Wealthier & Healthier

Derek Stewart

Food security is defined by the World Health Organisation as "when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life". However, the past year has been one of uncertainty within the global financial systems, leading to restricted economic development with consequential effects on food security. The financial crises have highlighted that, more than ever before, food is a global commodity and that changes in quantity, availability, quality and nutritive value will impact from global through to personal levels on economy, health and quality of life.

Our research at SCRI continues to deliver on the high aims of food security whilst ensuring that this is done within an economically sustainable framework. The following research snapshots highlight only some of the ways in which we do this. Soft fruit is increasingly accruing a portfolio of health benefits and to ensure its continued availability, disease free stocks for breeding and propagation are vital and this is highlighted for blackcurrant. The potential for the diversification of UK soft fruit crops is also showcased by the assessment of multiple blueberry varieties for UK fresh and processing markets.

Potato continues to be a key crop with respect to both the Scottish and global economies, and human nutrition *per se*. The important issue of mineral malnutrition may, in part, be soluble via the identification and exploitation of the genetic basis for mineral accumulation in potato. Furthermore, the underpinning mechanisms defining potato texture, a key quality determinant of cooked potato and continued customer preference have been elucidated. Both the mineral and textural studies have identified genes which can be exploited in breeding programmes and ultimately, in the form of new products.

Validation of an improved diagnostic test to accelerate the certification of blackcurrant (*Ribes nigrum* L.) plants for breeding and propagation

Alison Dolan, Stuart A. MacFarlane, Yvonne Pitkin and Wendy J. McGavin

Soft fruit production and processing represent a valuable sector within the Scottish and UK economy with, for example, the UK blackcurrant processing crop valued at >£200 million in 2009. This is underpinned by a regulated certification scheme to ensure the provision of high-health planting material sold in the UK to the industry.

To support these regulations, SCRI has a key role in maintaining the UK's sole nuclear stock collection of pest and pathogen-tested blackcurrant plants. We maintain and test a wide range of cultivars, both old and new, and supply high-health certified material to propagators, for subsequent release to growers, and to breeders and researchers worldwide. The pest and pathogen testing regimes follow RERAD, Defra and European and Mediterranean Plant Protection Organisation (EPPO) guidelines.

In addition, SCRI has a lead role in the provision of robust and validated detection methods to support the certification scheme. Reversion is one of the most serious diseases of blackcurrant, and is caused by a mite-transmitted virus, *Blackcurrant reversion virus*, BRV. The disease occurs in most areas where *Ribes* plants are found, except North America and Australia, and exists in two forms, the European (E) and the Russian forms (R), which have been recognised based on the severity of the symptoms displayed by the affected plant, with the latter (Fig. 1) being markedly more severe than the former.

Figure 1 Malformation of blackcurrant flowers associated with the R form of BRV resulting in a proliferation of the sepals from the usual five to ten.

In the most seriously affected plants, the disease causes sterility with a consequent complete loss of the crop, making BRV a serious impediment to profitable blackcurrant production. The current recommended test for detection of BRV in blackcurrants relies on grafting (Fig. 2).

Figure 2 Bottle with scion from plant under test grafted on to recipient plant.

For this, a shoot (scion) from the plant under test is grafted on to a recipient plant of a cultivar that is known to react to BRV by the formation of visual symptoms on its leaves, flowers and buds. The graft recipient is observed over a two-year period, highlighting this testing regime as costly and laborious. In addition, BRV accumulates only at low levels and has an erratic distribution in infected plants, raising concerns about the reliability of this testing method.

Diagnostic tests using reverse transcription-polymerase chain reaction (RT-PCR) methodologies have many benefits; notably that they are highly specific, and are relatively high-throughput at relatively low cost. However, if they are to replace the conventional tests that are currently recommended in certification guidelines, then they must be assessed carefully to show that they perform at least as well as, if not better than, the tests that they might replace. To this end we have developed and validated a robust RT-PCR diagnostic test for BRV in blackcurrant certification (Fig. 3).

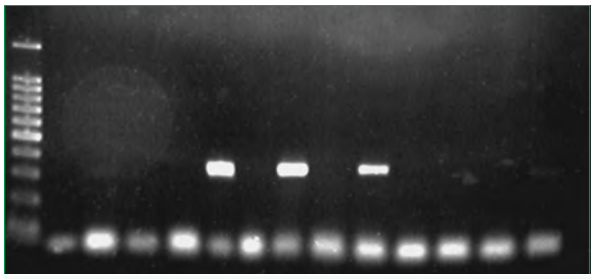


Figure 3 RT-PCR products resolved by electrophoresis through a 1.5 % agarose gel, and visualised by ethidium bromide staining and UV illumination.

From 17 initial sets of PCR primers, spanning the complete BRV genome, two were selected that gave the strongest, most reproducible amplification of BRV sequences from infected blackcurrant plants. Three sets of BRV-susceptible recipient plants (of cv. Baldwin, Ben Lomond and Ben Tirran) were grafted with scions from known infected donor plants, and the recipients were examined by RT-PCR and visually for reversion symptoms in leaves and buds over a four-year period.

Leaf symptoms alone were found to be an unreliable method for detection of BRV. The R form of the virus was detected in the buds both visually and by RT-PCR more readily than the E form. RT-PCR detection was determined to be as effective as post-grafting visual inspection in detecting BRV, with one of the primer sets detecting infection earlier in Ben Tirran than when using the conventional visual test. This primer set also detected BRV within two years after infection and for both forms of the virus in the three cultivars. The results support its use as an optimised, reliable, effective and, most importantly, validated PCR test for BRV. This testing method is now being considered as an alternative to the existing graft method currently used within the UK certification scheme and EPPO guidelines.

Developing molecular tools for the British blueberry industry

Susan McCallum, Mary Woodhead, Rex M. Brennan & Julie Graham

Fruit consumption in the UK, particularly of berry fruits, is expanding rapidly. In 2009, sales of soft fruit increased by 17.2% in the UK with a combined retail sales value for strawberries, raspberries, blueberries and blackberries close to £700 million. Consumer demand for blueberries (*Vaccinium* spp.) is at record levels, partly due to their perceived health benefits but also their convenience and enjoyable flavour. Blueberries account for 17% of fruit sales but it is estimated that only 3% of the blueberries purchased in the UK are grown here. Imports, primarily from Argentina, Chile, Spain and Poland, supply the significant shortfall. However, this is beginning to change, with growers across the whole of the UK from the south of England to the north of Scotland beginning to produce blueberries. It is anticipated that the combination of this geographic spread, together with the temperate climate, will allow marketing of UK-grown blueberries from early July through to late October.

Blueberries are a challenging crop to grow, being shallow rooted and requiring well drained, acidic soil (pH 4.5-5.5) with high organic matter content. Although it can take up to five years before plants reach full production, a well maintained plantation can be productive for at least 20 years and beyond. UK growers are currently planting mixtures of existing varieties, mainly from the USA, but the long-term performance, fruit quality and consumer acceptance of these varieties in the UK is largely unknown. It is known that environmental cues significantly affect the performance of different blueberry varieties and informed variety selection is a key component for success. To address this, a project funded by

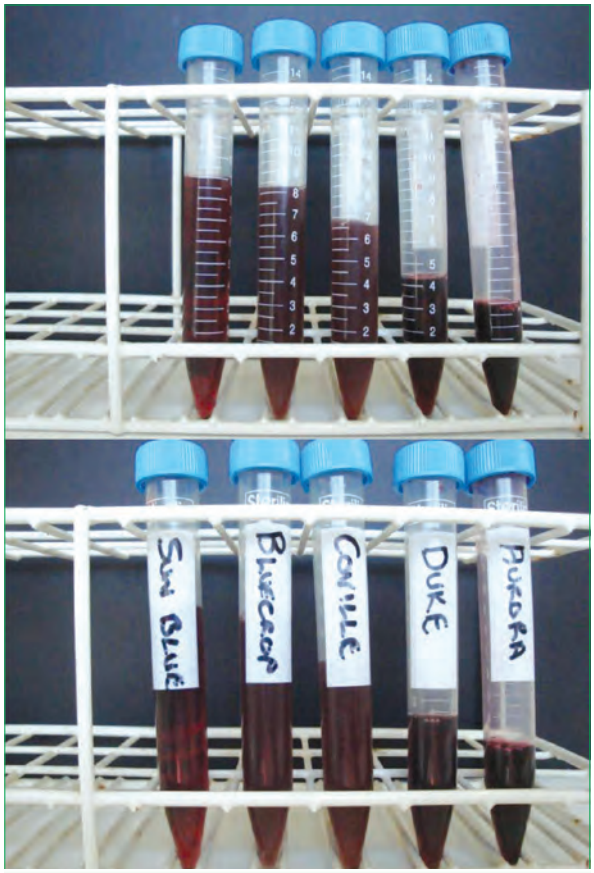


Figure 4 Variations in blueberry fruit juice obtained from a range of samples

Horticulture LINK has begun to look at the establishment, seasonality and machine harvestability of 40 different varieties across five locations in the UK. Fruit composition (sugars, acids, antioxidants) and other measures of quality (fruit size, colour, juice content and sensory characteristics) are being examined so that cultivars with the most appropriate qualities can be identified for the UK fresh and processing markets (Fig. 4).

The project has focused on the identification and collection of diverse germplasm (Fig. 5) enriched in sensory and health promoting compounds for future breeding programmes while allowing the determination of year to year variation and hierarchy maintenance of health-promoting compounds in different genetic backgrounds.

A genetic framework for future crop improvement is required to develop a thriving and sustainable industry. The genetic component of this project builds on the statistical developments derived from the BioSS software programme, TetraploidMap, to identify fruit quality, health and agronomic related quantitative trait loci (QTL) in tetraploid blueberry for marker assisted breeding. A mapping population developed from two key US blueberry cultivars segregating for a number of important phenotypic traits (for example time to fruiting, plant habit, and fruit quality) and a selection of molecular markers have been used. Data from 100 markers have been analysed and found to show segregation patterns consistent with the simplest model for meiosis, random chromosomal segregation, allowing the production of a draft tetraploid blueberry linkage map. For a genetic map to be useful for marker assisted selection it is necessary to generate a good coverage of molecular markers (Fig. 6). As blueberry has many chromosomes (12 chromosomes with 4 alleles = 48), approximately 350–500 markers will be required.

Knowledge of cultivars and establishment will allow the UK industry to be based on the best germplasm for the growing conditions and requirements. This

Figure 5 A subset of blueberry cultivars under analysis; Liberty, Duke, Chandler and Rubel.

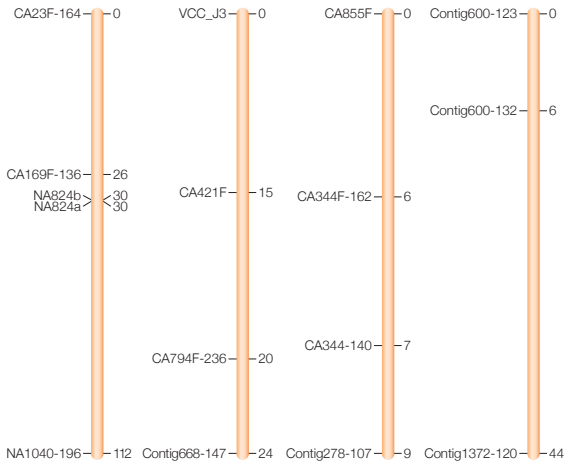


Figure 6 Chromosome map for blueberry.

project represents the first systematic study of the combined impact of genetics and environment on fruit phytochemistry in relation to the sensory and potential health properties of blueberry fruit. By linking the phenotype to genotype, a genetic framework for future crop improvement using marker assisted breeding will be established and this should greatly increase the speed and precision of blueberry breeding.

Breeding potatoes to address mineral malnutrition

Nithya K. Subramanian, Glenn J. Bryan, Christine A. Hackett, Gavin Ramsay & Philip J. White

Mineral malnutrition is one of the most serious challenges to human health, and it is estimated that up to two-thirds of the world's population might be at risk of deficiency in one or more essential mineral element. The mineral elements most commonly lacking in human diets are iron, zinc, calcium, iodine and selenium. Concentrations of these elements can be increased in plant produce through the judicious application of mineral fertilisers, and the selection of crop genotypes that acquire mineral elements more effectively from the soil and distribute them to edible portions. In collaboration with Martin Broadley and John Hammond at the University of Nottingham, we are identifying genetic factors affecting the concentrations of mineral elements essential for human nutrition in potato tubers that will allow breeders to select genotypes for improved nutritional quality.

Potato is one of world's most important food crops. It is of increasing importance in developing countries because of its high yield potential and its nutritional qualities. Its price is relatively buffered from the vagaries of world commodity markets and it is ideal for local consumption. Potatoes are an important source of mineral elements in human diets. For example, a single, medium-sized potato weighing 200 g can provide about 18% of the US Dietary Reference Intake (DRI) of iron and about 6% of the DRI for zinc. However, it will provide only 2% of the DRI for calcium. Potatoes can also be a significant source of iodine and selenium if mineral fertilisers containing these elements are applied to the crop. In addition, the bioavailability of iron and zinc is high in potato tubers, because they have relatively high concentrations of organic compounds that stimulate the absorption of these elements in the gut and low concentrations of phytate and oxalate that inhibit their absorption.

Although tubers develop underground, they are surrounded by periderm tissue which is impregnated with hydrophobic material that prevents the indiscriminate entry of mineral elements from the soil solution to the flesh. Indeed, most mineral elements are delivered to tubers through the phloem from the shoot. The inability of mineral elements to enter the maturing tuber from the soil solution, together with differences in the mobility of mineral elements in the phloem, results in distinct spatial distributions of mineral elements within the tuber (Fig. 7). It is commonly observed that calcium, which is immobile in the phloem, is concentrated in the skin and peripheral layers of the flesh, whereas elements like potassium, which are mobile in the phloem, are distributed throughout the tuber. Both iron and zinc are present at higher concentrations in the skin than in the flesh of potato tubers. These distributions affect the nutritional significance of culinary decisions, such as whether to consume potato skins.

There is significant, heritable variation in tuber mineral concentrations. This is apparent both between

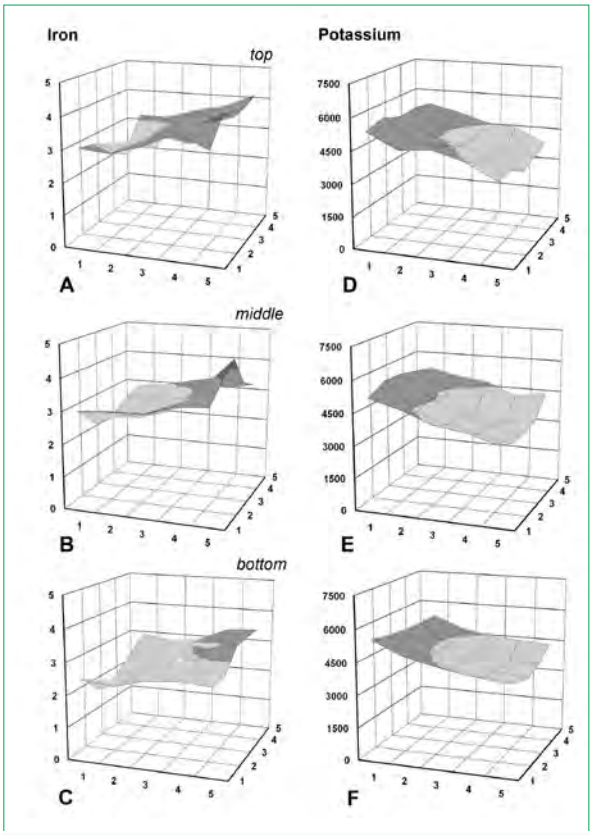


Figure 7 Distributions of iron (a, b, c) and potassium (d, e, f), from the bud (left) to stem (right) end, in the top, middle and bottom slices of recently-harvested, peeled tubers of the table variety Stirling.

commercial varieties and among the genotypes of genetic mapping populations. This variation can be used to identify chromosomal quantitative trait loci (QTL) that affect tuber mineral concentrations. Using a genetic mapping population derived from a cross between the processing clone 12601ab1 and the table cultivar Stirling, we have identified robust QTL affecting tuber iron, zinc and calcium concentrations. Several of these QTL are associated with chromosomal loci known to determine the time taken for the crop to reach maturity, which determines their market and agronomic characteristics. Other QTL are not associated with maturity classification, and can be used to select for genotypes with greater concentrations of mineral elements essential for human nutrition within the same maturity class. The latter QTL contain genes encoding ferric reductases, cation transport proteins, nicotianamine synthases and metal-chelate transporters, which are responsible for the uptake and movement of iron and zinc within the plant. This genetic information will allow breeders to select for genotypes to address mineral malnutrition.

The role of pectin methyl esterase in determining potato tuber textural quality

Heather A. Ross, Wayne L. Morris , Laurence J.M. Ducreux, Robert D. Hancock, Susan R. Verrall, Jenny A. Morris, Derek Stewart, Peter E. Hedley, Gordon J. McDougall & Mark A. Taylor

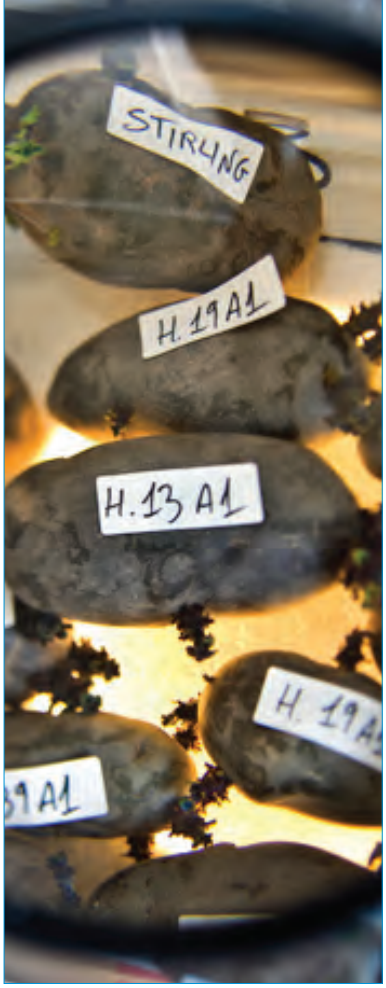
Potato tuber texture is a key quality determinant of cooked potato and a major trait that influences consumer preference. Tuber texture is also a key issue in potato processing and is known to be affected by pre-processing procedures such as blanching, peeling and storage. Although it is possible that a number of factors may be responsible for cooked potato texture, the relative importance of these is not yet clear. Several studies have described potato germplasm that produces tubers with markedly different textural properties. In particular, members of *Solanum tuberosum* group Phureja have been identified which exhibit a boiled tuber texture described as extremely floury or crumbly. The cooking (by steaming) time of Phureja tubers is generally in the order of half that taken for typical *Solanum tuberosum* group Tuberosum tubers at the same developmental stage.

In order to identify the factors that account for textural differences between the Phureja and Tuberosum types, we compared gene expression in tubers using a potato microarray. The Potato Oligo Chip Initiative (POCI) consortium recently developed the microarray, which contains gene probes based on 42,034 potato unigene sequences, and uses the custom Agilent platform. Using this microarray we were able to identify consistent differences in gene expression profiles between Phureja and Tuberosum cultivars, including genes likely to impact on texture. In particular there was a ten-fold higher expression level of a gene encoding pectin methyl esterase (PME). Pectin is a major component of the cell wall and its structure is likely to be an important factor in texture in potato tubers. PME demethylates pectin components which are then free to strengthen

the cell wall structure by cross-linking through interaction with calcium ions, liberated during the cooking process. Thus low PME activity may account for a softer cooked texture in Phureja tubers.

We set out to test this theory using a number of different approaches. Measurements of PME activity demonstrated that the differences in gene expression levels were also seen in the activity of the PME enzyme. Biochemical analysis of tuber cell walls also showed a reduced degree of pectin methylation in the Tuberosum samples compared with those from Phureja. Conclusive evidence in support of our hypothesis came from transgenic studies, in which we increased the expression level of the PME gene. Over-expression resulted in significant changes in textural properties (a firmer texture was measured in cooked tuber samples from transgenic lines with higher PME expression), associated with a significant increase in PME activity and reduced cell wall pectin methylation. Thus there is a clear link between PME activity, pectin methylation and processed tuber textural properties.

Our conclusion was that we had discovered a gene with an important role in determining cooked tuber texture. In current work we are aiming to identify the reasons why the Phureja version of the PME gene is expressed at much lower levels than in Tuberosum. This is done by comparing sequences that may control the degree to which the genes are switched on. Ultimately breeder-friendly markers may be developed to enable the conventional breeding of potatoes which produce tubers with specific textural properties.



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