Report

FV 351 (LK 09126)

Understanding Quality Determinants in Pea Seeds to improve market opportunities that promote sustainable agriculture

(QDiPS: drivers of a sustainable agriculture)

Annual 2012

Disclaimer

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

HDC
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel – 0247 669 2051

-

Project Number:	FV 351 (LK 09126)
Project Title:	Understanding Quality Determinants in Pea Seeds to improve market opportunities that promote sustainable agriculture (“QDiPS”)
Project Leader:	Claire Domoney, John Innes Centre
Report:	Second annual report - February 2012
Publication Date:	20 June 2012
Start Date:	January 18th 2010
End Date:	July 17th 2013
Project Cost (total project cost):	£0 - HDC in-kind support only

Sustainable Arable LINK Programme
Annual Project Report

Date	February 2012
Report No.	Second annual report
Project Title	Understanding Quality Determinants in Pea Seeds to improve market opportunities that promote sustainable agriculture (QDiPS: drivers of a sustainable agriculture)
Project Code (LK09XX)	Defra code = LK09126
Start Date	January 18th 2010
End Date	July 17th 2013
Last Project Management Meeting Date	January 26th 2012
Lead Participant	John Innes Centre
Project Leader	Dr C. Domoney

Summary of Project Progress

Comment on the progress towards achieving the objectives for the project as articulated in the original project proposal. Describe the scientific highlights and preliminary results. Include reasons for the change(s), and indicate how these have been agreed with sponsors and industry partners.

Analysis of seed compositional changes (macromolecules and metabolite) relevant to food use

A. Genetic mapping and metabolite analysis: vining peas (*r* and *rb* genotypes)

In the first annual report, we described how data acquired for vining pea samples from the industry at a range of tenderometer (TR) values could be used to determine the equivalent TR stages of seeds from laboratory genetic stocks. The relationship between TR value and dry matter accumulation in vining cultivars allows us to stage developing seeds of mapping populations, without the necessity to grow the large plots that are needed for direct measurements using a tenderometer. Three mapping populations that are densely populated with genetic markers were grown in microplots at JIC and at PGRO, using 100 vining type (*r* or *rb*) recombinant inbred lines (RILs) plus parent lines. At JIC, $3m^2$ plots were sown for immature harvest and separate $1m^2$ plots for mature seed harvest. The latter were planned to yield seeds at maturity for metabolite analysis and to supply seeds for 2012 field plots at both PGRO and JIC. Metabolite analysis of seeds from mapping populations will allow us to determine the genetic location of genes that influence the content of metabolites that are significant to the industry; these genetic loci can be exploited by breeders within marker assisted selection programmes.

TR values reflect, to a large extent, water content and the relationship between dry matter and water content suggested that the former should be a good predictor of TR in developing seeds. Based on mature seed weight data for 20 cultivars, and determination of dry weight in corresponding freeze-dried TR100 samples, the dry matter accumulated in seeds at TR100

was determined to be approximately 44.6 (± 3.8) % of that at maturity. Figure 1 shows examples of the immature stages collected from RILs grown in field plots at JIC in 2011, and their mean dry weights in comparison with the dry weights predicted for stages equivalent to TR100. For all the JI 15 x JI 1194 RILs, the desired stages corresponded to seeds within medium (M) or large (L) seed samples (Fig. 1).

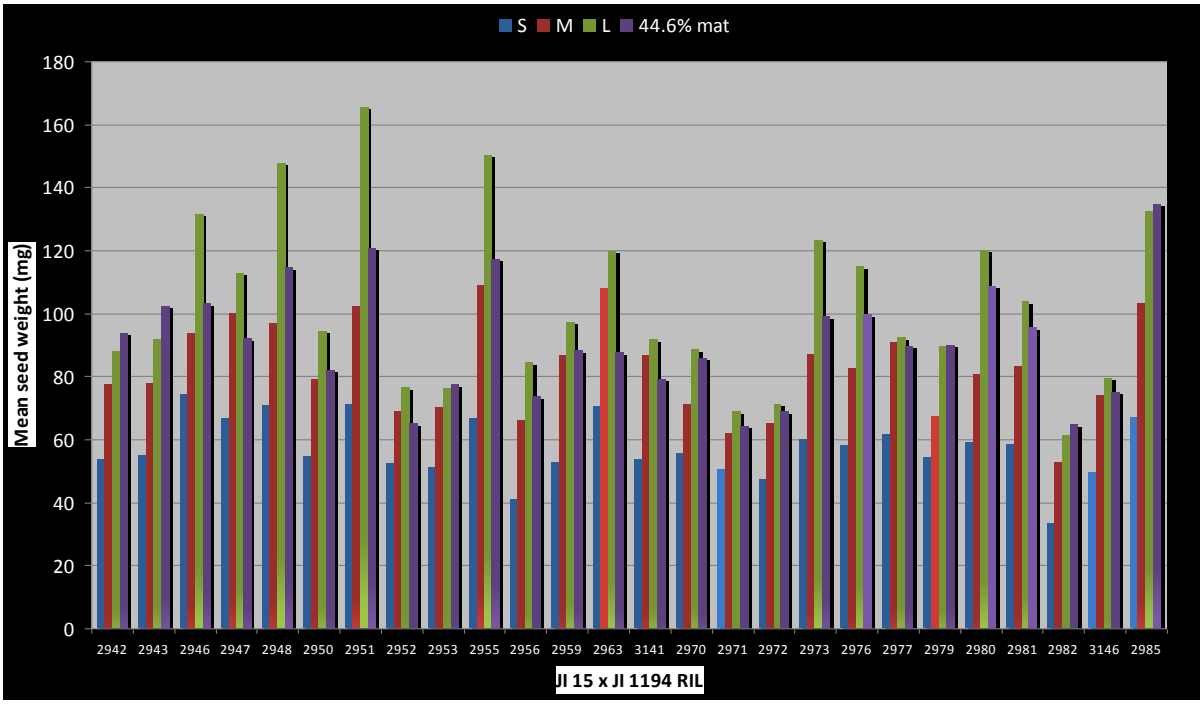

Figure 1: Three stages of development (S, M, L) were harvested and dry matter determined for immature seeds of every RIL, in comparison with that predicted for TR100 of every line (44.6% of mature seed weight). Twenty-six RILs are shown. For clarity, error bars are not displayed.

At PGRO, TR100 samples were collected from a subset of RILs for sensory evaluation, as well as mature seed samples from all RILs and parents.

Immature and mature seed samples from both field sites have been delivered to Fera for quantitative metabolite analysis and these data will be used to identify quantitative trait loci based on the genetic maps that are available. Seeds for the 2012 plot trials have been delivered to PGRO.

B. Genetic mapping and metabolite analysis: marrowfat peas (*Lox-3/Lox-2* genotypes)
Many off-flavours in foods have been attributed to co-oxidation products of the lipoxygenase (Lox) pathway. Low Lox genotypes have been identified in pea, where Lox activity is normally highest during late embryogenesis and hence this enzyme and its products are likely to be most relevant to the food industry using mature seed products. Three back-crosses have been carried out between the marrowfat parent lines (commercial cultivars, Princess, Kahuna

and Samson) and the low *Lox-3/Lox-2* genotype, H53 (a RIL derived from a cross involving a mutant, JI 1345). The F3 seeds from the F2 homozygous plants carrying the mutation were multiplied by Limagrain in a glasshouse in summer 2011, and both F3 and F4 seeds were sent to Premier for canning. (Note: since July 2011, the Premier canning business has been acquired by Princes). The F3 seed batches were small and provided limited quantities for taste evaluation at Princes, Long Sutton (JIC, Limagrain, PGRO and Princes staff). The F4 seeds are anticipated to allow sensory quantitative descriptive profiling (sQDA) by Campden BRI, in collaboration with Princes.

For 2012, a second low Lox genotype, L-3, having reduced amounts of Lox-3 enzyme only, will be multiplied for three-way comparison with parent and H53 genotypes. Genetic markers are available to follow these alleles in crosses, and this information has been made available to Limagrain breeding programmes. (The variants and markers may be exploited similarly in vining crops).

Seed samples of parent, H53 and L-3 backcross lines have been delivered to Fera for comparisons of their metabolite profiles, expected to show quantitative differences in lipid oxidation products that will link with sQDA analysis.

C. Novel genetic variants affecting seed composition and metabolite profiles: novel natural variants

In pea seeds, the most common mutations affecting the balance between sweetness and starch biosynthesis are *r* and *rb*, with lesions in starch-branching enzyme I and ADP-glucose pyrophosphorylase, respectively. DNA screens based on the mutations that have been widely exploited (in commercial cultivars) have been developed within QDiPS. These screens can be exploited within marker-assisted breeding programmes and will facilitate the early identification of *r* and *rb* genes in crosses, and the combination of individual mutations in breeding programmes where phenotype will not readily distinguish these.

The molecular screens also allow novel sources of genes influencing sweetness to be identified. The wrinkled seeded cv. Kebby, misclassified as a *rb* genotype, is actually neither *r* nor *rb* and crosses with the EMS mutagenised genotypes, *rug3* and *rug4*, suggest that the cv. Kebby carries a novel mutation. However, scoring seed phenotypes in crosses involving cv. Kebby is difficult and several experiments now suggest that the mutation may be maternally determined, affecting predominantly the testa phenotype, which is also subject to environmental effects.

Figure 2: Phenotype of F1 seeds derived from a cross between a rug4 *mutant and cv. Kebby (JI 2110)*

Figure 2 shows some of the F1 seeds derived from crosses between cv. Kebby and *rug4* (sucrose synthase) mutants. Although the majority of the F1 seeds show complementation (having round seeds), occasional F1 seeds appear wrinkled, suggesting some interaction between the two mutations, which is being investigated further. To facilitate mapping the mutation in cv. Kebby, a cross has been established with JI 281, a parent of one of the main mapping populations at JIC and IBERS. This cross is expected to provide genetic markers rapidly.

The DNA screens that identify the commercially exploited *r* and *rb* mutations can now be adapted to screen the wider germplasm collection for novel variation, thus allowing the relationship between starch, sugar and yield to be manipulated, alongside the approach adopted in D below.

D. Novel genetic variants affecting seed composition and metabolite profiles: novel induced variants

Novel variants for *r* and *rb* genes were identified among mutagenised genetic stocks having a wrinkled-seeded phenotype. In total, eight new variants are available for *r*, and three for *rb*. The positions of the mutations have been located in the corresponding genes (Figure 3), allowing molecular marker methods to be employed in screening crosses derived from these lines.

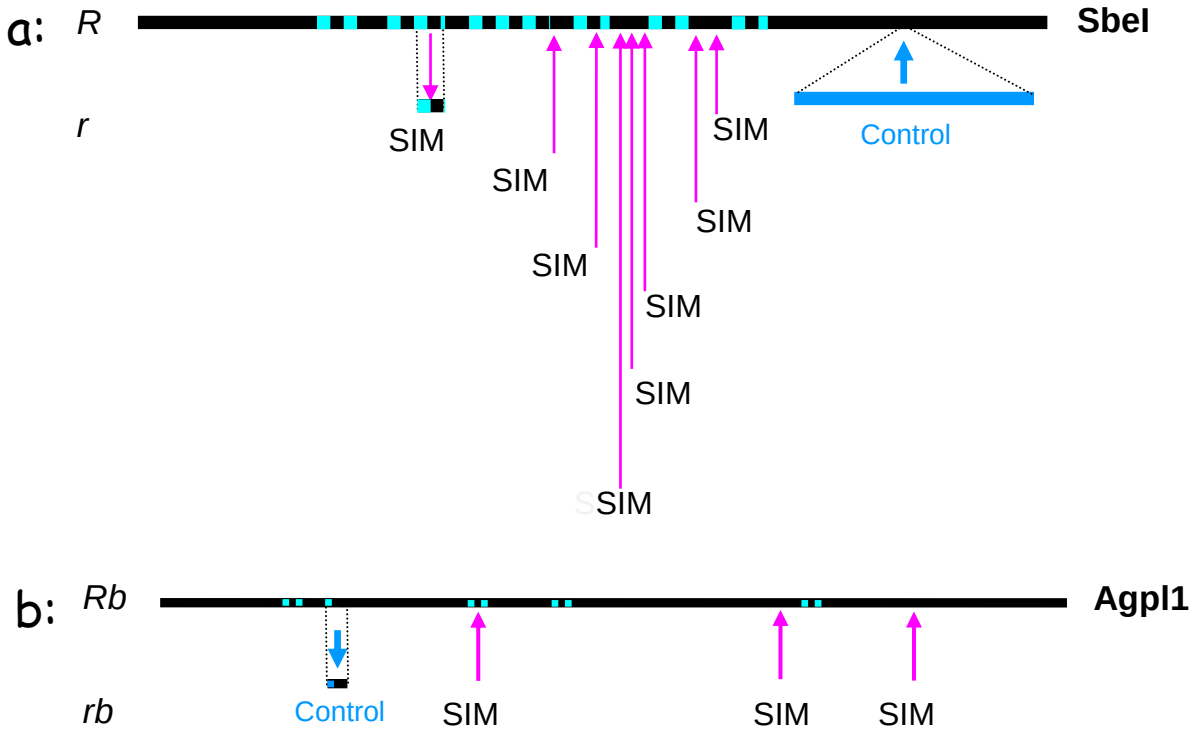

Figure 3: Diagrammatic representation of the positions of induced mutations (SIM) in r *and* rb *genes, encoding starch-branching enzyme I (SbeI) and ADP-glucose pyrophosphorylase (Agpl1), respectively, in comparison with controls (the natural mutations that have been commercially exploited).*

Preliminary metabolite data for the novel mutants, provided by GC-MS analysis, suggest that some mutants have altered seed composition relative to corresponding control lines (not shown). The seeds of all mutants have been bulked during winter 2011 to allow for these seed analysis to be repeated with replication, and to provide sufficient seeds for field plots in 2012, where the lines will be evaluated by the industry.

E. First comparison of maturity stage profiles with TR readings

1. Metabolite analysis of peas of differing TR value analysed by ^{1}H NMR spectroscopy.

^{1}H NMR spectroscopy was used to determine metabolites present in pea seeds that correlated with maturity as implied by the tenderometer (TR) value. Using the extraction methodology described and validated in the first QDiPs Annual Report, 79 freeze-dried pea seed samples and 16 in-house reference samples were analysed. Detailed information regarding the samples analysed is presented in Supplementary Table 1. A typical ^{1}H NMR spectrum acquired from the pea seed sample Bikini-1 is shown in Figure 4.

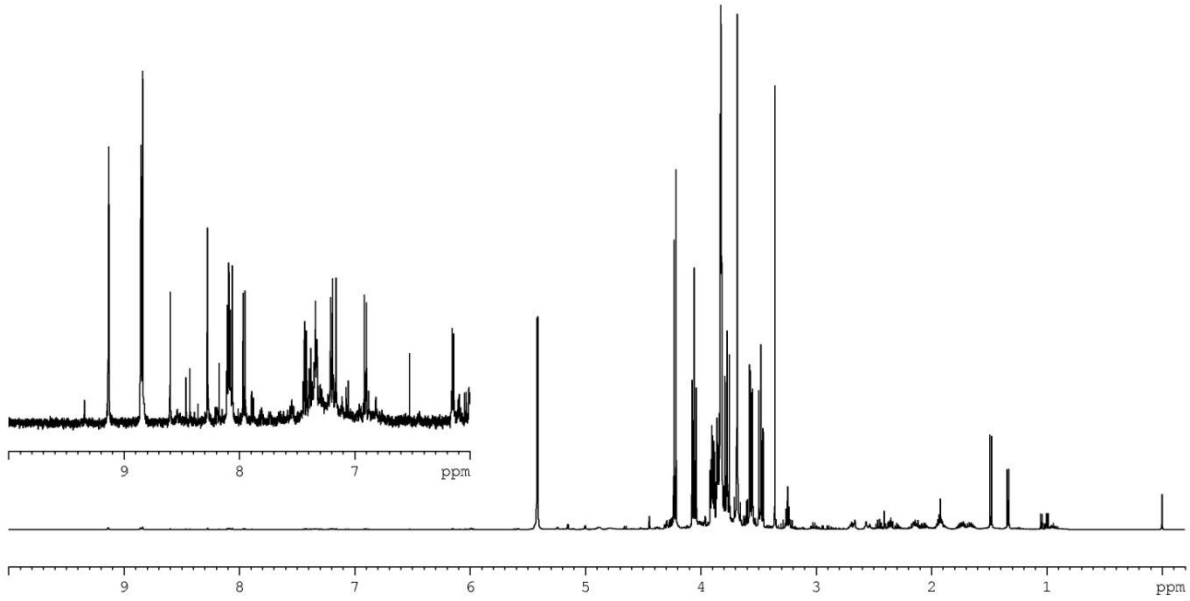

Figure 4. ^{1}H NMR spectrum acquired from sample Bikini-1. The region δ = 10 – 6 ppm has been vertically scaled to show several metabolites present at a lower intensity.

To determine the presence of underling trends in the ^{1}H NMR spectroscopic data they were analysed using principal components analysis (PCA). The PCA scores plot shown in Figure 5 determined that three groups of samples were significantly different from all other varieties analysed: Princess, Kahuna, and Samson, all marrowfat peas that were harvested at maturity.

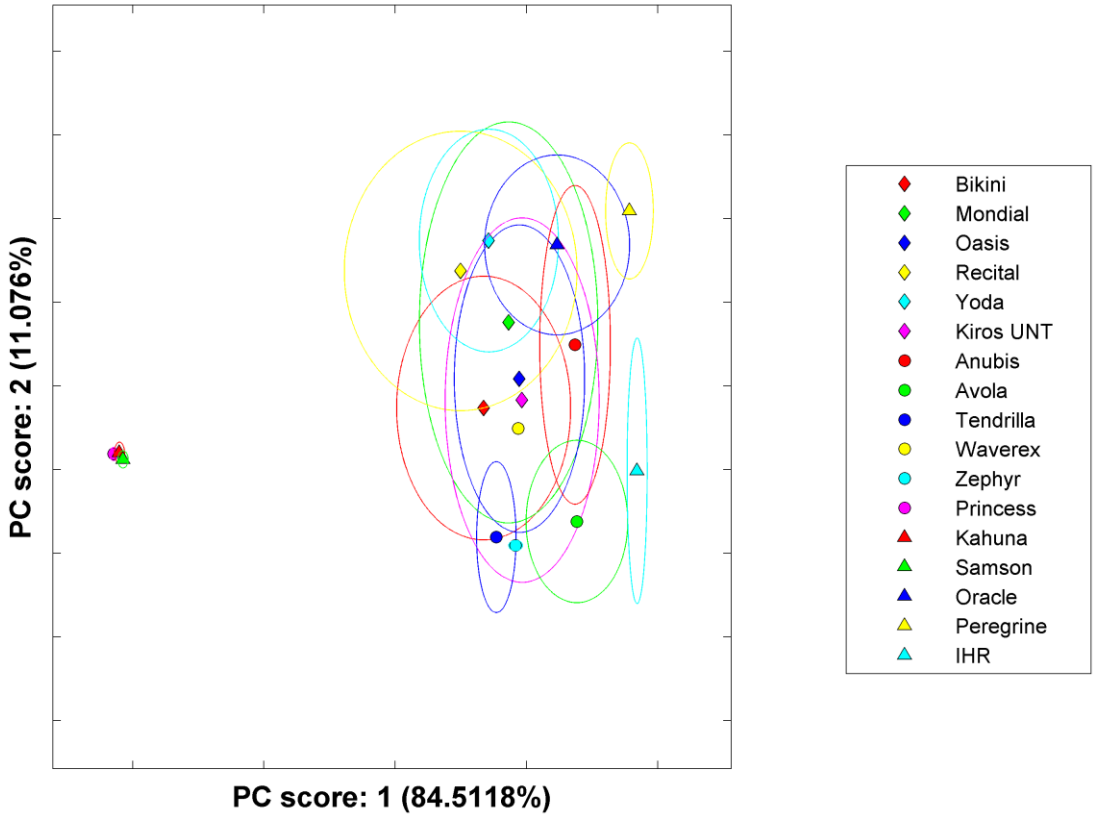

Figure 5. PCA scores plot (1 vs 2) of the ^{1}H NMR spectroscopic data of all pea samples analysed. Group centroids and standard deviations are shown.

Examination of the ^{1}H NMR spectroscopic profiles showed that the marrowfat samples differed significantly from all other pea samples analysed. In particular, it was noted that marrowfat peas were higher in raffinose, lower in sucrose and lower in a range of amino acids when compared to the immature pea seeds analysed. The marrowfat and in-house reference material samples were removed from the dataset and a second PCA performed coding the data using the TR values (Figure 6).

The PCA analysis showed a trend associated with TR value. To determine the presence of metabolites that correlated with TR value, linear regression analysis was performed. The ^{1}H NMR spectra were binned using an adaptive binning algorithm [1] to reduce the dataset size and to compensate for any minor changes in chemical shift between spectra. The binning algorithm reduced each NMR spectrum to 1017 resonances. Linear regression, correlating the intensity of the binned NMR resonances to the accurate TR value, was performed. Resonances with an R^2 value greater than 0.5 were selected as those showing a correlation with TR and this resulted in the identification of 90 resonances. Compound identification was performed using an in-house database of metabolites. In total, 85 of the resonances were identified and these corresponded to 11 compounds. The compounds that were shown to correlate with TR value were: alanine; arginine; gamma-aminobutyric acid (GABA); glutamate; glutamine; homoserine; isoleucine; leucine; lysine; sucrose and valine. The highest R^2 value of each compound is provided in Table 2. Figures 7 and 8 show the correlation of the

intensity of one resonance from valine, and one resonance from isoleucine with TR value, respectively.

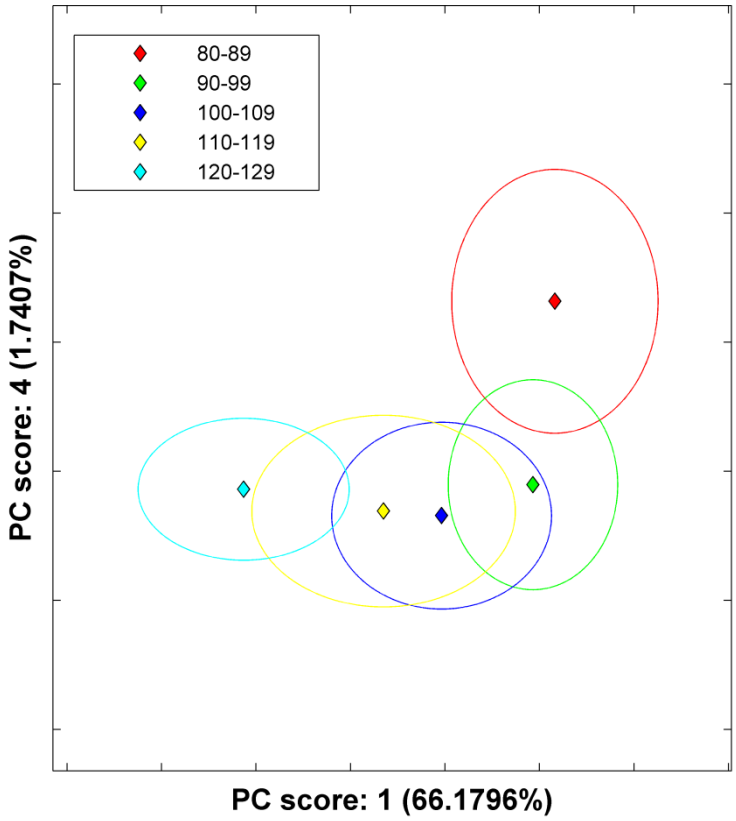

Figure 6. PCA scores plot (1 vs 4) of the ^{1}H NMR spectroscopic data of all immature pea seeds. Samples are coloured by their TR range described in the legend.

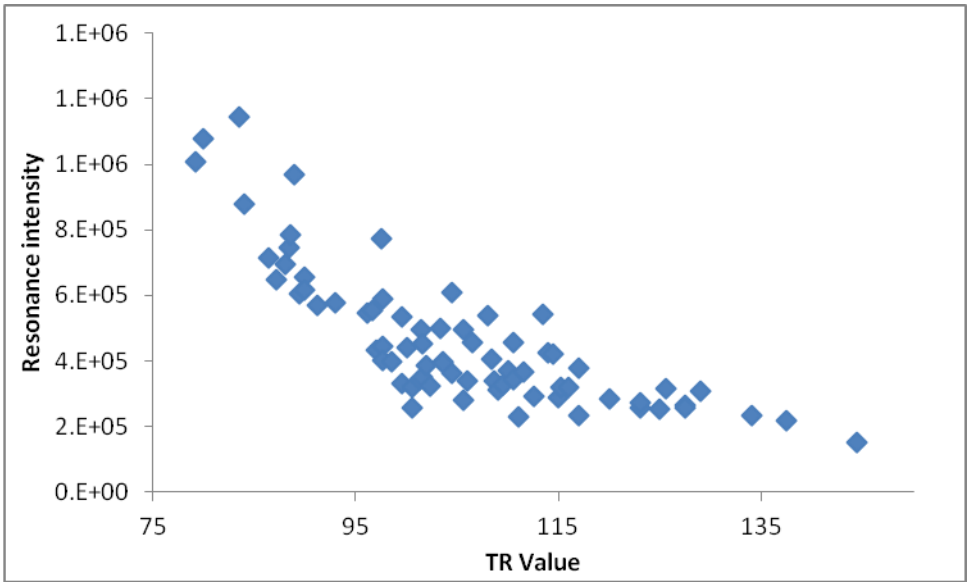

Figure 7. Intensity of a valine resonance (0.980 – 0.995 ppm) plotted against TR value (n=73)

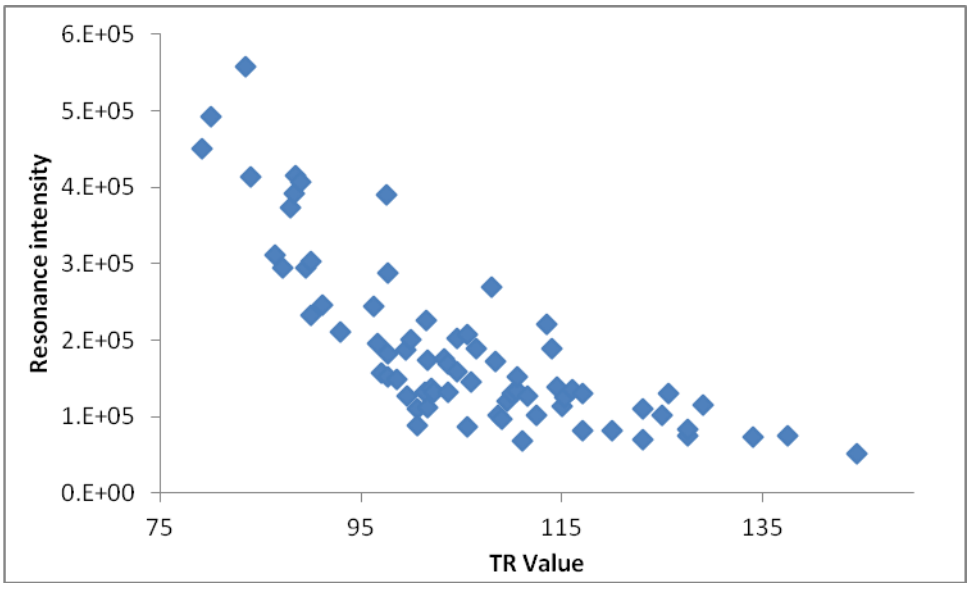

Figure 8. Intensity of an isoleucine resonance (1.014 – 1.029 ppm) plotted against TR value (n=73)

All identified metabolites showed a reduction in concentration as TR values increased. Examination of data acquired by a second analytical technique, liquid chromatography high resolution mass spectrometry (LC-HR-MS; experimental conditions are described in the first annual report) confirmed the identity of the compounds identified in Supplementary Table 2. LC-HR-MS identified an extra 11 features which are potentially associated with TR. The molecular formula of these features and a tentative identification (based on a metabolite database as part of the software package MAVEN[2]) is presented in Supplementary Table 3. These compound identifications require further analysis for verification.

2. Saponin analysis of pea seed samples

Of the compounds that may contribute to seed quality, saponins are candidates for bitterness, mouthfeel and sweetness. The two predominant saponin types in pea reported in the literature [3] are Saponin B and DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one). Their structures are shown in Figure 9. It has been suggested that DDMP could be the native saponin in peas and that DDMP is converted to Saponin B.

The aim of the following work was to measure relative concentrations of the two saponins by High Performance Liquid Chromatography-High Resolution Mass Spectrometry (HPLC-HR MS). Total saponin content was then related to the maturity of the pea, i.e. its TR value. Representative samples acquired from year 1 were analysed. The optimised extraction methodology for saponin analysis (see Supplementary protocols) is summarised in Figure 10. High Performance Liquid Chromatography (HPLC) was carried out on a Thermo Fisher Accela LC system. High Resolution Mass Spectrometry (HPLC-HR MS) was carried out on a Thermo Fisher Exactive MS. (For conditions employed, see Supplementary Tables 4 and 5). The range of samples analysed for saponins B and DDMP is given in Supplementary Table 6. An in-house reference sample (commercial frozen peas) was analysed with every batch.

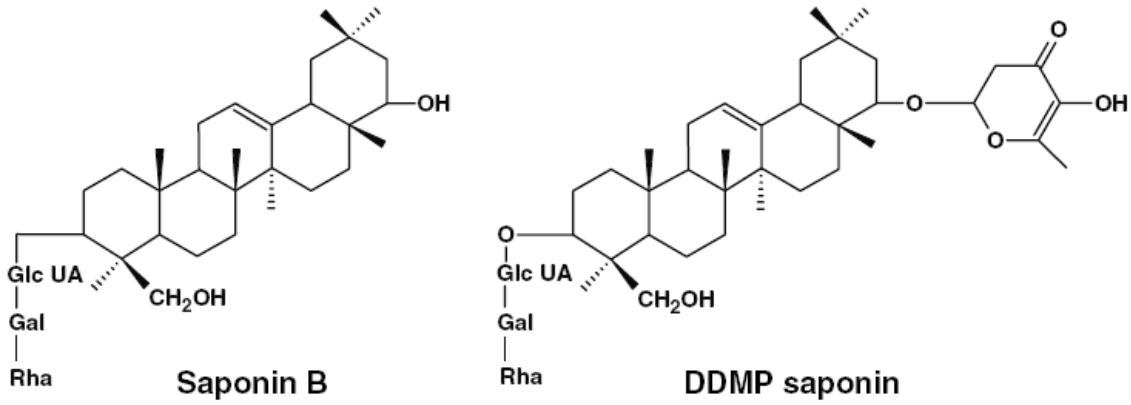

Figure 9. Structures of saponin B and DDMP saponins. 'Glc UA', 'Gal' and 'Rha' represent glucuronic acid, galactose and rhamnose, respectively [3].

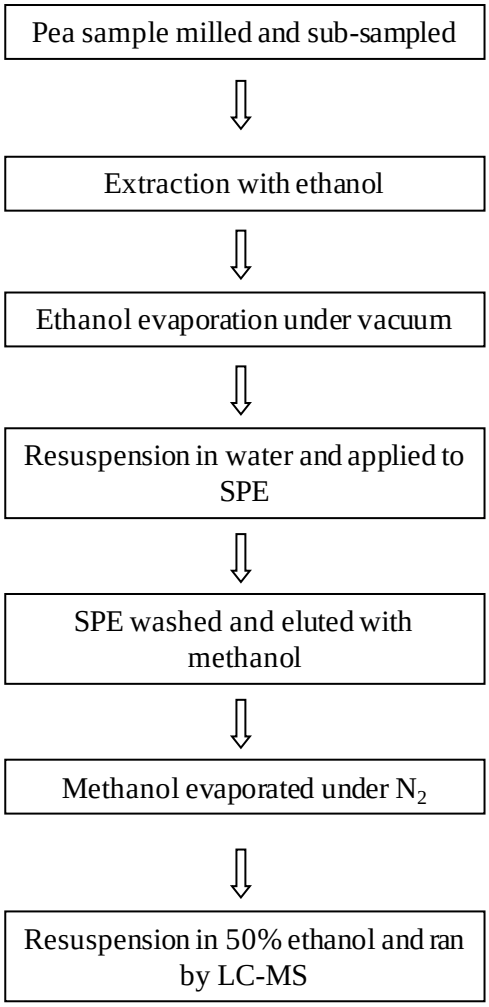

Figure 10. Summary of optimised extraction method for saponins in peas

Following initial analysis, it was clear that the mature marrowfat seeds contained a higher concentration of saponins compared with the vining pea samples. Figure 11 shows the relative concentration of saponins in the marrowfat samples, and Figure 12 the relative concentration in the remainder of the samples.

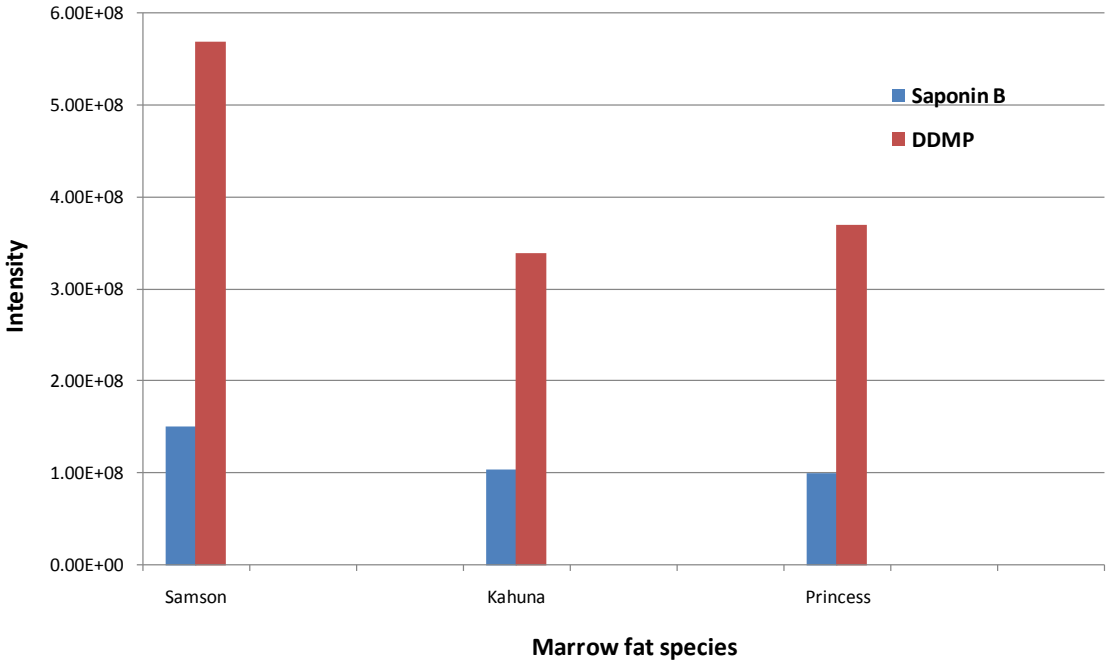

Figure 11. Relative mean intensity of Saponin B and DDMP in marrowfat peas

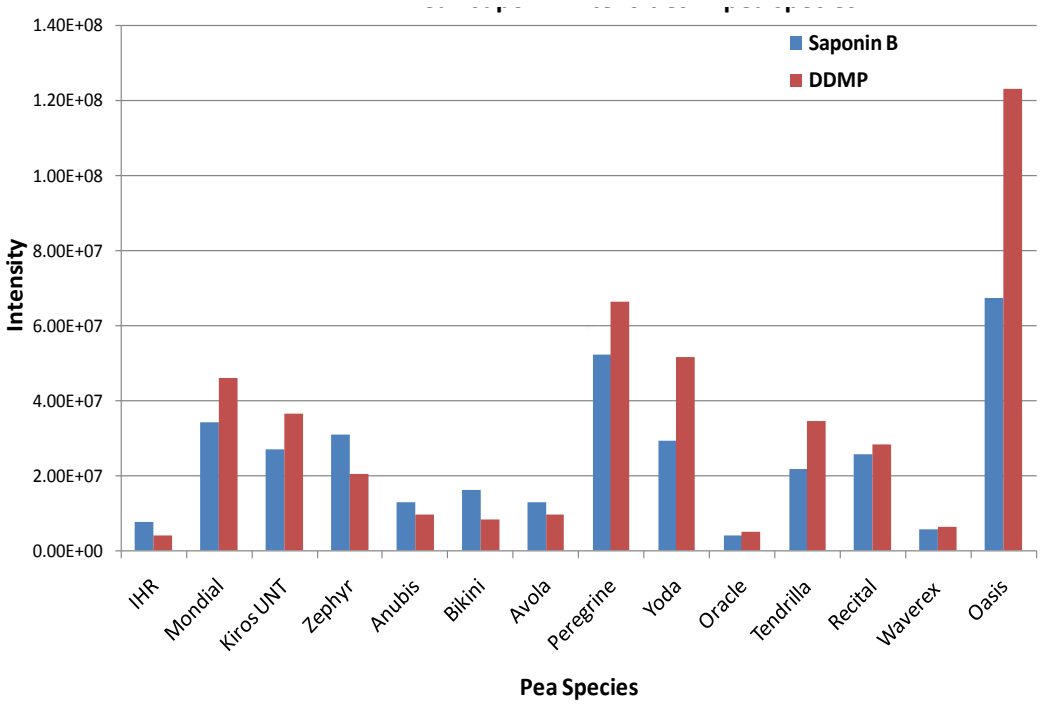

Figure 12. Relative mean intensity of Saponin B and DDMP in vining peas

The two saponin compounds were identified from mass spectra based on their theoretical

monoisotopic accurate mass. For saponin B and DDMP, their positive ions (M+H) have masses of *m/z* 943.52607 and *m/z* 1069.55776, respectively. Using the HPLC method described above, the retention times of saponin B and saponin DDMP were 13.8 and 17.5 minutes, respectively. A typical LC-HRMS extracted ion chromatogram (EIC) for each saponin is shown in Figure 13.

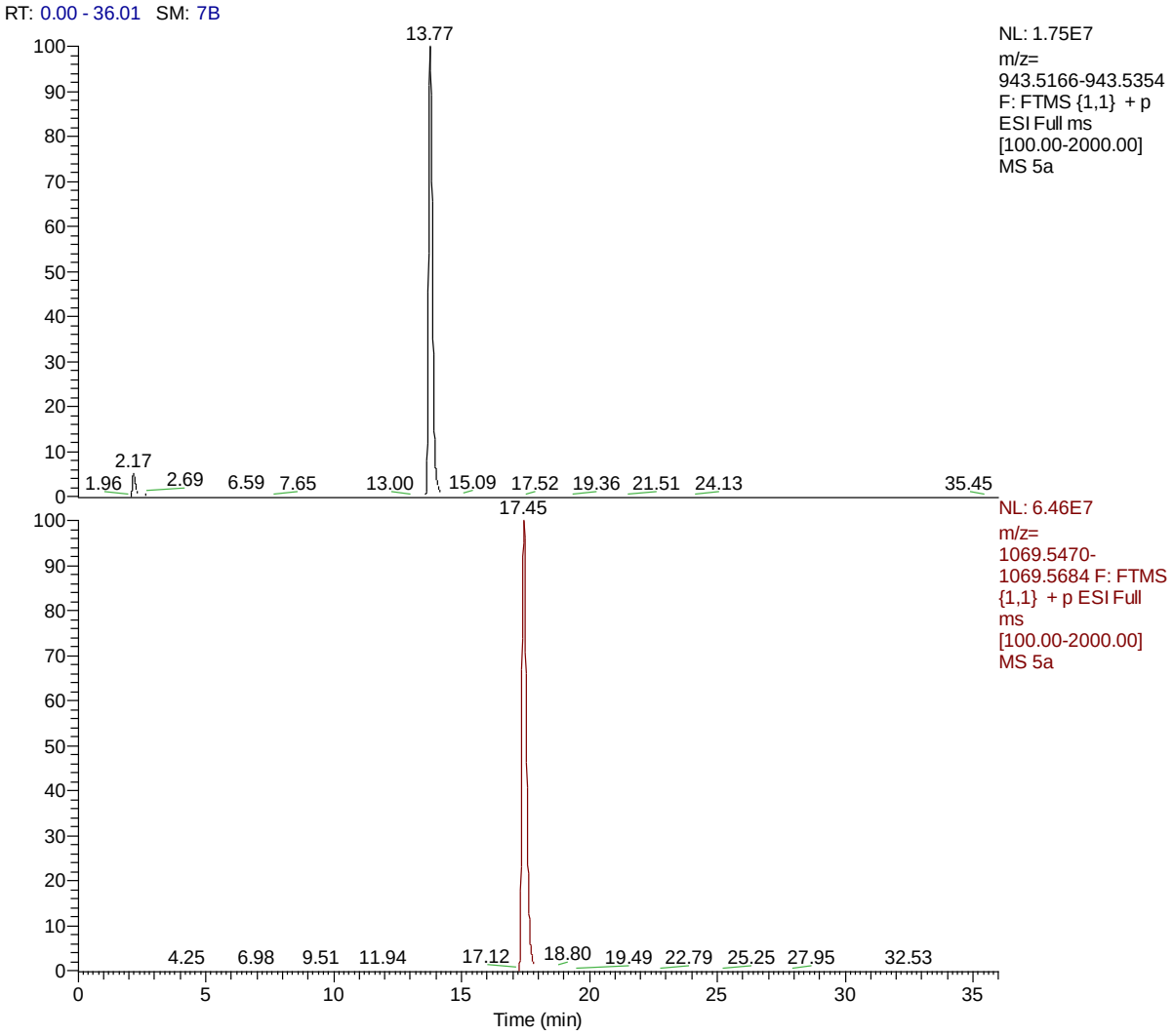

Figure 13. Extracted ion chromatograms of Saponin B (top) and DDMP (bottom) in Samson pea extract

Further compound identification was established using an MS/MS approach. Using in source fragmentation, the sugar moieties of the compounds fragment from the parent M+H ion (Figure 14).

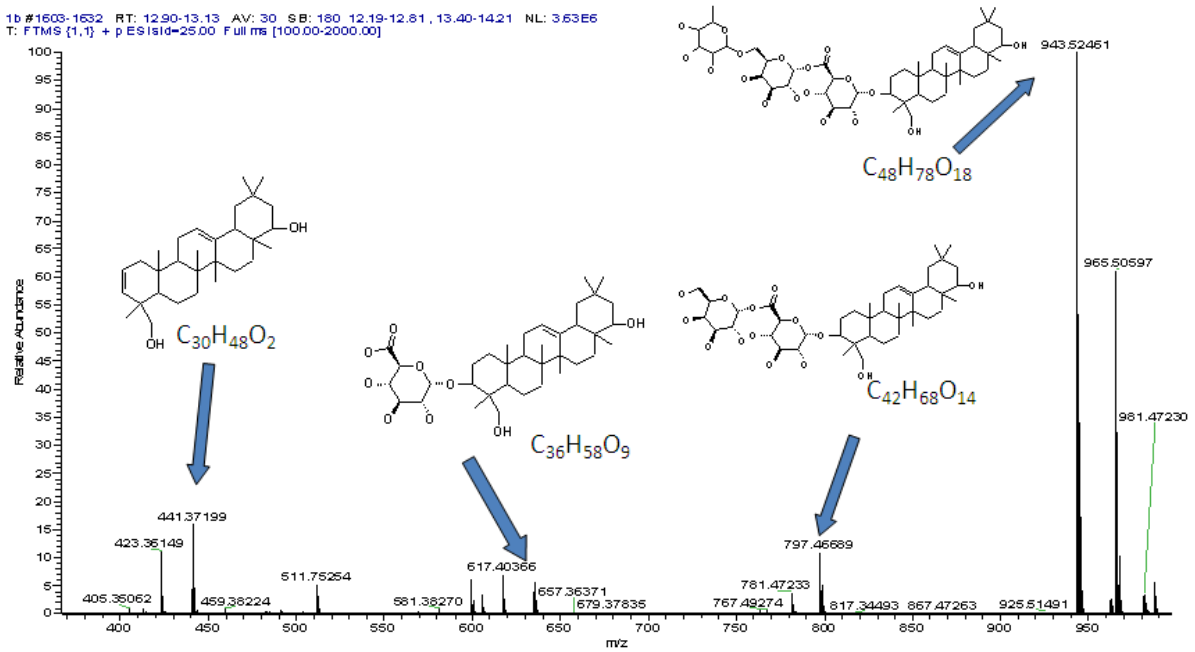

Figure 14. Parent ion of Saponin B (m/z 943.5246, $C_{48}H_{78}O_{18}$) and associated fragments by high resolution MS

Figure 15 examines the relationship between mean saponin intensities and TR in the different vining pea cultivars (excluding marrowfat samples). No clear relationship between TR and saponin concentration was apparent across different pea varieties.

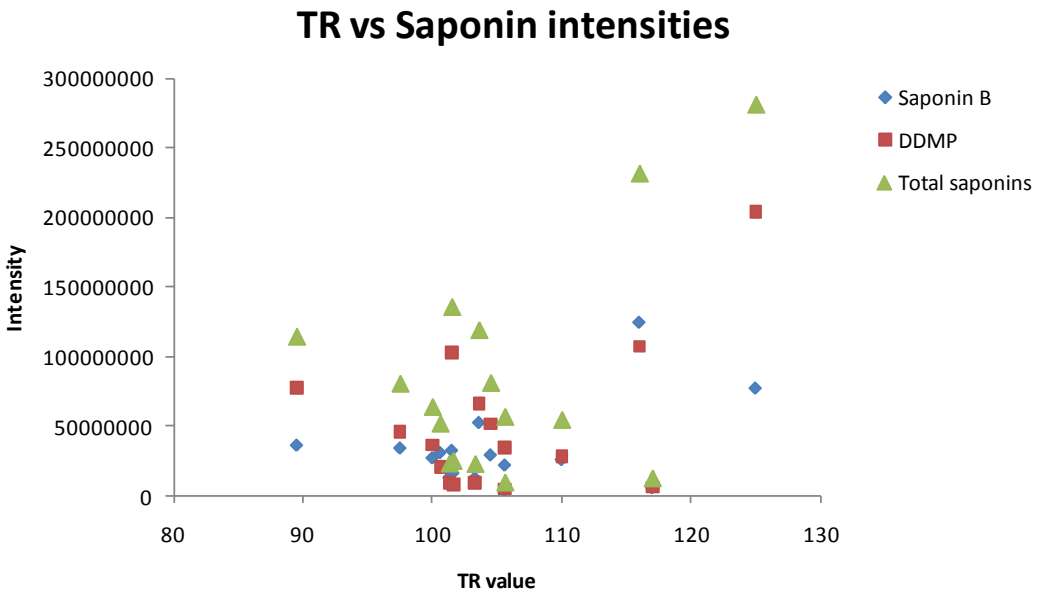

Figure 15. Relationship between TR value and mean saponin compound intensity from 16 different vining pea samples

However when saponin intensities are compared across TR values for Oasis (one of the developmental series available for vining seeds), a relationship between total saponin content and TR value is apparent. This is shown in Figure 16.

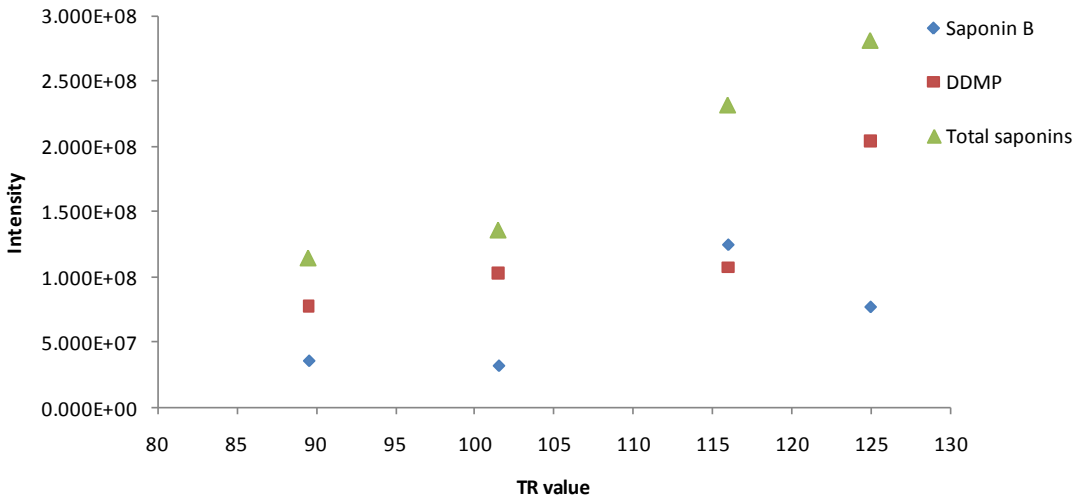

Figure 16. Relationship between TR value and mean saponin compound intensity from Oasis (n=4). R^2 = 0.957 for total saponins.

3. GC-OPD analysis of peas

To complement LC-MS and NMR analysis, Gas-Chromatography-Mass Spectrometry (GC-MS) was undertaken with a fitted Olfactory Detection Port (ODP). GC analysis captures the more volatile compounds that account for many flavour and odour components in foods. Running an ODP port simultaneously alongside MS analysis allows the use of the human nose as a separate detection mechanism. Automatic voice recording software documents "real-time" comments and therefore allows a single analyst to run the analysis. When a smell is emitted from the column into the nose cone, this is recorded along with the analyst's description of the smell, and the equivalent MS spectra can be scrutinised to try to identify the compound associated. Figure 17 shows a schematic of the system.

For GC-OPD analysis, 5 ml of dichloromethane was added to 1 g of frozen pea material and homogenised for 2 minutes by ultra turrax. 1 g of magnesium sulphate was added to the extract as a drying agent. The extract was then vortex mixed for 30 seconds before centrifugation at 4500 rpm for 10 minutes. The supernatant was filtered through a 0.45µm PTFE syringe filter and analysed by GC-MS/ODP.

Three samples across maturity ranges were chosen for preliminary analysis alongside an in-house reference material (frozen commercial peas), with the GC-MS/ODP final conditions given in Supplementary Table 7. MS spectra were evaluated against the AMDIS and NIST libraries. Preliminary results, presented in Supplementary Table 8, show ODP/MS results for sample S10-017602 (Oracle, TR = 105.6). All compounds shown are tentatively identified based on the highest match factor of the NIST or AMDIS library. Figure 18 shows the Total Ion Chromatograph (TIC) for the Oracle sample. The green lines indicate where the analyst

has registered a smell from the ODP (as recorded in the table).

The data suggest that a robust analytical method has been developed for the detection of volatile components of pea samples. However, Fera has little experience with organoleptic analysis such as this, and any compound identified so far has only been tentatively associated with each olfactory perception. It is suggested that, to further this work, the ODP analysis is performed on pea samples that have been through taste panels or analysed by taste experts, in order to fully identify the compounds associated with taste quality.

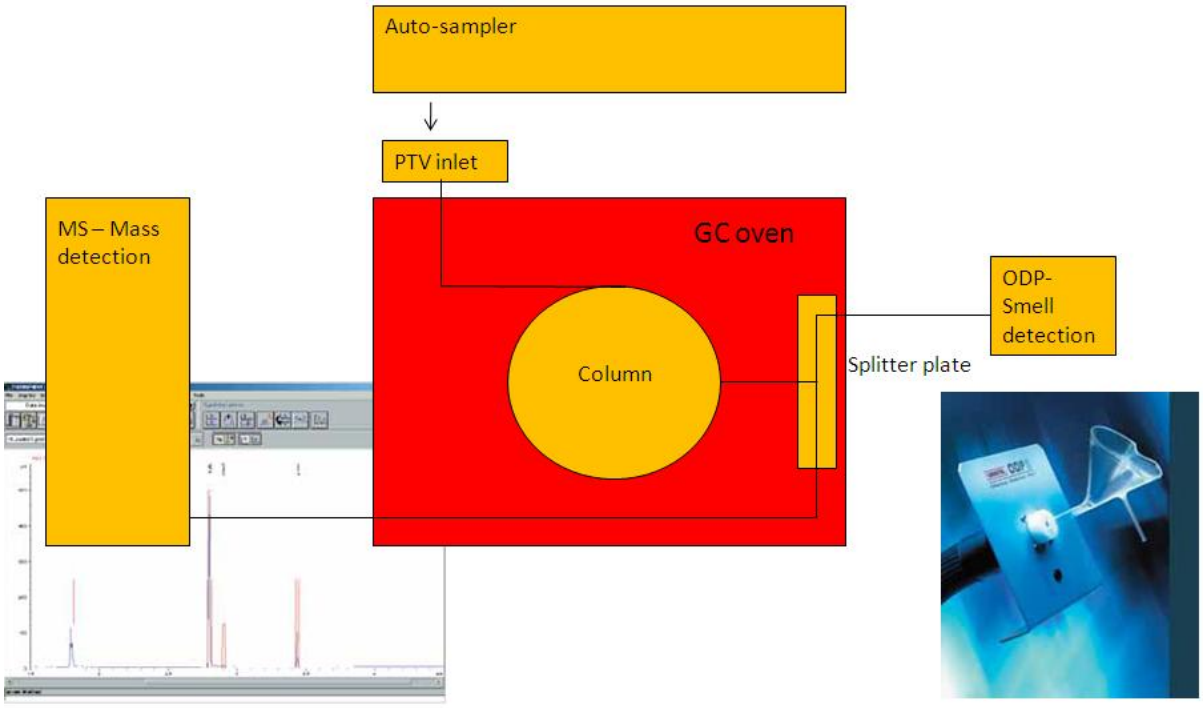

Figure 17. GC-MS/OPD schematic

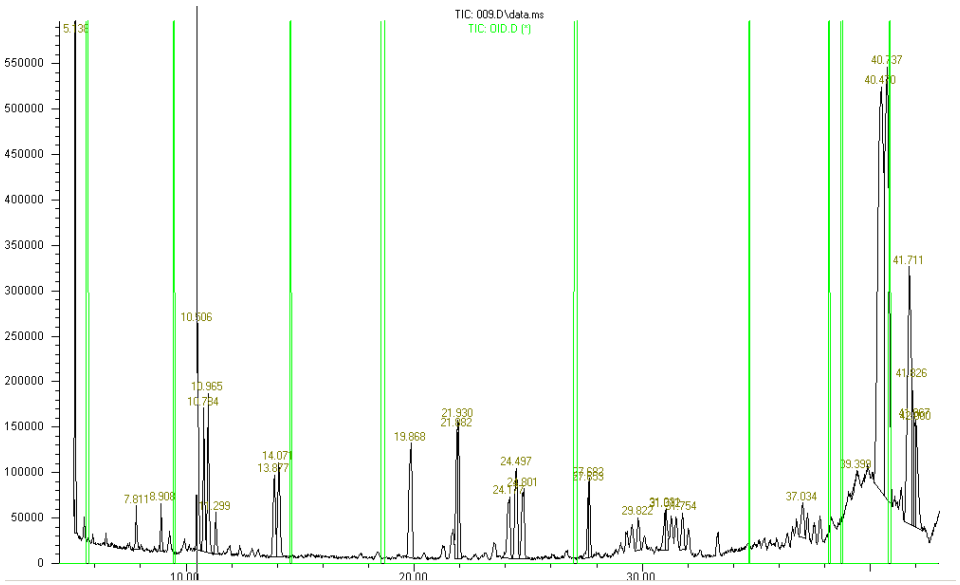

Figure 18. TIC of sample S10-017602. Green lines indicate a registered ODP smell

F. Economic and environmental analysis

In line with project objectives, the primary focus of this part of the project is to:

a) Assess the impact of improvements to systems for quality assessment for peas, in terms of meeting current and increased market demands, on UK sustainable agriculture; and
b) Predict consequences of changes to rotations in relation to climate change, and in particular possible change in nitrogen fertiliser use associated with changes in area cropped for peas.

Analysis of pea markets is almost completed and is summarised below. Overall figures have suggested that there is considerable scope within the UK to increase production among all markets for dry pea (and other pulse) crop products (source PGRO & BEPA). Our discussions with the industry, chiefly PVGA, indicate that the fresh and vining markets offer little scope for expansion, being limited by regional production, proximity to factories and overall volume.

1) For human consumption markets within the UK, a combination of factors, including long term trends in average consumption and substitution among various pea products in retail, have been explored. The copious price promotion strategies used by the UK industry indicate that consumption of most vegetable products requires sustained promotion campaigns. Introduction of new varieties with higher quality and productivity may potentially promote overall consumption. In particular, if higher quality attributes and technical improvements in quality assessment methods delivered by the QDiPS project can decrease average TR readings across the vining pea varieties toward TR106, then introduction of improved varieties may lead to further differentiation of retail pea products and potentially increase demand.

2) For export vegetable pea markets, improved yield is likely to enhance UK competitiveness. However, there are difficulties with unravelling the statistics that pertain to export and import volumes. Combining pea data as a whole include feed and vegetable (food) peas. While the available figures for the latter category suggest that, within this category, imports have overtaken exports, our discussions with PGRO, BEPA and industries suggest that the import figures require investigation as to their source and validation. This investigation is in progress; if the figures are validated, here there is a clear target for UK produced crops.

3) For feed markets, availability of soya and favourable world prices for rapeseed (which is the main competing break crop) appear to limit expansion of feed markets for pea. Improved yield and nutritional profile of peas emerged as key factors for future expansion of this market, though inconsistency of supply has a major negative impact.

A framework for analysis for objective b (above) has been developed and the analysis is due to start shortly. The main focus of this analysis will be to model the impact of legume

cropping using a marginal abatement cost curve (MACC) approach. This will allow legume cropping to be viewed alongside other GHG reducing activities in agriculture. The agriculture MACC produced by the Committee on Climate Change (CCC) has subsequently fed into the published targets for GHG reductions by the agriculture sector.

Project Rationale

What new factors have arisen which might affect the original rationale for the project? State your current view of the project? Do you see any potential challenges or opportunities that might affect the future progress of the project?

The growing and harvesting of the RILs in 2011 provided a challenge, due to their variable phenotypes. The experience should assist with the 2012 trials but clearly each season brings its own set of challenges.

Staff changes at JIC have been raised as an issue with both BBSRC and Defra, and solutions suggested and/or agreed.

Technology Transfer, Uptake and Exploitation,

Describe any transfers of technology (movements of people or artefacts, including software, between partners). Describe the extent of progress towards exploitation, including products, processes or materials, as well as patents. Include potential developments, in terms of new/improved products, processes, equipment and services, such as those at the prototype or concept stage, or any other significant developments that have resulted from involvement in the programme.

Two meetings were held with Premier (Princes) to discuss the processing, canning and the replicated sensory evaluation of marrowfat lines carrying the low lipoxygenase (Lox-3 and Lox-2); seeds at F3 and F4 were canned in accordance with current regulations. The second meeting involved Canadian breeders and industrial collaborators of Premier (Princes).

Novel mutants and their application have been discussed with Limagrain and Birds Eye and, through PGRO, presented to the industry; some of the novel mutants will be trialled in 2012 as a collaborative exercise subject to appropriate MTAs. The marker information for mutations has also been discussed, as will be appropriate later on within marker-assisted selection. It is envisaged that these mutations will be relevant to vining and also to canning and marrowfat end uses. Increasing constraints on salt, sugar and other additives to canned foods mean that there is an even greater demand on seed products to have a high level of endogenous sweetness and flavour.

Dissemination and Communications

List all publications, stories or acknowledgments in the scientific, commercial and popular press (including the media, television and on websites). Note when and where Consortium members have presented the project at events or conferences. Mention clubs or networks that have been formed through or because of LINK collaboration. Where possible, please attach any copies of dissemination and publicity outputs. Describe what has been done to

ensure the results exploitation to date and any anticipated upcoming project dissemination or communications outputs.

The following have been logged as QDiPS dissemination activities in 2011:

- Articles: HDC Field Vegetables Review, Campden BRI newsletter, Vegetable Magazine 2011
- Presentation to non-legume seed company, March 2011
- Stakeholder event, PGRO, June 2011 with QDiPS PGRO plot visit
- Limagrain open day, June 2011
- Presentation at BEPA meeting, JIC, June 2011
- Presentation to chairman, Global Food Security Programme Strategy Board, June 2011
- Presentation to UK Plant Genetic Resources Group, JIC, June 2011
- Presentation to Campden BRI Agri-Food Panel meeting, May 2011
- Presentation & meeting with Pro-Veg, August 2011
- Meeting with farm visit at Coop, August 2011
- Presentation to BAGCD, November 2011
- Presentation to VAA, November 2011
- Meeting & presentations to Princes, August 2011 & December 2011
- Novel Metabolomics Approach for Legume Breeding - Understanding Quality Determinants in Pea Seeds: Poster at Metabomeeting, Helsinki, Finland, 25th-28th September 2011

Alignment to the Sustainable Arable LINK Programme Objectives

Identify how the project to date has impacted on one ore more of the following areas:

a) The resource productivity of the UK arable sector
b) Promotion of sustainable, diverse, modern and adaptable farming
c) Improving rural economies
d) Reducing environmental impacts of the arable sector
e) Enhancing biodiversity and the rural environment.

Identify the environmental indicators selected for this project and the impact of the results to date on achieving these objectives. Please indicate how the results in general will influence sustainable farming. Provide information about actual / potential wealth creation and improvements to the quality of life that have resulted from the participation of the consortia in the LINK project.

The main indicators within the project relate to: maturity stage determination, breeding programmes, and crop choice in rotation related to nitrogen use.

As discussed, the replacement of current practices for determining stages of maturity will require downstream development of a kit to measure easily the amount of a key compound or group of compounds that are shown to be important by metabolomic analyses. The development of such kit will require close liaison between Fera, instrument manufacturers and

the vining pea industry. Developments in technology that are being exploited for field use in plant and animal husbandry will be relevant here. The development of more accurate predictors of seed development and the rate of developmental change offers a way to improve the reliability and accuracy of harvest time, which will ultimately be linked to the delivery of the premium prices available for high quality. The same arguments apply to the canning industry, where samples are harvested at a later TR also linked to quality, and to the dried marrowfat products where currently there are no predictors of organoleptic quality. Improvements to these processes will contribute substantially to the competitiveness of the industry.

The development of genetic markers linked to seed quality provides the opportunity for breeding programmes to screen plants at early generations, leading to potential savings of glasshouse space, energy, labour, and reducing the cost of taste panel evaluation. A comprehensive set of markers linked to agronomic and quality traits will provide this impetus, when balanced against cost savings. Novel screens have already become available within the project.

The impact of the project outcomes on rotation choices and land use are being studied within the project, based on a detailed and comprehensive analysis of all available datasets. This work is placed within Fera, where supervision of environmental and economic analysis is available as an in-kind contribution. Discussions have principally involved PGRO, Princes, PVGA and BEPA (via Wherry & Sons) but are being extended to include all partner industries.

Key Issues Raised by the Project Monitor or Project Officer

Name of Project Monitor: Prof. David Pink, Harper Adams University College
Name of Project Officer: Dr Farhana Amin, Farming and Food Science, Defra

State any key issues or concerns raised by the Project Monitor or Project Officer.

None have been raised.

Budget

Declare what project resources have been used to date (stated) in (£).

Budget summary (£)

Project Year	Government Sponsorship	Industrial contribution		Total Outgoings	Balance
		Cash	In-kind		
1	149,700.00	0.00	340,500.00	448,889.30	41,310.70
2*	227,400.00	0.00	226,900.00	442,497.42	2,173.51
3+	254,000.00	0.00	108,200.00	0.00	362,200.00
4+	97,900.00	0.00	80,000.00	0.00	177,900.00

* = Current year
+ = Projected spend

Outgoings summary for current year for Research Partners

Project Cost	Outgoing (£)
Pay costs	37,147.34
Consumables	18,786.50
Capital Depr	0.00
Travel	916.53
Overheads	56,001.88
Sub-contracts	102,745.17
Other costs	226,900.00
TOTAL	442,497.42

(NB. Reports are to be submitted directly to the Programme Secretariat in Defra, with a copy to the appointed PMC Monitor and Project Officer). All Consortium members should approve annual reports and, wherever possible, express agreement for the report to be disseminated. Reports can be submitted as Word or Adobe Acrobat documents and the main body should be concise (not exceeding a maximum of 20 pages, including appendices.) Please feel free to attach an appendix summarising appropriate data.

Contact

Please send your interim reports to the Sustainable Arable LINK Programme at the following address:

Defra
Area 8A LMB
17 Smith Square
London SW1P 3JR
Direct line 0207 238 1537
Fax 0207 238 1540
Email: sustainable.arable@defra.gsi.gov.uk

References

1. Davis, R.A. et al. (2007) *Adaptive binning: an improved binning method for metabolomics data using the undecimated wavelet transform.* Chemometrics and Intelligent Laboratory Systems 85:144-154
2. Clasquin, M.F., Melamud, E. and Rabinowitz, J.D. (2002) *LC-MS data processing with MAVEN: a metabolomic analysis and visualization engine*, in *Current Protocols in Bioinformatics*, John Wiley & Sons, Inc.
3. Heng L. et al. (2006) *Bitterness of saponins and their content in dry peas.* Journal of the Science of Food and Agriculture 86:1225–1231

QDiPS annual report 2011: SUPPLEMENTARY TABLES & PROTOCOLS

Table 1. Pea samples analysed by ^{1}H NMR spectroscopy and their associated metadata

Sample name	Fera LIMS	Date collected	TR Value	Date received	TR Range	Generic name
Bikini-1	S10-009452	04/07/2009	90	6/24/2010	80-90	Bikini
Bikini-2	S10-009453	07/04/2009	99.5	6/24/2010	90-100	Bikini
Bikini-3	S10-009454	07/07/2009	120	6/24/2010	110-120	Bikini
Bikini-4	S10-009455	06/07/2009	123	6/24/2010	120-130	Bikini
Mondial-1	S10-009456	07/07/2009	88.5	24/06/2010	80-90	Mondial
Mondial-2	S10-009457	08/07/2009	97.5	6/24/2010	90-100	Mondial
Mondial-3	S10-009458	09/07/2009	108	6/24/2010	100-110	Mondial
Mondial-4	S10-009459	11/07/2009	123	6/24/2010	120-130	Mondial
Oasis-1	S10-009460	07/07/2009	89.5	6/24/2010	80-90	Oasis
Oasis-2	S10-009461	08/07/2009	101.5	6/24/2010	100-110	Oasis
Oasis-3	S10-009462	07/09/2009	116	6/24/2010	110-120	Oasis
Oasis-4	S10-009463	10/07/2009	125	24/06/2010	120-130	Oasis
Recital-1	S10-009464	07/01/2009	83.5	6/24/2010	80-90	Recital
Recital-2	S10-009465	07/04/2009	110	6/24/2010	100-110	Recital
Recital-3	S10-009466	07/04/2009	113.5	6/24/2010	110-120	Recital
Recital-4	S10-009467	07/06/2009	134	6/24/2010	130-140	Recital
Yoda-1	S10-009468	07/04/2009	89	6/24/2010	80-90	Yoda
Yoda-2	S10-009469	06/07/2009	104.5	24/06/2010	100-110	Yoda
Yoda-3	S10-009470	07/07/2009	114.5	6/24/2010	110-120	Yoda
Yoda-4	S10-009471	08/07/2009	127.5	6/24/2010	120-130	Yoda
Kiros UNT-1	S10-009472	n/a	88	6/24/2010	80-90	Kiros UNT
Kiros UNT-2	S10-009473	n/a	100	6/24/2010	90-100	Kiros UNT
Kiros UNT-3	S10-009474	n/a	106	6/24/2010	100-110	Kiros UNT
Kiros UNT-4	S10-009475	n/a	115	24/06/2010	110-120	Kiros UNT
Anubis-1	S10-009476	n/a	99.6	6/24/2010	90-100	Anubis
Anubis-2	S10-009477	n/a	101.3	6/24/2010	100-110	Anubis
Anubis-3	S10-009478	n/a	101.6	6/24/2010	100-110	Anubis
Avola-1	S10-009479	n/a	97	6/24/2010	90-100	Avola
Avola-2	S10-009480	n/a	103.3	6/24/2010	100-110	Avola
Avola-3	S10-009481	n/a	103.6	24/06/2010	100-110	Avola
Bikini-1	S10-009482	n/a	101.6	6/24/2010	100-110	Bikini
Bikini-2	S10-009483	n/a	111	6/24/2010	110-120	Bikini
Oasis-1	S10-009484	n/a	96.6	6/24/2010	90-100	Oasis
Oasis-2	S10-009485	n/a	102.3	6/24/2010	100-110	Oasis
Tendrilla-1	S10-009486	n/a	105.6	6/24/2010	100-110	Tendrilla
Tendrilla-2	S10-009487	n/a	108.6	24/06/2010	100-110	Tendrilla
Waverex-1	S10-009488	n/a	117	6/24/2010	110-120	Waverex
Zephyr-1	S10-009489	n/a	100.6	6/24/2010	100-110	Zephyr
Zephyr-2	S10-009490	n/a	100.6	6/24/2010	100-110	Zephyr

Table 1. Continued

Sample name	Fera LIMS	Date collected	TR Value	Date received	TR Range	Generic name
Princess	S10-011097	n/a	n/a	7/13/2010	n/a	Princess
Princess	S10-011098	n/a	n/a	7/13/2010	n/a	Princess
Kahuna	S10-011099	n/a	n/a	13/07/2010	n/a	Kahuna
Kahuna	S10-011100	n/a	n/a	7/13/2010	n/a	Kahuna
Samson	S10-011101	n/a	n/a	7/13/2010	n/a	Samson
Samson	S10-011102	n/a	n/a	7/13/2010	n/a	Samson
Oracle	S10-017594	n/a	144.4	9/21/2010	140-150	Oracle
Oracle	S10-017595	n/a	125.6	9/21/2010	120-130	Oracle
Peregrine	S10-017596	n/a	108.4	21/09/2010	100-110	Peregrine
Peregrine	S10-017597	n/a	97.6	9/21/2010	90-100	Peregrine
Oracle	S10-017598	n/a	115.2	9/21/2010	110-120	Oracle
Oracle	S10-017599	n/a	97.6	9/21/2010	90-100	Oracle
Oracle	S10-017600	n/a	88.4	9/21/2010	80-90	Oracle
Peregrine	S10-017601	n/a	103.6	9/21/2010	100-110	Peregrine
Oracle	S10-017602	n/a	105.6	21/09/2010	100-110	Oracle
Peregrine	S10-017603	n/a	86.4	01/10/2011	80-90	Peregrine
Peregrine	S10-017604	n/a	79.2	01/10/2011	70-80	Peregrine
Peregrine	S10-017605	n/a	96.2	01/10/2011	90-100	Peregrine
Peregrine	S10-017606	n/a	87.2	01/10/2011	80-90	Peregrine
Peregrine	S10-017607	n/a	97.6	01/10/2011	90-100	Peregrine
Peregrine	S10-017608	n/a	80	10/01/2011	70-80	Peregrine
Peregrine	S10-017609	n/a	91.2	01/10/2011	90-100	Peregrine
Avola	S11-020612	18/06/2010	90	2/24/2011	80-90	Avola
Avola	S11-020613	21/06/2010	104.5	2/24/2011	100-110	Avola
Avola	S11-020614	21/06/2010	110.5	2/24/2011	110-120	Avola
Avola	S11-020615	21/06/2010	117	2/24/2011	110-120	Avola
Bikini	S11-020616	25/06/2010	84	24/02/2011	80-90	Bikini
Bikini	S11-020617	26/06/2010	102	2/24/2011	100-110	Bikini
Bikini	S11-020618	26/06/2010	110.5	2/24/2011	110-120	Bikini
Bikini	S11-020619	28/06/2010	137.5	2/24/2011	130-140	Bikini
Oasis	S11-020620	28/06/2010	93	2/24/2011	90-100	Oasis
Oasis	S11-020621	29/06/2010	98.5	2/24/2011	90-100	Oasis
Oasis	S11-020622	29/06/2010	111.5	24/02/2011	110-120	Oasis
Avola	S11-020623	06/07/2010	106.5	2/24/2011	100-110	Avola
Avola	S11-020624	07/06/2010	114	2/24/2011	110-120	Avola
Avola	S11-020625	07/07/2010	129	2/24/2011	120-130	Avola
Bikini	S11-020626	07/12/2010	109	2/24/2011	100-110	Bikini
Bikini	S11-020627	07/12/2010	127.5	2/24/2011	120-130	Bikini
Oasis	S11-020628	16/07/2010	109.5	24/02/2011	100-110	Oasis

Table 1. Continued

Sample name	Fera LIMS	Date collected	TR Value	Date received	TR Range	Generic name
Oasis	S11-020629	7/16/2010	112.5	2/24/2011	110-120	Oasis
IHR	IHR_1 to IHR_16	n/a	n/a	n/a	n/a	IHR

n/a not applicable or not known.

Table 2. Compounds indentified by ^{1}H NMR shown to correlate with TR

R^2 (TR vs resonance intensity)	Resonance start (ppm)	Resonance end (ppm)	Assignment
0.630	0.980	0.995	Valine
0.618	3.033	3.039	Unknown 1
0.594	1.014	1.029	Isoleucine
0.590	1.883	1.889	Arginine
0.583	3.039	3.047	Lysine
0.579	3.785	3.798	Sucrose
0.575	1.995	2.001	Homoserine
0.569	0.971	0.980	Leucine
0.562	3.021	3.033	GABA
0.549	2.106	2.111	Glutamate
0.534	2.088	2.093	Glutamine
0.529	3.106	3.122	Unknown 2
0.517	2.222	2.240	Unknown 3
0.505	1.851	1.856	Unknown 4
0.503	3.174	3.184	Unknown 5
0.503	1.484	1.521	Alanine

Table 3. Molecular features determined by LC-HR-MS analysis that differentiate high and low TR samples

Formula	Highest concentration	Tentative ID
$C_5H_4N_4O$	High TR	Xanthine
$C_4H_5N_3O$	High TR	Cytosine
$C_4H_4N_2O_2$	High TR	Uracil
$C_9H_{11}NO_3$	Low TR	Tyrosine
$C_4H_9NO_3$	Low TR	Threonine
$C_3H_7NO_3$	Low TR	Serine
$C_9H_{11}NO_2$	Low TR	Phenylalanine
$C_5H_{11}NO_2S$	Low TR	Methionine
$C_{10}H_{13}N_5O_5$	Low TR	Guanosine
$C_6H_{13}N_3O_3$	Low TR	Citrulline
$C_6H_{11}NO_4$	Low TR	alpha-Aminoadipic acid

Table 4. Optimised liquid chromatography conditions for saponin analysis

Parameter	Optimised setting
Column	Sunfire C18 (Waters, UK) 150 x 2.1mm, 3µm
Flow rate (ml/min)	0.25
Temperature (°C)	30
Injection volume (µl)	20
Mobile phase A	0.1% formic acid in water
Mobile phase B	0.1% formic acid in acetonitrile
Gradient	70% MPA to 100% MPB over 36 minutes, held for 6 minutes before returning to starting conditions

Table 5. Optimised HRMS parameters for saponin analysis

Parameter	Optimised setting
Ionisation mode	Electrospray positive
Sheath gas flow rate	60
Aux gas flow rate	10
Sweep gas flow rate	0
Spray voltage (Kv)	4.2
Capillary temperature. (°C)	350

Table 6. Details of pea samples analysed for saponins B and DDMP

Sample name	Fera LIMS no.	TR value
Oasis-1	S10-009460	89.5
Oasis-2	S10-009461	101.5
Oasis-3	S10-009462	116
Oasis-4	S10-009463	125
Mondial-2	S10-009457	97.5
Yoda-2	S10-009469	104.5
Anubis-2	S10-009477	101.3
Peregrine	S10-017601	103.6
Oracle	S10-017602	105.6
Kiros UNT-2	S10-009473	100
Recital-2	S10-009465	110

Avola-2	S10-009480	103.3
Bikini-1	S10-009482	101.6
Tendrilla-1	S10-009486	105.6
Waverex-1	S10-009488	117
Zephyr-1	S10-009489	100.6
Samson	S10-011101	n/a
Kahuna	S10-011099	n/a
Princess	S10-011097	n/a

Table 7. GC-MS/ODP settings

Parameter	Setting
Injection type	Gerstel CIS
Injection volume	5 µl
Injection initial temperature	40 ºC
Injection ramp	10 ºC / sec for 16 seconds, held for 1 minute
GC column type	Zebron (Phenomenex, Uk.) ZB-5MS 30 meter x 0.25mm x 0.25 µm
GC flow rate	1 ml/min
GC ramp:	
Initial temperature	40 ºC
Duration	1 minute
Ramp rate 1	3 ºC / min
Duration	20 minutes
Ramp rate 2	5 ºC / min
Duration	20 minutes, held for 2 minutes
ODP temperature	150 ºC
MS mode	Scan, 50-550 amu

Table 8. Summary of ODP comments and tentatively identified associated compounds, in sample S10-017602.

Retention time (minutes)	Smell description	Intensity	MS Library search	NIST / AMDIS match factor / 1000
5.6	Faecal	Medium	1,3,5 trimethyl cyclohexane	793
9.5	Burnt toast (burning)	Small	3-methyl nonane	875
14.6	Green / cut grass	Small	Tetradecanol	780
18.6	Green / cut grass	Large	Nothing found	n/a
27.0	Green / cut grass	Small	Tetradecane	871
34.7	Burnt toast (burning)	Small	Hexadecane	800
38.2	Sweet / Apple	Small	4,6,8-Trimethyl-1-nonene	724
38.7	Green / cut grass	Small	Nothing found	n/a

40.8	Green / cut grass	Small	Methyl8-pimaren-18-oate	767

Extraction methodology for saponin analysis

Each pea sample was stored at -20°C before being dried for approximately 48 h in a freeze-drier and then ground to a fine powder. From this powder a sub-sample of 18 mg was taken and 1.8 ml of 70% ethanol added. After shaking for 30 minutes, the extract was centrifuged for 10 minutes at 14,000 rpm, and a 1.6 ml aliquot removed, before evaporation under vacuum. After evaporation the sample was resuspended with 0.5 ml of HPLC water before further shaking and centrifugation for 5 minutes at 14,000 rpm. The supernatant was then applied to a pre-conditioned Solid Phase Extraction (SPE) cartridge (Bond Elut-C18, 200mg, 3ml), washed with 3 ml of HPLC water and eluted with 2 ml of methanol. The eluted sample was evaporated under nitrogen before resuspension with 0.5 ml of 50% ethanol and analysed by LC-HRMS.