Grower Summary

Selection and testing of putative ‘crumbly’ fruit molecular markers for both diagnostic and breeding purposes in *Rubus idaeus*

SF 167 (extension for marker development)

Extension 2020

Project title:	Selection and testing of putative ‘crumbly’ fruit molecular markers for both diagnostic and breeding purposes in *Rubus idaeus*
Project number:	SF 167 (extension for marker development)
Project leader:	Julie Graham – The James Hutton Institute
Report:	Final report
Previous report:	PhD thesis Luca Scolari (SF 167)
Key staff:	Luca Scolari
Location of project:	Invergowrie, Dundee, DD2 5DA, Scotland, UK
Industry Representative:	Louise Sutherland Freiston Associates Ltd.
Date project commenced:	December 2019

DISCLAIMER

While the Agriculture and Horticulture Development Board 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.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Name]

[Position]

[Organisation] Julie Graham Nov 2020

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

GROWER SUMMARY

Headline

The development of the Glen Moy x Latham raspberry linkage map using the Genotype by Sequencing (GbS) technology (Hackett et al. 2018), due to the higher markers density, allowed the identification of a new 'crumbly' QTL on linkage group 3 (LG3) as well as a more accurate and precise location for those two previously identified on LG1 and LG3. All three QTL proved to be robust across different seasons therefore there is potential to identify molecular markers located within these QTLs. Markers strongly associated with 'crumbly' fruit across a wider gene pool would be valuable for molecular markers assisted breeding and for diagnostic purposes.

Background

Genetic markers represent important progress with application in the crop science particularly in plant breeding (Kebriyaee et al. 2012). Genetic markers are genes or DNA sequences mapped on known chromosome positions and associated with specific genes and/or traits. They are like signs/flags for target genes to which are associated. Genetic markers can be grouped in two main categories, classical (i.e. morphological, cytological and biochemical markers) and molecular markers with the most common being: restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and single-nucleotide polymorphism (SNP) (Nadeem et al. 2018). In this work we focused on SNPs and SSRs markers.

Simple sequence repeats (SSRs) also called microsatellite are nucleotide motifs repeated in tandem. Such motifs can be mononucleotide (A), dinucleotide (GT), trinucleotide (ATT), tetranucleotide (ATCG), pentanucleotide (TAATC) and hexanucleotide (TGTGCA). The sequences flanking the SSRs are conserved and are used to design primers; by means of the polymerase chain reaction (PCR) potential polymorphisms can be easily assessed. SSRs are very abundant in the genome and are co-dominant allowing to distinguish between homozygous and heterozygous alleles (Nadeem et al. 2018).

SNPs are single base-pair changes found into the genome. They can be, depending on the nucleotide substitution, of two different types, transition (i.e. C/T or G/A) or transversions (i.e. C/G, A/T, C/A or T/G). SNPs are very abundant in plant genomes with a frequency ranging from 1 SNP in every 100-300 bp, they can be found in coding or non-coding sequence of

genes and are identified by analysing sequence data stored in database (Nadeem et al. 2018).

Summary

Two different kind of loci, for the identification of potential molecular markers, were considered during this study. Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSRs). Eight markers were selected in total. Five were SNPs and were of the different kinds, gene 'tags', located in non-coding regions of the genome, and target genes located in coding regions while the remaining three markers were SSRs.

The markers are all significantly associated with crumbliness in the Latham x Glen Moy population and in order to determine their wider applicability we required a validation population. The numbers in this population were smaller than ideal however due to being able to identify varieties that never show crumbly symptoms. A population of 63 different raspberry genotypes, with ¾ being selected as being prone to 'crumbliness' while the ¼ as never showing 'crumbly' symptoms was developed. DNA was extracted was from each of the 63 different genotypes and by means of the primers specifically designed to amplify the genome regions containing the selected markers.

Analysis of the allelic polymorphisms was conducted for each marker and potentially significant associations between alleles and 'crumbly' or not 'crumbly' were assessed; the goal was to identify at least one marker significantly linked with one of the two phenotypes through a Genome Wise Association Study (GWAS) using the selected 63 genotypes population as sample. Though not ideal this allows us to examine the allele status of the markers from the LxM population in a wider gene pool.

Financial Benefits

The James Hutton Institute (JHI) is the sole source of *Rubus* plant material for entry into the UK Plant Health Certification Scheme. Provision of disease-free true-to-type nuclear stock of certified varieties represents a statutory requirement and the propagation of high-health planting material is crucial to the establishing of commercial production throughout the UK. The current test of fruiting mother stocks and releasing only those that have passed, may not guarantee control of the condition and thus no foolproof testing method exists.

The identification of 'crumbly' molecular markers could pave the way for the development of faster diagnostic test to replace the current fruiting test; the same molecular knowledge could

be useful to the Raspberry Breeding programme based at James Hutton Limited allowing the selection of new varieties crumbly free or at least more resistant to this condition.

Action Points

Rubus 256e can be considered as a marker for crumbly fruit. Other regions have been identified but need further validation.