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# Developing Tools for Long-Term Breeding of Blueberry Germplasm for UK Production

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*Fruit consumption in the UK, particularly of berry fruits, is expanding rapidly and consumer demand for blueberries (*Vaccinium *spp.) is at record levels with UK growers unable to meet current demand. Consumers are aware that eating fruit can be pleasurable and with appropriate packaging, convenient, but blueberry remains unfamiliar to a wide cross section of UK consumers. There is great potential for UK growers to supply high quality fresh blueberries across a wide season so that consumption of this healthy fruit can be integrated into the British diet. 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 software program, TetraploidMap, to identify fruit quality, health, and agronomic-related quantitative trait loci in tetraploid blueberry for marker assisted breeding.*

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## INTRODUCTION

Blueberry is a highly heterozygous crop with a range of ploidy levels and a long juvenile period (Camp, 1945; Bruederle and Vorsa, 1994) making selection of key traits difficult and long term. Any investment in the development of a sustainable blueberry industry should focus on currently available germplasm and the development of tools to allow the accurate development of future varieties that meet UK requirements.

Significant genotype × environment interactions have been reported for many plant traits in highbush blueberry indicating the importance of selecting the varieties best suited for different regions (Finn et al., 2003). Previous work has shown that cultivar choice is not simply a case of importing cultivars adapted elsewhere. Once trialed in the UK, these cultivars often fail to establish quickly, ripen, or perform in the same way, which may in part be due to inappropriate environmental cues (Arora et al., 2003). Analyses of germplasm across different parts of the UK, including establishment, seasonality, sensory fruit quality, and machine harvestability are, therefore, required to properly assign the most appropriate cultivars to specific regions, markets, and cropping systems.

In the USA where blueberry is a major crop, genetic studies have largely concentrated on the identification of genes and biochemical pathways involved in cold tolerance and cold hardiness (Dhanaraj et al., 2004; Naik et al., 2007; Alkharouf et al., 2007) with many gene sequences now available in public databases. Genetic mapping in blueberry has been reported using dominant RAPD markers (Levi and Rowland, 1993; Rowland and Levi, 1994; Qu and Hancock, 1997) with further mapping work carried out using a small number of co-dominant markers including single sequence repeats (SSRs) and expressed sequence tag PCR (EST-PCR) markers (Rowland et al., 2003; Brevis et al., 2007). In other plant families, such as the *Rosaceae*, mapping is more advanced and genes involved in ripening (Graham et al., 2009) fruit quality including anthocyanin production (Chagne et al., 2007; Kassim et al., 2008), aroma production (Carbone et al., 2006), and sugar/acid metabolism have been characterized and mapped (Liebhard et al., 2003; Etienne et al., 2002). These provide a basis on which to begin mapping candidate genes that may influence blueberry characters important to UK consumers.

Highbush blueberry cultivars (*Vaccinium corymbosum* L.) have been recognized to occur at three ploidy levels, $2n = 2x = 24$, $4x = 48$, and $6x = 72$ (Camp, 1945; Megalos and Ballington, 1988). The major blueberry varieties cultivated around the world are autotetraploid (Qu et al., 1998; Boches et al., 2005). Autopolyploids (polysomic polyploids), such as

sugarcane (*Saccharum* spp.) and potato (*Solanum* spp.), contain genetically similar (homologous) genomes while allopolyploids (disomic polyploids) consist of distinct (non-homologous) genomes and include wheat (*Triticum* spp.) and rapeseed (*Brassica* spp.). In allopolyploids the association of two differentiated genomes by means of interspecific hybridization results in the observed chromosome doubling. Autopolyploids, on the other hand, are thought to derive from chromosome doubling of the same genome within a single parental species (Gallais, 2003).

Pairing during meiosis can occur in autopolyploids between randomly chosen pairs of homologous chromosomes (bivalents) or between more than two homologous chromosomes (multivalents). Each chromosome has the potential to pair randomly with any of its homologues leading to tetrasomic inheritance where all allelic combinations ($A_1A_2A_3A_4$) may be produced in equal frequencies (Soltis and Soltis, 1993). Allopolyploids, in contrast, result in pairing behavior during meiosis between two homologous pairs of chromosomes ($A_1A_2B_1B_2$) resulting in bivalent formation only and, hence, disomic segregation (Soltis and Soltis, 1993; Gallais, 2003). A further form of polyploidy, segmental allopolyploids has been described by Stebbins (1947) that results from parental genomes of partially divergent chromosome arrangements giving rise to intermediate polyploids with polysomic and disomic inheritance on the same chromosome (Gallais, 2003).

While statistical methods for linkage analysis and quantitative trait loci (QTL) mapping in diploid species are well-developed, polyploid analyses have advanced more slowly (Luo et al., 2001; Hackett et al., 2001). A theoretical model for tetraploid analysis was developed by Hackett et al. (1998) studying linkage analysis of dominant markers in an autotetraploid species. This approach was later adopted by Meyer et al. (1998) in the development of a linkage map in potato. Luo et al. (2000) developed a model for the prediction of marker genotypes for autotetraploid parents by analysing marker phenotypes from segregating data obtained from parents and progeny, the methodology of which is utilized in the software TetraploidMap (BIOSS, Dundee, Scotland; Hackett and Luo, 2003; Hackett et al., 2007).

TetraploidMap (BIOSS) is a software package that allows the construction of a linkage map based around marker data obtained from dominant and co-dominant (multiallelic) loci scored in parents and a full sib family in an autotetraploid species (Hackett and Luo, 2003). Multiallelic markers, such as microsatellites, are considerably more informative than single or double dose dominant markers as these allow homologous chromosomes to be deduced in both parents. The estimation of recombination frequencies which are based on microsatellite markers is also up to four times more informative than dominant markers (Luo et al., 2001). It may not always be possible, however, to identify parental genotypes directly from their phenotypes due to differences in allele dosage and they must, therefore, be deduced instead by parental and offspring phenotypes (Hackett and Luo, 2003).

A plethora of marker types including fully versus partially informative as well as dominant versus co-dominant markers can be found segregating in a tetraploid population simultaneously (Wu et al., 2001). The genotypes of diploid parents can be predicted by the segregating pattern of progeny but this is not always the case with tetraploids due to the possibility of multiple allele dosage and double reduction (Wu et al., 2001). Both the occurrence and frequency of double reduction in autopolyploids would be expected to affect the pattern of gene segregation. The value of double reduction frequency will itself be affected by the position of each locus on a chromosome with greater values found towards the distal-proterminal regions and almost null at loci located near centromeres (Welch, 1962).

To date, there is no publically available linkage map for tetraploid blueberry that has good coverage of co-dominant genetic markers that are specifically targeted towards identifying fruit quality attributes defined as important targets by UK consumers of both fresh and processed blueberry fruit. Generating a meaningful genetic map and locating key QTL for fruit phytochemicals and quality traits, would provide the framework for knowledge-based crop improvement which is the future towards developing a successful and sustainable UK blueberry industry. The research objectives of this project will therefore focus on (1) identifying current cultivars best adapted to the UK climate and (2) developing a tetraploid linkage map that can provide the basis for a robust marker assisted breeding programme.

## MATERIALS AND METHODS

Two tetraploid plants, cultivars 'Draper' and 'Jewel', which segregate for a number of key phenotypic traits used to develop an F1 population comprised of 105 individuals (kindly provided by Michigan State University) were used in the construction of a genetic linkage map. 'Draper' is an early to mid-season Northern highbush cultivar producing high quality, firm and sweet fruit with superior shelf-life released by Michigan State University in 2002. 'Jewel' is an early to mid-season Southern highbush cultivar from a high yielding plant producing very large, slightly tart, and moderately firm fruit, which was released by the University of Florida in 1999. Of course, environment has a significant influence on all these traits, particularly flavor.

A diverse range of 40 cultivars (Table 1) primarily derived from tetraploid *V. corymbosum* planted at a number of sites (both field grown and under protection), were selected for analysis across the UK for a range of phenotypic and sensory factors across seasons.

The traits to be examined on both the named cultivars and the mapping population will be: establishment, agronomic traits (plant growth, height, spread, branch number, and branch strength), seasonality, sensory

**TABLE 1** Individual Cultivars Analyzed; Diverse Highbush Cultivars, Primarily Derived from Tetraploid *V. corymbosum*, Were Planted at a Number of Sites Both Field Grown and Under Protection

| Cultivars analyzed | | | |
|---|---|---|---|
| Aurora | Chanticleer | Hardyblue | Nui |
| Berkeley | Collins | Heerma I | Ozarkblue |
| Bluecrop | Coville | Heerma II | Patriot |
| Bluegold | Darrow | Herbert | Powderblue |
| Bluehaven | Draper | Ivanhoe | Puru |
| Bluejay | Duke | Jersey | Reka |
| Blueray | Earliblue | Legacy | Rubel |
| Bluetta | Elliott | Liberty | Spartan |
| Brigitta | Goldtraube 53 | Nelson | Sunshineblue |
| Chandler | Goldtraube 71 | Northland | Toro |



characters, fruit composition (both fresh and processed), yield, fruit size, machine harvestability (cultivars only), and natural infection by *Monilinia vaccinii-corymbosi* (mummy berry), *Godronia cassandrae* (canker), and other diseases. Further data will be available from the same mapping population planted across the USA as part of a Specialty Crop Research Initiative-funded project to develop genomic tools for blueberry (Rowland et al., 2012). Sensory and phytochemical composition studies will identify a range of desirable flavor, composition, and appearance attributes that contribute to fruit quality and consumer appeal for both fresh and processed markets across different seasons and environments.

## Genetic Material and DNA Isolation

Young blueberry leaves were collected from the two parental cultivars, 'Draper' and 'Jewel', along with their 105 progeny. DNA was isolated using a DNeasy mini plant kit (Qiagen, 2006). For all samples, DNA isolation was made following the manufacturer's instructions (Qiagen, Hilden, Germany) and the DNA was eluted in 50 $\mu$l of sterile distilled water. Leaves/buds were collected from a range of named *Vaccinium* cultivars and DNA extracted using the same DNeasy mini plant kit protocol (Qiagen, Hilden, Germany).

## Development of SSR Markers

SSR markers used in this project were derived from two expressed sequence tag (EST) libraries developed from cold acclimated (CA) and non acclimated (NA) floral buds (Rowland et al., 2003, 2008; Dhanaraj et al., 2004) from the cultivar 'Bluecrop', and are described by Rowland et al. (2011). A limited number of additional SSRs were developed from a small number of the 'Bluecrop' ESTs and from a microsatellite enriched genomic library (Boches et al., 2005).

## PCR Conditions

Total DNA from parents and progeny were used as templates for polymerase chain reactions using the following conditions. A typical 25 $\mu$l reaction contained 25 ng of template DNA, 1.0 $\mu$M of forward and reverse primer, 0.2 mM dNTPs, 1× PCR buffer (containing 10 mm Tris-HCl, 1.5 mm $MgCl_2$, 50 mm KCl, pH 8.3) and 0.1 units Taq polymerase (Roche, Mannheim, Germany). PCR was performed using a Perkin Elmer 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) as follows: 5 min at 95°C, then 30 sec at 95°C, 30 sec at 57°C, and 45 sec at 72°C for 40 cycles followed by 10 min at 72°C before PCR products were resolved and visualized on a 1.5% agarose gel.

## Detection and Analysis of ssr Segregation through Genemapper

PCR products were analyzed on an ABI 3730 DNA sequence analyzer (Applied Biosystems, Foster City, USA) using Rox 500 (Applied Biosystems, Foster City, CA, USA) as an internal size standard. Allele sizes were visualized using GeneMapper software Version 3.7 (Applied Biosystems). Band polymorphisms were scored in individuals as present (1), absent (0), or missing data (9), where markers failed to amplify in an individual.

# RESULTS AND DISCUSSION

Phenotypic analyses have been initiated on the mapping population currently growing in Scotland. The potted plants are 2 years old and are held in an unheated glass house with initial analyses focusing on agronomic characteristics (plant growth, spread, etc.) and fruit traits (size, total soluble solids, color) on individuals that began producing ripe fruit from mid June 2010. The named cultivars replicated across Scotland had not reached the ripe fruit stage (as from the middle of July) so only agronomic traits (plant architecture and seasonality) have been observed so far.

## Marker Analysis

A total of 70 genomic SSR primer pairs were examined for polymorphism between the parents of the mapping population, 25 in this study and 45 previously analyzed (Brevis et al., 2007). Seven were monomorphic, 11 homozygous in both parents, and 8 primer pairs did not amplify products. A total of 44 polymorphic co-dominant SSR markers were identified. Seventeen EST-SSR primer pairs were assessed, 13 of which proved polymorphic (Brevis et al., 2007). For the dominant markers, (SSR markers were treated as individual loci) 16 scorable products were obtained from 6 additional SSR primers. When all SSRs were treated as individual dominant

**TABLE 2** Marker Segregation Ratio of Parental Bands with SSRs Treated as Individual Dominant Markers (Parental Marker Source, Segregation Ratio, Loci Significance Number with $p > 0.01$, Number Between $0.001 < p < 0.01$, and Number with $p < 0.001$)

| Source | Ratio | Identified | $p > 0.01$ | $0.001 < p < 0.01$ | $p < 0.001$ |
|---|---|---|---|---|---|
| Draper only | 1:1 | 55 | 51 | 2 | 2 |
| | 5:1 | 18 | 13 | 5 | 0 |
| Jewel only | 1:1 | 61 | 53 | 4 | 4 |
| | 5:1 | 13 | 13 | 0 | 0 |
| Both | 3:1 | 31 | 27 | 1 | 3 |
| | 11:1 | 10 | 10 | 0 | 0 |
| | 35:1 | 10 | 10 | 0 | 0 |
| Total | | 198 | 177 | 12 | 9 |

markers, 198 segregating alleles were obtained, the ratios of which are shown in Table 2.

With co-dominant markers, like SSRs, it is easy to detect the presence of double reduction (two sister chromatids recovered from a single gamete). However, there were no offspring genotypes identified in this data set which could be obtained only through double reduction.

## Linkage Map Construction

Segregation analysis was carried out on the parents and 90 of the 105 progeny produced. Segregation data derived from polymorphic SSRs in the 'Draper' × 'Jewel' cross were analyzed using TetraploidMap for out-breeding populations (Hackett and Luo, 2003). Markers were grouped into linkage groups using cluster analysis as described in Luo et al. (2001).

Linkage groups were then ordered using a multipoint analysis that included a two point analysis to estimate recombination frequencies and LOD scores between all pairs of markers, followed by a ripple search. Due to the small sample size used in this study, it was not possible to order markers accurately with a recombination fraction of 3 cM or less.

The preliminary genetic linkage maps generated consisted of 14 groups ranging from 2 to 71 cM covering 274 cM in parent 1 ('Draper') and 16 linkage groups from 2 to 150 cM in length covering 375 cM for parent 2 ('Jewel').

# CONCLUSIONS

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

Progress has been made in the construction of a tetraploid blueberry linkage map using both SSR and EST-SSR markers. The marker data analyzed to date suggests that blueberry has the simplest model for meiosis, random chromosomal segregation.

Once the linkage map has been developed, QTL mapping of data from the key traits assessed in the project may proceed. Additional work focusing on increasing the mapping population and analyzed markers will produce a useful and robust genetic tool for use in marker-assisted selection and breeding, thus, accelerating the development of new high quality blueberry cultivars.



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