10 ºC. This was rapidly heated to 240 ºC and the trapped volatiles injected onto the GC column.

Molecular Analysis

DNA was extracted from all samples using standard plant DNA extraction methodologies. For AFLP analysis, DNA was digested with enzymes that cut DNA at specific 4 and/or 6 base pair sequences (two different enzyme combinations were used: PstI/MseI and EcoRI/MseI). The resulting fragments were tagged with radioactivity and run through a gel to separate them on the basis of size. The result is a series of bands on gels (a DNA "fingerprint") that represent distinct pieces of DNA. These "fingerprints" are unique for each clone. SSR analysis involves the selective amplification of specific sequences in the genome that show 'length variation' between the two parents. In the case of both marker systems, the banding pattern for both parents is known beforehand so that the pattern of inheritance by the progeny can be followed. In the current study, this principle is employed to follow the inheritance of characteristic from the sensory and volatile analyses that were shown to be related to flavour.

Data Analysis

For the data from the CPC material and known Tuberosum cultivars, each measured variate was checked for normality of distribution using boxplots. In order to get a feel for the possibly informative relationships between variates, pair-wise scatter-plots were drawn and a correlation matrix produced. Pair-wise correlations between all 22 sensory attributes were calculated. Regression of 'acceptability' on each sensory variate was performed. The Mann-Whitney U test was used to look for differences between the means for the *S. phureja* and *S. tuberosum* samples – this test makes no distributional assumptions nor does it require that the samples are of the same size.

Linkage and QTL analysis

Linkage analysis was performed using Joinmap 3.0. QTL analysis using the program MapQTL) was a problem in the FT.4 population as (i) the population was quite small, (ii) due to the crossing scheme and limited number of markers there was not good coverage of the contribution of both parents to all of the the chromosomes. A marker regression method was favoured over interval mapping. Basically trait values for a population are regressed onto 1/0 (presence/absence) marker scores, in a way analogous to 'line fitting' exercise to quantitative data, the main difference being that there are only two 'x-values' for each marker (i.e. 0 and 1) This approach can easily be done with Genstat, or in MapQTL. Given the number of traits it is important that QTLs detected by this method are not purely due to chance. To address this issue a 'permutation test' (based on 1000 permutations of a trait) can be carried out. These analyses provide figures for the 95% and 50% 'cut off' values for the statistics (KW) generated from the QTL analysis. Marker-trait associations exceeding the 95% point of the permutation test are deemed 'significant' and those exceeding the 50% point are described as 'noteworthy'. A more detailed explanation of these procedures is given in the Results section.

Results

Agronomic traits

There was considerable variation in vigour, and yield between the four backcross populations. Initially the backcrosses gave rise to different numbers of healthy seed (a target of 200 clones of each population was initially chosen, although FT26 gave only 195 seeds). Percentage germination also differed and, consequently, different numbers of plants obtained (FT.1 = 218, FT.4 = 257, FT.24 = 143, FT.26 = 113). The mean vigour scores for the plants from each population were: FT.1 = 4.08, FT.4 = 5.27, FT.24 = 3.76, FT.26 = 4.27. In terms of absolute number of vigorous clones, however, the Phureja backcross populations were better than the Tuberosum populations with FT.4 being the most vigorous. That is, 212 clones of FT.4 were given a vigour score of 5, 6 or 7 while the corresponding numbers for FT.1, FT.24 and FT.26 were 123, 58 and 52 respectively.

Backcross	No. seeds obtained	No. seeds sown
FT1	395	250
FT4	600 +	250
FT24	600+	250
FT26	195	195

TABLE 1. NUMBER OF SEEDS OBTAINED AND SOWN FOR EACH BACKCROSS.

Analysing the data from 50 clones of each population included in the first ware trial, it was clear that, in terms of yield and mean tuber size (characteristics important for the ability to carry out sensory and volatile analysis), FT.4 was the better of the two backcross populations to the Phureja parent, and FT.26 was the better of the backcrosses to the Tuberosum parent (Fig. 2). Yield ranged from 0 kg to 10 kg (overall mean values were 3.41, 5.39, 2.29 and 3.52, respectively, for FT.1, FT.4, FT.24 and FT.26), and tuber number from 0 to 200. Tuber yield from plants of the backcross population FT.4 was significantly greater than that from clones of the other three populations ($p = 0.005$, 0.001, 0.001 for comparisons with FT.1, FT.24 and FT.26, respectively). Statistically significant differences were also recorded for mean tuber weight. Tubers from clones of FT.4 were generally larger than those from the other three populations (0.001 for all three comparisons).

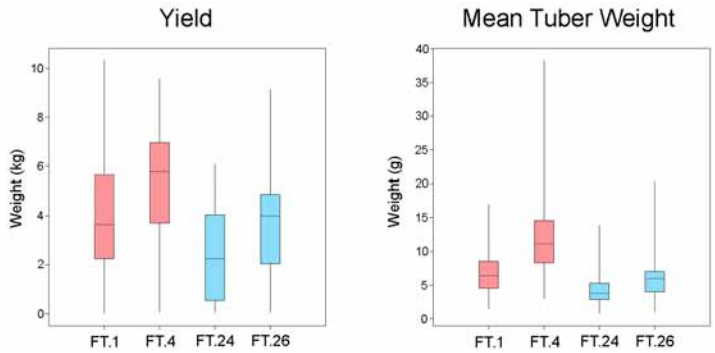

FIGURE 2. TOTAL YIELD (KG) AND MEAN TUBER WEIGHTS PER CLONE FOR THE FOUR BACKCROSS POPULATIONS. FT.1 AND FT.4 WERE THE POPULATIONS FROM THE PHUREJA BACKCROSS; FT.24 AND FT.26 WERE FROM THE TUBEROSUM BACKCROSS.

There was also considerable variation in skin colour (Fig. 3) and tuber shape (Fig. 4). More of the tubers from the clones of the Phureja backcross populations were elongated in shape than tubers from the Tuberosum backcross. None of the tubers from clones of the FT.1 population were scored in categories 0 and 1 while 26.2% were scored in categories 4 and 5. For the clones of FT.4, FT.24 and FT.26 the respective scores were: 22.0% and 6%; 27.6% and 2.1%; 62.0% and 0% (Fig. 4).

FIGURE 3. SKIN COLOUR VARIED CONSIDERABLY IN THE BACKCROSS POPULATIONS

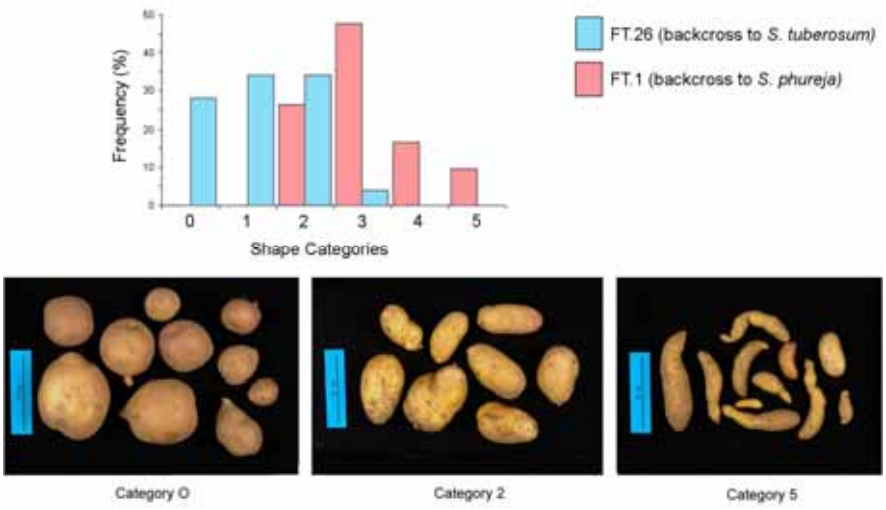

FIGURE 4. VARIATION IN TUBER SHAPE. TUBERS FROM THE VARIOUS CLONES RANGED FROM ALMOST ROUND IN SHAPE (CATEGORY 0) TO BEING UP TO FIVE TIMES AS LONG AS WIDE (CATEGORY 5). THE HISTOGRAM SHOWS THE FREQUENCY DISTRIBUTION FOR THE TWO MOST EXTREME POPULATIONS (FT.1 AND FT.26) WITH RESPECT TO THIS CHARACTER.

Cooking times

Cooking times were distinctly different for the two species groups (Table 2). The range of cooking times for Phureja potatoes was 7.5 – 11.0 minutes (average of 9.1 minutes) while that of the Tuberosum potatoes was from 13.0 – 16.5 minutes (average of 14.0 minutes). For all of the subsequent sensory and volatile analysis the potatoes were cooked according to these empirically determined times.

Clone	Mean Cooking Time
80.CP.23	7.5
PHU.950 (412)	8.0
71.P.10	8.5
DB.207 (35)	8.5
DB 333(16)	8.0
842.P.75	8.0
DB161 (10)	8.0
DB.244 (37)	8.0
DB.226 (70)	9.0
71.T.46	9.8
DB.168 (11)	10.3
DB.257 (28)	10.0
81.5.66	10.0
DB.337 (37) – Mayan Gold	10.0
DB.378 (1)	11.0
DB.271 (39)	11.0
ESTIMA	13.0
ANYA	13.3
RECORD	13.0
M PIPER	14.0
HERMES	14.5
NADINE	16.5

TABLE 2. MEAN COOKING TIMES (BASED ON 2 TRIALS) IN RANK ORDER. THE S. TUBEROSUM VARIETIES ARE HIGHLIGHTED IN LIGHT-GREY.

Cooking times ranged from 6 minutes (clone 64) to 16 minutes (clone 187). For those clones and cultivars for which we had replicates there was considerable variation in determined cooking times: 2DH40 = 14m and 8m 30s; 99.FT.1B = 10m 30s and 6m; Hermes = 12m 30s, 19m and 13m 30s; clone 12 = 13m and 9m 30s; clone 85 = 8m and 10 min; clone 179 = 12m 30s and 8m 30s.

Sensory analysis

Phureja/Tuberosum trial

In comparisons between the two species groups, S. phureja and S. tuberosum, highly significant differences in means were recorded for the colour attributes yellowness ($p = 0.001$) and whiteness ($p = 0.001$). There was also a highly significant difference for acceptability ($p = 0.003$) and flavour intensity ($p = 0.002$), with the S. phureja clones generally having higher scores for these attributes than the S. tuberosum varieties. Notable differences in after-taste intensity ($p = 0.010$), and sweet ($p = 0.020$) and salt ($p = 0.023$)

flavour were also seen. Except for colour, flavour intensity was the character that most distinctly distinguished S. phureja varieties from S. tuberosum (Fig. 5). Ranking all varieties with respect to acceptability, four of the six S. tuberosum varieties were in the bottom five (Table 3). Anya, the S. tuberosum variety with the best score for acceptability, only ranked ninth. Record scored by far the poorest for this character. Although there were differences between the species with respect to sensory attributes, in PCA analysis, no coordinate unambiguously separated them. The colour attributes, explaining 62.6% of the variability in the data set, had a strong influence. The second component (20.3%) was dominated by the mouth-feel attributes floury and dry. Removing the appearance attributes and acceptability, the mouth-feel characters (floury, moist/dry) dominated the first component (61.6% of the variability explained) and earthy flavour and aroma the second component (14.8% of variability).

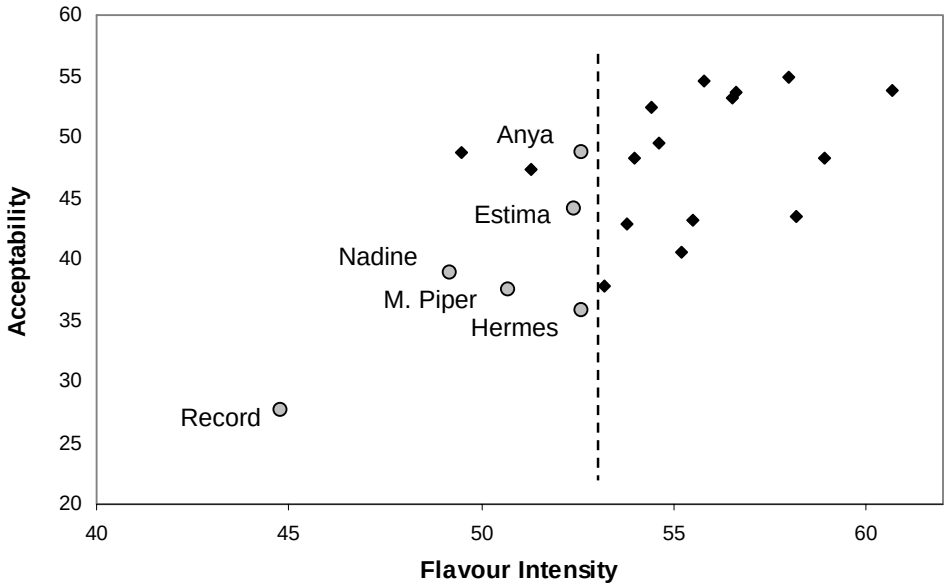

FIGURE 5. SCATTER PLOT OF FLAVOUR INTENSITY AND ACCEPTABILITY SHOWING THAT S. PHUREJA CLONES GENERALLY HAVE A MORE INTENSE FLAVOUR THAN CULTIVARS OF S. TUBEROSUM.

The six S. tuberosum varieties are named. The variety "Record", scored particularly badly for both flavour intensity and acceptability. Both Acceptability and Flavour Intensity were scored on a scale from 1 – 100.

For an initial look at the data set from the sensory panel, it was thought interesting to try and identify the sensory attributes that account for the difference in acceptability between the various clones and varieties. For the initial sensory analysis, attention was principally paid to scores for acceptability and the attributes that were strongly correlated to it.

In order to identify any potentially interesting relationships, correlation matrices were produced (Tables 4 and 5). In the Phureja/Tuberosum trial, the characters that most strongly correlated positively with acceptability were creamy flavour (r = 0.77) and flavour intensity (r = 68). Sweet and salt flavours also correlated to some extent with acceptability (0.58 and 0.54 respectively). The correlation of the attribute creamy aroma with acceptability was

heavily dependent on the outlier, Nadine that had a high score for creamy aroma but scored poorly for acceptability - removing Nadine from the analysis resulted in a correlation of 0.73. Negative characters were metallic after-taste (-0.53) and metallic flavour (-0.52). However, the negative correlation between metallic after-taste and acceptability was very strongly influenced by the outlier, Record – removal of this from the data set resulted in there being no correlation. Indeed, Record was a distinct outlier in many of the relationships (Fig. 5) and the analysis was repeated in its absence in order to see whether correlations remained significant. In the data set 'minus Record', sticky mouth-feel became a significant attribute (correlation with acceptability of -0.62).

In the two backcross populations (only 22 clones analysed from each population), there were no strong positive correlations with acceptability. However, metallic flavour (-0.65), metallic after-taste (-0.72) and bitter after-taste (-0.71) correlated negatively with acceptability. These values were -0.52, -0.53 and -0.35, respectively, for the Phureja/Tuberosum trial.

In order to appreciate the relative contribution of the various sensory attributes to overall acceptability, multiple linear regression analysis was performed. Only the highly correlated attributes were entered into the analysis. It was possible to explain 71.3% of the variability in the data set regressing acceptability on creamy flavour ($p = 0.001$), yellow appearance ($p = 0.013$) and sticky mouth-feel ($p = 0.001$). Although flavour intensity was highly correlated with acceptability it proved not to significantly contribute to it. This apparently counterintuitive result is explained by the fact that flavour intensity is highly correlated, and accounted for, by other attributes such as creamy, sweet and salty flavour.

Clone	**Acceptability**
DB 257 (28)	55.0
DB 378	54.6
DB 207 (35)	53.8
DB 168 (11)	53.7
DB 161 (10)	53.2
DB 226 (70)	52.5
842.P.75	49.5
71.P.10	48.8
ANYA	48.7
DB 244 (37)	48.3
DB 337 (37) - Mayan Gold	48.3
81.S.66	47.4
ESTIMA	44.1
DB 271 (39)	43.5
71.T.46	43.3
80.CP.23	42.9
DB 333 (16)	40.6
NADINE	39.0
PHU 950412	37.8
MARIS PIPER	37.5
HERMES	35.9
RECORD	27.7

TABLE 3. RANK ORDER OF BOILED POTATOES WITH RESPECT TO "ACCEPTABILITY". SCALE RANGES FROM 0 (LEAST POSITIVE) - 100 (MOST POSITIVE). THE TUBEROSUM VARIETIES ARE HIGHLIGHTED IN LIGHT-GREY.

	Appearance				Aroma			Flavour						Atfter-taste				Mouth-feel					Acceptability
	White	*Yellow*	*Grainy*	*Shiny*	*Intensity*	*Creamy*	*Earthy*	*Intensity*	*Creamy*	*Earthy*	*Sweet*	*Salt*	*Metallic*	*Intesnsity*	*Metallic*	*Bitter*	*Floury*	*Sticky*	*Moist*	*Smooth*	*Chalky*	*Acceptability*
APPWhite	1.00																					
APPYellow	-0.85	1.00																				
APPGrainy	-0.08	-0.22	1.00																			
APPShiny	-0.23	0.33	-0.84	1.00																		
AROMIntensity	0.04	-0.06	0.20	-0.21	1.00																	
AROMCreamy	0.16	-0.08	-0.55	0.48	-0.22	1.00																
AROMEarthy	-0.10	0.09	0.22	-0.25	0.73	-0.11	1.00															
FLAVIntensity	-0.50	0.43	-0.17	0.32	0.32	0.29	0.48	1.00														
FLAVCreamy	-0.27	0.28	-0.48	0.53	-0.05	0.66	0.09	0.68	1.00													
FLAVEarthy	0.04	-0.05	0.33	-0.42	0.43	-0.17	0.76	0.17	-0.13	1.00												
FLAVSweet	-0.57	0.60	-0.37	0.55	-0.08	0.38	0.08	0.82	0.67	-0.10	1.00											
FLAVSalt	-0.40	0.38	-0.19	0.30	0.10	0.26	0.31	0.68	0.55	0.10	0.55	1.00										
FLAVMetallic	0.24	-0.25	0.39	-0.61	-0.02	-0.45	-0.04	-0.41	-0.56	0.04	-0.55	-0.47	1.00									
ATIntensity	-0.48	0.41	0.16	-0.06	0.42	-0.15	0.48	0.70	0.17	0.26	0.45	0.40	0.09	1.00								
ATMetallic	0.27	-0.26	0.22	-0.42	-0.09	-0.32	-0.13	-0.41	-0.51	-0.21	-0.51	-0.38	0.91	0.03	1.00							
ATBitter	-0.25	0.01	0.32	-0.17	-0.17	-0.44	-0.14	-0.05	-0.34	-0.15	-0.07	-0.37	0.39	0.30	0.33	1.00						
MFFloury	0.08	-0.07	0.64	-0.82	0.22	-0.50	0.28	-0.28	-0.45	0.32	-0.54	-0.21	0.65	0.28	0.57	0.14	1.00					
MFSticky	0.25	-0.19	-0.37	0.48	-0.20	0.33	-0.17	0.04	0.17	-0.11	0.27	-0.06	-0.36	-0.35	-0.33	0.01	-0.73	1.00				
MFMoist	0.01	-0.03	0.64	-0.77	0.13	-0.59	0.06	-0.36	-0.53	0.13	-0.53	-0.28	0.64	0.26	0.57	0.28	0.95	-0.74	1.00			
MFSmooth	0.02	-0.09	-0.44	0.56	-0.37	0.38	-0.55	0.24	0.34	-0.64	0.45	0.22	-0.37	-0.10	-0.16	0.02	-0.62	0.38	-0.48	1.00		
MFChalky	-0.08	-0.01	0.71	-0.74	0.11	-0.64	0.10	-0.32	-0.57	0.23	-0.52	-0.29	0.70	0.26	0.55	0.33	0.84	-0.60	0.89	-0.51	1.00	
Acceptability	-0.43	0.49	-0.33	0.29	0.28	0.39	0.31	0.70	0.76	0.05	0.57	0.60	-0.52	0.41	-0.52	-0.32	-0.15	-0.25	-0.19	0.08	-0.34	1.00

TABLE 4: CORRELATION MATRIX FOR THE 22 SENSORY ATTRIBUTES FOR THE CPC S. PHUREJA AND FOR THE 6 CULTIVATED VARIETIES OF S. TUBEROSUM: STRONG POSITIVE CORRELATIONS (GREATER THAN 0.6) ARE HIGHLIGHTED IN PALE PINK; STRONG NEGATIVE CORRELATIONS (-0.6) ARE HIGHLIGHTED IN PALE YELLOW.

	Appearance				*Aroma*			*Flavour*						*Atfter-taste*				*Mouth-feel*				*Acceptability*
	White	*Yellow*	*Grainy*	*Shiny*	*Intensity*	*Creamy*	*Earthy*	*Intensity*	*Creamy*	*Earthy*	*Sweet*	*Salt*	*Metallic*	*Intensity*	*Metallic*	*Bitter*	*Floury*	*Sticky*	*Moist*	*Smooth*	*Chalky*	*Acceptability*
APPWhite	1.00																					
APPYellow	-0.86	1.00																				
APPGrainy	0.20	-0.49	1.00																			
APPShiny	-0.43	0.45	-0.34	1.00																		
AROMIntensity	-0.31	0.37	-0.08	0.22	1.00																	
AROMCreamy	-0.10	0.26	-0.51	-0.14	-0.03	1.00																
AROMEarthy	-0.12	0.06	0.23	0.17	0.60	-0.49	1.00															
FLAVIntensity	-0.28	0.34	-0.15	0.56	0.61	-0.27	0.56	1.00														
FLAVCreamy	0.33	-0.22	-0.17	0.17	-0.33	0.08	-0.07	0.03	1.00													
FLAVEarthy	-0.17	0.05	0.24	0.42	0.45	-0.50	0.77	0.59	-0.16	1.00												
FLAVSweet	-0.24	0.22	-0.13	0.49	0.21	-0.17	0.35	0.63	0.36	0.33	1.00											
FLAVSalt	-0.04	0.05	0.09	0.31	0.28	-0.21	0.42	0.50	0.15	0.46	0.14	1.00										
FLAVMetallic	-0.08	-0.16	0.47	0.43	0.13	-0.62	0.38	0.27	-0.24	0.61	0.05	0.41	1.00									
ATIntensity	-0.32	0.22	0.10	0.38	0.53	-0.29	0.46	0.77	-0.17	0.61	0.57	0.40	0.40	1.00								
ATMetallic	-0.03	-0.26	0.53	0.27	0.10	-0.53	0.42	0.17	-0.24	0.64	0.05	0.28	0.85	0.36	1.00							
ATBitter	0.02	-0.32	0.55	-0.01	-0.12	-0.41	0.19	-0.13	-0.11	0.23	-0.06	0.01	0.53	0.04	0.67	1.00						
MFFloury	0.37	-0.37	0.56	-0.71	-0.11	-0.09	-0.04	-0.37	-0.19	-0.14	-0.55	0.00	-0.09	-0.31	0.00	0.04	1.00					
MFSticky	-0.29	0.13	-0.16	0.72	0.01	-0.24	0.11	0.38	0.21	0.35	0.58	0.10	0.42	0.40	0.37	0.22	-0.79	1.00				
MFMoist	0.36	-0.38	0.69	-0.55	-0.09	-0.30	0.04	-0.29	-0.18	-0.03	-0.44	0.11	0.14	-0.22	0.11	0.09	0.92	-0.61	1.00			
MFSmooth	0.12	-0.30	0.82	-0.15	0.08	-0.59	0.25	-0.02	-0.42	0.37	-0.21	0.16	0.58	0.16	0.53	0.34	0.53	-0.11	0.73	1.00		
MFChalky	0.13	-0.10	0.14	-0.70	-0.10	0.37	-0.26	-0.52	-0.42	-0.40	-0.69	0.25	-0.35	-0.44	-0.26	-0.06	0.71	-0.83	0.50	0.15	1.00	
Acceptability	0.03	0.31	-0.47	-0.13	0.23	0.38	-0.06	0.23	0.30	-0.28	0.16	0.07	-0.65	0.01	-0.72	-0.71	0.03	-0.34	-0.09	-0.47	0.11	1.00

TABLE 5: CORRELATION MATRIX FOR THE 22 SENSORY ATTRIBUTES FOR THE BACKCROSS POPULATIONS FT.4 AND FT26: STRONG POSITIVE CORRELATIONS (GREATER THAN 0.6) ARE HIGHLIGHTED IN PALE PINK; STRONG NEGATIVE CORRELATIONS (-0.6) ARE HIGHLIGHTED IN PALE YELLOW.

Reassessment of First Years' Data

In year one, sensory testing was carried out on tubers of 16 Phureja clones and 6 Tuberosum cultivars: each of the 22 varieties was sampled from two field plots. Because of the practical constraints of handling hot products (the requirement for cooking and maintenance of samples at required temperature), the following design was used for the tasting panel. Tubers from any given field plot were only evaluated on a single day but tubers of the cultivar Record were included as a control in the sensory profiling on every day. This enabled variety means to be adjusted for day-to-day differences in scoring by individual assessors, assuming that the samples of Record were uniform.

A more complex model allowing for the possibility of a component of this day-to-day variation being common to all assessors has now been evaluated. The presence of a statistically significant variance component for day indicates that there is some day-to-day variation common to assessors. The precise reason for this is not clear, but its identification is useful. However, it is probably unwise to attempt to adjust variety comparisons for such identified effects. For example, if there had been a slight difference in cooking time in the control between two days resulting in a small day-to-day effect in the control, it is likely that the other varieties were not affected and hence not requiring adjustment.

Sensory analysis of FT.4 population

Sensory data for the FT.4 population were generated in the second and third years of the project. After the first set of sensory data was collected, an analysis was performed. In year 3 of the project, it was possible to perform a joint analysis of the sensory data gathered over two years for the FT.4 population. A total of 119 clones were trialled in both years along with Mayan Gold, Hermes and Record and grandparent clone 2DH40(3). Variance components for the different sources of variation in the combined analysis of adjusted means of clones in years 2 and 3 are shown in Table 6. From these variance components it is possible to compute 'broad-sense' heritabilities.

Attribute	σ^2_{Year}	σ^2_{Clones}	$\sigma^2_{Residual}$	Clones P value	Heritability (broad sense)
White	3.22	280.24	98.00	P<0.001	0.85
Yellow	0.00	205.97	64.38	P<0.001	0.86
Grainy appearance	0.36	12.77	28.37	P<0.001	0.48
Shiny appearance	0.81	10.71	10.30	P<0.001	0.68
Odour intensity	7.86	1.92	10.99	0.052	0.27
Creamy odour	0.14	0.54	2.28	0.018	0.32
Earthy odour	0.25	2.35	12.69	0.044	0.27
Flavour intensity	6.67	1.20	5.45	0.024	0.30
Creamy flavour	11.21	1.84	7.69	0.017	0.32
Earthy flavour	1.01	3.48	13.66	0.013	0.34
Salty flavour	1.97	1.80	8.89	0.033	0.29
Metallic flavour	0.49	2.74	11.55	0.018	0.32
Sweetness	3.73	9.43	13.13	P<0.001	0.59
Acceptability	0.00	13.99	28.62	P<0.001	0.49
After-taste intensity	1.14	0.96	5.98	0.067	0.24
Metallic after-taste	0.06	1.80	9.01	0.035	0.29
Bitter after-taste	0.83	0.27	1.81	0.078	0.23
Floury	53.43	55.61	61.81	P<0.001	0.64
Sticky	0.61	1.88	11.21	0.058	0.25
Moist/dry	7.07	51.31	56.55	P<0.001	0.64
Smooth/grainy texture	10.59	10.47	39.93	0.011	0.34
Chalky texture	5.53	1.95	4.60	P<0.001	0.46

TABLE 6: VARIANCE COMPONENTS FOR SENSORY ATTRIBUTES OF FT.4 CLONES IN COMBINED ANALYSIS OF YEARS 2 & 3.

Encouragingly, 18 of 22 attributes showed a significant (P<0.05) difference among the clones tested. It was evident that for most attributes discrimination between clones was still possible despite the comparatively large differences between years and the residual variation in some cases.

Spearman rank correlations between acceptability, determined by the Sensory Scotland panel, and the other 21 attributes assessed are shown in Table 7. It should be noted that acceptability is a very subjective assessment (and is likely to vary between different parts of the UK depending on the respective bases of regional preferences) unlike the other 21 attributes. Hence considerable caution should be used when considering acceptability scores. For the Sensory Scotland panel in Ayr, there was a very strong positive correlation between acceptability and creamy flavour and a very strong negative correlation between acceptability and stickiness. There were also highly significant positive correlations between acceptability and both creamy odour and flavour intensity. There were highly significant negative correlations between acceptability and metallic flavour, metallic after-taste, bitter after-taste and smooth/grainy texture (i.e. acceptability decreased as grainy texture increased).

Attribute	Correlation	P value
White	-0.240	0.009
Yellow	0.274	0.003
Grainy appearance	0.107	0.247
Shiny appearance	-0.171	0.062
Odour intensity	0.059	0.523
Creamy odour	0.372	P<0.001
Earthy odour	0.032	0.728
Flavour intensity	0.302	P<0.001
Creamy flavour	0.636	P<0.001
Earthy flavour	-0.206	0.025
Salty flavour	0.114	0.218
Metallic flavour	-0.423	P<0.001
Sweetness	0.192	0.036
After-taste intensity	-0.054	0.558
Metallic after-taste	-0.340	P<0.001
Bitter after-taste	-0.368	P<0.001
Floury	0.246	0.007
Sticky	-0.539	P<0.001
Moist/dry	0.205	0.026
Smooth/grainy texture	-0.302	P<0.001
Chalky	0.091	0.323

TABLE 7: SPEARMAN RANK CORRELATIONS BETWEEN SENSORY SCOTLAND ACCEPTABILITY SCORES AND OTHER SENSORY ATTRIBUTES FOR CLONES IN FT.4 POPULATION.

Volatile analysis

Phureja/Tuberosum trial

From the headspace volatile analysis of boiled potatoes, 68 compounds were identified, with a further forty others yet to be characterised. The analysis of this data set was rendered difficult by the fact that, in many cases, replicates were very different from each other. Where an obvious error was found in one of the replicates this was removed and the result from the other was maintained. However, in some cases replicates for a particular clone/compound were very different but both seemed valid. For the sake of this analysis, both were removed and were treated as missing data. Some of these differences in replicates could be due to the long entrainment periods used in the first year, whereby it is possible that volatiles from the surrounding laboratory could have been 'pulled' into the volatile collection vessel. The use of much shorter entrainment periods in later years should address this issue.

Approximately 30 compounds that differed in concentration between the two species groups were detected. The broad spectrum of volatile compounds produced by the two potato species were sufficiently different that, in principal component analysis, they were to some extent distinct. The Tuberosum cultivars showed very little variation with respect to the first two principal components lying in a tight cluster close to one extreme of the graph (Fig. 6). The exception to this was Record that was an outlier to the rest of the group. The compounds having most influence on the principal axis were hexanal and α-copaene, with, on average, the first being significantly greater in Tuberosum cultivars and the latter significantly higher in

Phureja clones. Hexanal and α-copaene also predominated in the second principal coordinate, but methional, nonanal and decanal also contributed significantly to this axis.

However, it is likely that these compounds have little influence on the sensory properties of the cooked potatoes. That is, they do not impinge on the human senses at the concentrations found in these samples.

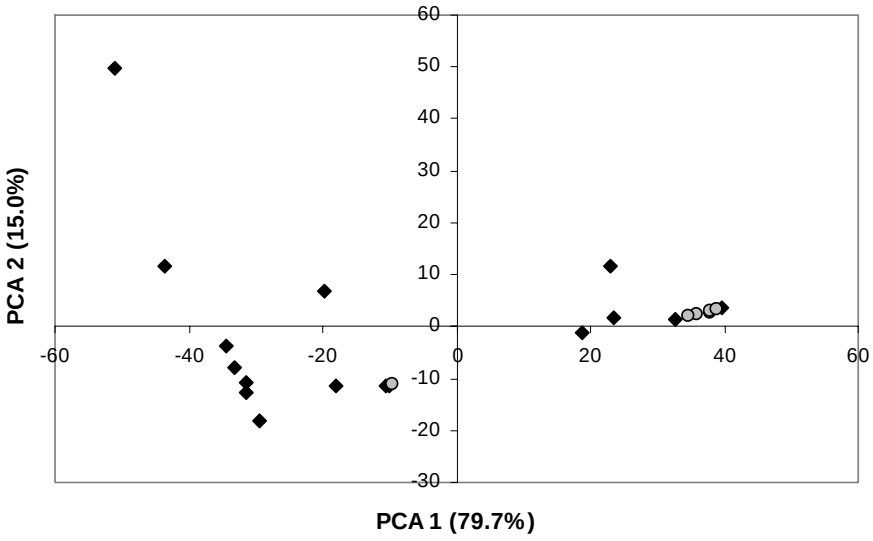

FIGURE 6. PRINCIPAL COMPONENT PLOT OF VOLATILE DATE. THE BLACK LOZENGES REPRESENT THE PHUREJA CLONES, THE GREY CIRCLES ARE THE TUBEROSUM CULTIVARS.

More than thirty other compounds were significantly different between the S. phureja clones and the *S. tuberosum* varieties. However, these were present in very small amounts and did not have a major influence on to first two principal axes that, together, explain almost 95% of the variability in the data set. None of these was correlated with acceptability.

Given that differences between Tuberosum and Phureja were seen in both sensory and volatile analysis, correlations were sought between the two data sets – initially all correlations greater than 0.5 (-0.5) were considered. Only two compounds, pentanal and methyl-hexanal, correlated with acceptability and both were negative with respect to this attribute. Using simple linear regression pentanal accounted for 29.2% of variance ($p = 0.006$) and methyl-hexanal 23.5% of variability ($p = 0.013$). The compound α-copaene, although not correlating with acceptability, was positively correlated with intensity of aroma and flavour. Many of the sensory attributes that correlated in any way with volatile compounds were to do with Earthiness (2,3-pentanedione, 2-pentanal, 2,4-octadienal, 2,4-heptadienal) and metallic flavour and aftertaste (2-methylbutanol, pentanal, dimethyl disulphide, dimethyl trisulphide).

Other compounds that showed lower degrees of correlation with acceptability were Pentane, Pentanol, and dimethyl trisulphide. Again, these were all negatively correlated, and in simple linear regression accounted for 32.5% ($p = 0.003$), 13.6% ($p = 13.6$) and dimethyl trisulphide 19.5% (0.023). In multiple linear regression, it was possible to explain 34.4% if the variability with respect to acceptability with the compounds Pentane, Pentanal, Pentanol, Methyl hexanal and dimethyl trisulphide.

However, the compounds that distinguished the two species were not those that were related to acceptability. There were many correlations between the various volatile compounds. These, as yet, have not been analysed.

Volatile analysis of FT.4 population

In year one of the project, an established method was used for the entrainment of volatile compounds released from cooked potato tubers. An 'improved' method was adopted for the analysis of the FT.4 population. It was decided that all future entrainments would be carried out at 50°C over the course of 2 h. This entrainment time gave chromatograms of comparable quality to 24 h entrainment at ambient temperature. Then a new gas chromatograph-mass spectrometer was commissioned immediately prior to the analysis of the FT.4 backcross population replicate in year three. The increased sensitivity of the new instrument required that volatile entrainment was minimized to prevent saturation of the largest peaks on the gas chromatograms. Therefore, for year three's analysis, the entrainment conditions were carried out at 50ºC for half an hour.

The problem of expressing volatile concentrations as relative to overall volatile profile were addressed by adding fixed amounts of non-intrinsic standards to the sample matrix. The standards chosen were 2-Pentanone and β-Ionone, neither of which coincides with intrinsic volatiles. This allowed concentrations to be expressed relative to the standards, giving results that, while not absolute, were more representative and could be better related to the sensory evaluation and genetic analyses. In year two, β-Ionone and 2-Pentanone were used at a volume of 5 µl each. In year three, only β-Ionone was added as a standard as it was felt that the use of two standards was unnecessary, and the volume added to the mashed potato matrix was reduced to 1 µl, again to minimise the signal to prevent saturation.

Peaks on the GC-MS chromatograms were assigned by correlating retention times and mass spectra with those of known standards. A total of 41 volatile compounds (see Fig. 7 and Table 5) were quantified and normalised against the β-Ionone peak. These values were calculated for a total of 114 samples from the FT.4 backcross population, plus controls.

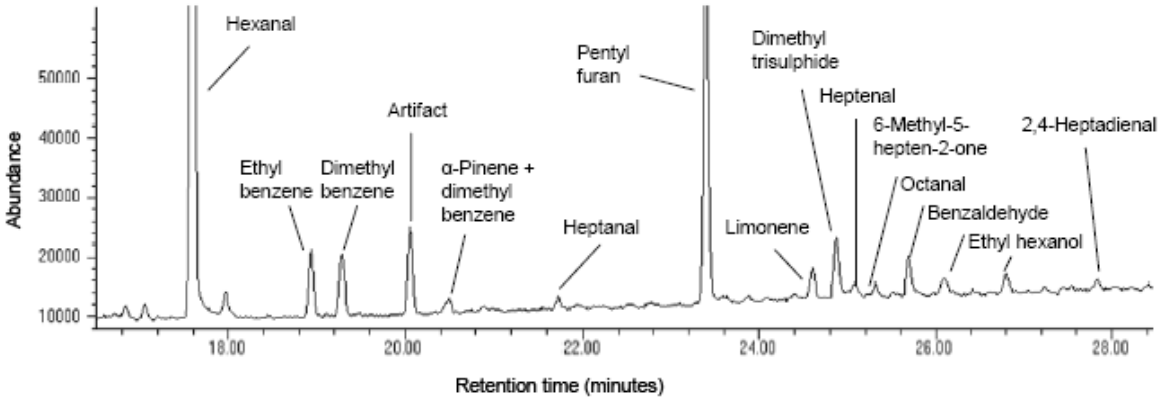

FIGURE 7: REPRESENTATIVE SECTION OF ATD-GC/MS TOTAL ION CHROMATOGRAM TRACE OF COOKED PHUREJA/TUBEROSUM HYBRID POTATO TUBERS, SHOWING A NUMBER OF FLAVOUR-RELATED VOLATILE ORGANIC COMPOUNDS.

Propanal	Ethyl benzene
2-Propanone	Dimethyl benzene
2-Methyl Propanol	Butyl furan
3-Methyl Furan	2-Methyl-2-Butenoic acid methyl ester.
2-Methyl Furan	5-Methyl hexanal
Butanal	2-Hexenal
2-Butanone	Heptanal
3-Methyl butanal	Pentyl furan
2-Ethyl furan	Methional
2-Methyl butanal	2,3-Octanedione
Pentanal	Dimethyl trisulphide
2,3-Pentanedione	2-Heptenal
Octane	Artifact/octanal
Dimethyl disulphide	Benzaldehyde
Methyl benzene	Ethyl hexanol
2-Methylbutanoic acid, methyl ester	Octenal
3-Methylbutanoic acid, methyl ester	Nonanal
Pentenal / Pentanol	Decanal
Unknown compound a	β-Cyclocitral
Hexanal	α-Copaene
Unknown compound b	

TABLE 8: VOLATILES SCORED IN FT4 BACKCROSS POPULATION, ARRANGED BY GC RETENTION TIME.

Molecular analysis

The FT.4 backcross population comprising 122 clones and both parents of the cross [99.FT.1(5) and DB337(37)] were initially analysed with a set of 21 AFLP and 16 SSR primer pairs yielding a total of 219 segregating markers. Linkage mapping, using these segregating markers, identified 11 of the 12 expected linkage groups. Out of these, 5 linkage groups were successfully assigned to their respective chromosomes, however, the size of chromosome 2 appeared distorted. To further resolve the linkage mapping and assign all linkage groups to chromosomes, screening and analysis of additional 5 SSR markers was performed during year 3. The final molecular analysis of AFLP and SSR data (21 primer pairs of each type), involved 239 segregating markers. These data allowed the assignment of all 12 linkage groups to their respective chromosomes (Figure 8).

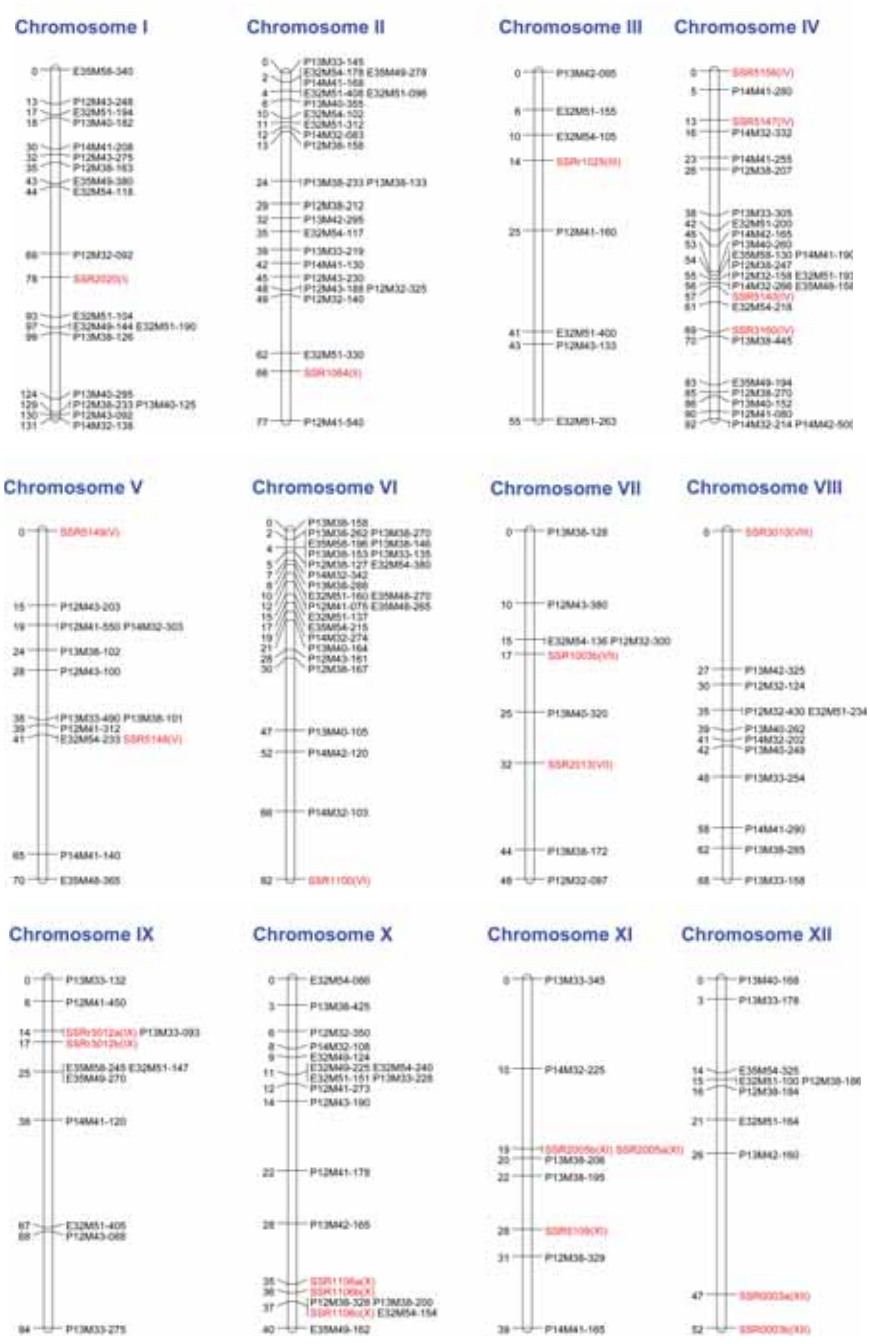

FIGURE 8. TWELVE LINKAGE GROUPS THAT HAVE BEEN ASSIGNED TO THE TWELVE POTATO CHROMOSOMES. SSRS USED TO ASSIGN LINKAGE GROUP TO A CHROMOSOME ARE INDICATED IN RED.

QTL analysis has performed on the sensory and volatile data. QTL interval mapping using the program MapQTL was a problem in the FT.4 population as (i) the population was quite small (ii) due to the crossing scheme and limited number of markers there was not good coverage of the contribution of both parents to all of the the chromosomes. Another important consideration was to examine the 'power' of the QTL analysis, which is tantamount to estimating the probability of detecting a QTL effect of a given size. For a QTL explaining 10% of the trait variance and a single marker segregating in a 1:1 ratio at the same position as the trait, we could use a test with significance level 0.05, and the probability of detection is 0.947 for a population of 122. For many markers, we need to use a more stringent significance level to avoid too many false positives. For a 'noteworthy' effect, the probability of detection is reduced to 0.765 and for an effect that is significant by a permutation test, the probability of detection is 0.480. Increasing the population size to 250 plants would increase the probability of detecting a QTL explaining 10% to 0.938. The probability of detection is reduced if the marker is further from the QTL, or if it segregates in a 3:1 ratio. As phenotyping large populations for volatile and sensory traits is problematic, both on the grounds of cost and experimental design, future projects could use approaches such as selective phenotyping.

These considerations led us to conclude that the safest approach is a marker regression method, either by a non-parametric approach such as the Kruskal-Wallis test, which occurs in MapQTL, or a regression of the trait on the marker. This can easily be done in Genstat, as can the Kruskal-Wallis test. However, these types of analysis raise a problem with the level of significance to use. A permutation test (based on 1000 permutations of a single trait, Salty) was carried out in Genstat to establish levels of significance for this population. The quantiles are:

Proportion	Quantile
0.5000	7.99
0.7500	9.57
0.8000	10.02
0.8500	10.65
0.9000	11.33
0.9500	**12.64**
0.9900	15.28

Marker-trait associations that exceed the 95% point of the permutation test (i.e. KW statistic > 12.6) are described as 'significant' (bold in Table 9), and those that exceed the 50% point (i.e. KW > 8.0) are described as 'noteworthy'. In Table 9 the most significant marker is shown for each QTL effect, but it should be noted that there will be other significant markers linked to this and that the QTL location will have a large confidence interval.

Due to the large number of zero values giving distributional problems in the 2005 volatile data, only the 2006 data has been used for QTL analysis. This was analysed after a log (trait + c) transformation, where c is a small constant equal to 0.01* (min value > 0). A robust analysis using the Genstat ROBSSPM procedure was used to weight outlying observations to reduce their influence.

A large number (120) QTL effects were detected in the Kruskal-Wallis analyses (Table 9). Overall 21 marker loci are in the significant category in that they are associated with at least one trait with a KW statistic greater than 12.6. A further 34 marker loci were associated with at least one of the traits at a lower significance level (KW > 8.0).

Chromosome	Map pos	Marker	Trait
I	0.00	E35M58-340	F-Creamy06 F-earthy06
	44.04	E32M54-118	2_3_Octanedione06
	97.35	**E32M49-144**	**Shiny06 (Grainy06, Floury, Moist_dry06)**
	128.93	P12M38-233	F-Intensity06
	130.41	P12M43-092	2_Butanone06, Benzaldehyde06
II	5.68	P13M40-355	2_Heptenal06
	9.79	E32M54-102	Earthy
	11.17	E32M51-312	F-Metallic
	11.76	P14M32-083	Methional06
	24.12	**P13M38-233**	**Shiny, Floury, (Grainy, Chalky05)**
	29.38	**P12M38-212**	**White, Yellow, (O-creamy05)**
	38.67	P13M33-219	5_Methyl_hexanal06
	47.51	P12M43-188	Sweet, Chalky06, Pentenal/pentanol06, 2_3_Octanedione06
	62.20	**E32M51-330**	**Moist_dry05, (White, Yellow, Sticky05)**
III	**9.89**	**E32M54-105**	**White, Yellow, (F-earthy05)**
	25.46	P12M41-160	Salty05, Sweet05
IV	92.17	P14M42-500	F-Metallic05
V	**15.05**	**P12M43-203**	**O-earthy05**
	19.11	P14M32-303	2-Heptenal06
	27.67	**P12M43-100**	**Salty05, (Acceptability05)**
	38.63	P12M41-312	Hexanal06, 5_Methyl_hexanal06
	64.69	P14M41-140	Acceptability06
	69.64	E35M48-365	White, Yellow
Va	**0.00**	**E35M58-220**	**2-Heptenal06, (Propanal06, Pentanal06, Hexanal06)**
	24.99	**P13M38-165**	**F-Metallic, A-Metallic, Bitter, A-Intensity06, (Acceptability05)**
VI	11.95	P12M41-075	2_Methyl butanal06
	15.18	E32M51-137	Floury05
	17.14	P14M32-274	F-Intensity06, O-Intensity06
	60.81	**P14M42-120**	**2-Heptanal06, 2_Octenal06, (Butanal06, Butyl furan06, 2_Hexenal06, 2_3_Octanedione06, 2-Octenal06)**
VII	0.00	P13M38-128	O-creamy05
	24.92	P13M40-320	Dimethyl_trisulphide06, 2_3_Octanedione06
	44.36	P13M38-172	Moist_dry05, 2_Octenal06
	48.34	**P12M32-097**	**Sticky, (Floury)**
VIII	0.00	SSR3010(VIII)	2_Methyl_Propanal06
	27.06	P13M42-325	109_124a06
	30.35	P12M32-124	O-earthy05,2_Ethyl_furan06, 5_Methyl_hexanal06, 2_Heptenal06
	34.62	P12M32-430	O-creamy06
	67.87	**P13M33-158**	**Alpha copaene06**
IX	**13.97**	**P13M33-093**	**Alpha copaene06**
	24.88	E32M51-147	Grainy05, Shiny05, Floury05,Moist_dry05

	93.56	P13M33-275	F-Intensity05
X	**6.36**	**P12M32-350**	**Grainy05, (Shiny05)**
	11.283	P13M33-228	F-Creamy06, Floury05
	12.03	**P12M41-273**	**Moist_dry06, (Chalky05)**
	13.87	P12M43-190	Butanal06, 2_Heptenal0
	22.05	**P12M41-178**	**Acceptability (O-Creamy06, F-Metallic06)**
	36.12	**SSR1106b(X)**	**Butyl furan06, (F-Intensity06, Pentenal/pentanol06**
	36.68	P12M38-328	2_Butanone06
XI	24.29	SSR2005a(XI)	F-Intensity06
	25.437	P13M38-206	Beta_cyclocitral06
XII	**14.05**	**E35M54-325**	**F-Intensity06, (O-Intensity06, O-earthy06)**
	21.04	**E32M51-164**	**Sweet, (Floury, Moist_dry05, Chalky06, Grainy05)**
	25.84	P13M42-160	Salty06
?	**0.00**	**P14M32-306**	**2-Methyl Propanal06, 2-Butanone06, Benzaldehyde06, (Salty06, 2_Methyl_butanal06, Ethyl_hexanol06, Nonanal06)**
	21.11	**E32M51-264**	**White, Yellow**

TABLE 9. MAIN QTLS EFFECTS DETECTED IN THE KRUSKAL-WALLIS ANAYSIS OF SENSORY AND VOLATILE DATA. MARKERS SHOWN ARE THOSE EXPLAINING LARGEST TRAIT EFFECT.
QTL LOCI IN BOLD TYPE ARE THOSE WITH SIGNIFICANT EFFECTS (I.E. KW STATISTIC > 12.6)

Discussion

This project was designed with ambitious and groundbreaking objectives. Previous attempts to gain an understanding of the genetics of potato flavour have not made much progress, as evident from the almost complete lack of publications on this topic, save for a few metabolite profiling experiments on conventional cultivars (e.g. Beckmann et al. 2007). The main scientific objective of this project was to gain a fundamental understanding of the genetic and biochemical control of flavour characteristics in potato using well-established, but previously unquantified, differences between conventional Tuberosum potatoes and the more extreme Phureja type of potato. The commercial objectives were: i) the generation of Phureja potato products with consumer appeal; ii) improved Tuberosum breeding material carrying desirable Phureja flavour and "mouth-feel" characteristics; iii) the development of molecular and biochemical markers that can be used for marker-assisted breeding (MAB). It is clear that the project has progressed most of these areas to a considerable extent.

We have identified some (~30) essential, and possibly highly significant volatile differences between Phureja and Tuberosum cultivars and breeding clones. The compounds having most influence on a PCA were hexanal and α-copaene, the first being significantly greater in Tuberosum cultivars and the latter in Phureja clones. Other significant differences were seen for methional, nonanal and decanal. These results have given the project team ideas about likely candidate genes that impact on these volatile differences, although it is possibly that some or all of these compounds are not related to sensory properties of the cooked tubers. Many of the compounds found to be different between the Phureja and Tuberosum material, were present in extremely small amounts, so we do not know at this point if these differences are likely to impinge on sensory characters.

Sensory analysis of the core set of Phureja and Tuberosum clones found significant differences between the two species groups, for several traits: yellowness, whiteness, flavour intensity, after-taste intensity, sweetness, saltiness, and acceptability being the most significant. Colour aside, flavour intensity was the character that most distinctly distinguished Phureja and Tuberosum material. If the clones surveyed are ranked on the basis of the 'catch-all' trait of acceptability, four of the six Tuberosum varieties were in the bottom five. The highest ranking Tuberosum variety, Anya, only ranked ninth of the 22 clones. Record was by far the lowest scoring variety for this character. Although there were differences between the 'species' with respect to sensory attributes, no individual coordinate unambiguously separated them. An attempt to try to identify the sensory attributes that account for the main differences in acceptability revealed that creamy flavour (r = 0.77), flavour intensity (r = 68), sweet (r = 0.58) and salt (r = 0.54) were most strongly correlated positively with acceptability. Negatively correlated characters were metallic after-taste (-0.53) and metallic flavour (-0.52). Some of the correlations were accepted by the presence of the extreme outlier Record. An interesting observation was that flavour intensity, while highly correlated with acceptability, did not contribute to it significantly. Although this might appear counterintuitive, it is explained by the fact that flavour intensity is accounted for by other attributes such as creamy, sweet and salty flavour.

Given that differences between Tuberosum and Phureja were seen in both sensory and volatile analysis, correlations were sought between the two data sets. Two compounds, pentanal and methyl-hexanal, were found to be negatively correlated with acceptability. The compound α-copaene, although not correlating directly with acceptability, was positively correlated with intensity of aroma and flavour. Significant correlations were found between several volatiles and the 'earthy' sensory characteristics. these included 2-pentenal, which is described as having a green, apple-like odour, as well as 2,3-pentanedione, 2,4-octadienal and 2,4-heptadienal, each of which have oily, musty odours that would contribute to earthiness. Several volatiles correlated with metallic flavour and aftertaste, including 2-methylbutanol, pentanal, dimethyl disulphide and dimethyl trisulphide. Of these, the sulphides are of particular interest, as sulphides, in general, are known to cause metallic off-tastes. Other compounds showing smaller correlations with the general acceptability characteristic were Pentane, Pentanol, and dimethyl trisulphide (all negative). The volatile compounds that distinguished the tuberosum cultivars with the phureja clones were not those that were related to acceptability.

Genetic analysis of sensory and volatile traits focussed on the FT.4 population. It was chosen from a set of four populations, due to its high levels of vigour, good tuber yields, and seemingly high levels of phenotypic variation. This population was subjected to various types of phenotypic analysis (sensory and volatile analyses, as well as image analysis and general agronomic phenotyping) as well as genotypic analysis with molecular markers. By performing the genotyping we were able to construct a linkage map of the FT.4 population and could identify all twelve of the potato chromosomes. This process was greatly assisted by the use of microsatellite markers which, being of known chromosomal location, serve as 'anchors', for the potato chromosomes. The linkage map serves as a framework on which to base the QTL analysis of the volatile and sensory data for the FT.4 population.

Various factors prevented the use of a QTL 'interval mapping' approach, so instead a 'marker regression' approach was adopted. This necessitated the use of a permutation test to establish levels of significance for the FT.4 population. Marker-trait associations that exceed the 95% point of the permutation test (i.e. KW statistic > 12.6) were deemed 'significant', and those that exceed the 50% point (i.e. KW > 8.0) as 'noteworthy'.

A large number of QTL effects (~120) were detected in the QTL analyses. These have tended to occur in clusters of up to seven different traits (see Table 9). Linkage groups I, II, V, VIII and X appear to be particularly 'QTL-rich'. Chromosome II contains QTL effects for a large number of both sensory and volatile traits, whereas chromosome V contains mostly sensory trait QTLs. Chromosome VIII appears to contain a large number of primarily volatile effects, including a significant effect for α-copaene. Interestingly, chromosome IX also contains a significant QTL for α-copaene. Chromosome X appears to harbour four discrete loci with significant QTL effects, three impacting on sensory and one on both types of trait. Given the high correlations between some of the sensory traits and acceptability (e.g. intensity, creamy) it is interesting to consider the locations of QTLs for these. Chromosomes I (F-intensity), V (Acceptability), VI (F-intensity), IX (F-intensity), X (F-intensity, F-creamy, acceptability) and XII (F-intensity) contain the majority of QTLs that impinge on acceptability or the traits most highly correlated with it. Therefore loci on these chromosomes are possibly the ones that we should focus upon with regard to marker assisted breeding and candidate gene analysis. However it should be borne in mind that the trait 'acceptability' is based on preference of a small set of evaluators from a geographically limited area, and who had been trained to score for specific traits. It is possible that their assessment of acceptability may not reflect that of the general consumer, particularly from a wider geographical distribution. Another aspect is the location of genes which act on the volatile trait 'α-copaene', present to differing extents in the two types of potato examined. Significant QTLs for this trait map to locations on chromosomes VIII and IX. This is a trait where, in principle, a candidate gene approach could be adopted. This compound, one of a family of sesquiterpenoids, results from a biosynthetic pathway that is quite well understood, and an initial approach would focus on the role of sesquiterpene synthases, which may well be one of the primary determinants of α-copaene levels in potato tubers. Similarly QTLs for 'hexanal', another key volatile in the Phureja-Tuberosum analysis, map to chromosomes V and VI. Identification of candidate genes responsible for the formation of this volatile, possibly involved in lipid oxidation, should also be feasible.

In the results section the most significant marker is shown for each trait, but it should be noted that there will be other significant markers linked to this and that the QTL location will have a large confidence interval. This means that size of the chromosomal regions that may contain the genes corresponding to the various QTLs may be quite large, so gene isolation will not be a realistic possibility, of course unless good candidate genes can be identified which map to the QTL location. In such cases proof of function will necessitate a GM approach, whereby the candidate gene is over-expressed in a suitable genetic background.

Conclusions

In this project significant advances have been made in the analysis of flavour in cooked potatoes. Essential differences between volatiles and sensory results have been catalogued for conventional cultivars and the more extreme Phureja type of potatoes. By using a genetic cross between the two types of potato, a picture of the complex genetics of sensory traits in potato has begun to emerge. Despite this complexity there are sufficient indications that will allow us to refine these analyses in the future.

We have a strong indication of the importance of sesquiterpenoids, α-copaene in particular, in potato flavour. Phurejas are generally more abundant in these compounds than conventional potatoes. Future work should focus on establishing the genes and gene products involved in the synthesis of α-copaene in potato tubers. Similarly identification of the genes underlying the 'umami' trait (Morris et al 2007) which appears to correlate highly with some of the sensory

scores obtained in this study, albeit based on a very small sample, is a key target of future work. In the near future as part of core and EU-funded research we will, resources permitting aim to carry out the following:

- Make transgenic plants for an up-regulated copy of a potato sesquiterpene synthase in a genetic background that does not produce any α-copaene (ongoing, Dr Mark Taylor) to assess whether this has the effect of increasing the amounts of α-copaene in the tubers.

- Analyse the umami trait on the FT.4 population. Preliminary work in Mark Taylor's group suggests that this trait can be measured quite accurately and relatively cheaply. Tuber material has been prepared for this analysis, which will take place in spring/summer 2008. QTL analysis of the resultant data will permit us to determine to what extent, if any, QTLs for any of the sensory traits overlap with QTL effects for 'umami-ness'. Such relationships would lead us into research areas involving trying to identify the causative factors behind the umami trait, thought to be due primarily to synergies between the presence of glutamate (an amino acid) and associated glycoconjugates with 5'-ribonucleotides, generated by RNA breakdown. One hypothesis that umami may, in part, be determined by textural differences allowing 'better access' to the substrates for the nucleic acid breakdown processes.

- Textural analysis of the FT.4 population will allow us to gain an understanding of the relationship, if any, between flavour and texture. SCRI has developed a robust method for analysing cooked potato texture and these measurements are ongoing as part of an EU-funded study.

- Identify gene expression differences between typical Tuberosum cultivars and Phureja clones. To this end we have, in collaboration with the group of Dr Mark Taylor, performed a microarray experiment to establish gene expression differences between Phureja and Tuberosum tubers at a series of different developmental stages. These experiments have identified a set of candidate genes, some of which have been prioritised for future work (mapping, targeted expression analysis, transgenics etc). This analysis is being prepared for publication, ad will be submitted in the near future.

References

Beckmann M, Enot D P, Overy D P, Draper J (2007) Representation, Comparison, and Interpretation of Metabolome Fingerprint Data for Total Composition Analysis and Quality Trait Investigation in Potato Cultivars. J Agric Food Chem 55: 3444-3451

De, Maine M J, Carroll C P, Torrance C J W. 1993. Culinary quality of tubers derived from *Solanum-Phureja* and *Solanum-Tuberosum* X *Solanum-Phureja* hybrids. Journal of Agricultural Science 120: 213-217

Dobson G, Griffiths D W, Davies H V, McNicol J W. 2004. Comparison of fatty acid and polar lipid contents of tubers from two potato species, *Solanum tuberosum* and *Solanum phureja.* Journal of Agricultural and Food Chemistry 52: 6306-6314.

Griffiths DW, Dale MFB, Morris WL, Ramsay G. 2007. Effects of Season and Postharvest Storage on the Carotenoid Content of *Solanum phureja* Potato Tubers. J Agric Food Chem 55: 379-385

Morris WL, Ross HA, Ducreux LJM, Bradshaw JE, Bryan GJ, Taylor MA (2007) Umami compounds are a determinant of the flavour of potato (*Solanum tuberosum* L.). J Agric Food Chem 55: 9627-9633

3. Project deliverables

- Methods for volatile analysis of cooked potatoes.
- Methods and experimental designs for sensory analysis of large numbers of cooked potato samples.
- Lists of volatile compounds that differ in concentration between Phureja and Tuberosum tubers.
- Sensory data on Phureja and Tuberosum clones and cultivars.
- QTLs for sensory and volatile profiles of cooked potatoes.
- Molecular markers linked to QTLs for sensory and volatile traits which may be used in breeding work.
- Diploid breeding clones with improved flavour attributes over conventional Tuberosum material.
- Project final report
- Publications based on the analyses presented in the project final report (in preparation May 2008).