Final Report

Controlling Dormancy and Sprouting in Potato and Onion

Ref: 11140028

Reporting Period: 1/9/2013-31/8/2017

Report Authors:

Glenn Bryan, Sanjeev Kumar Sharma and Mark Taylor (James Hutton Institute)

Leon Terry, Carmen Alamar, Fady Mohareb, Ikenna Ohanenye and Andrew Thompson (Cranfield University)

Colin Turnbull and Rosa Lopez Cobollo (Imperial College)

Richard Colgan and Debbie Rees (University of Greenwich)

Date report submitted: March 2019

POTATOES

© Agriculture and Horticulture Development Board 2019

© Agriculture and Horticulture Development Board 2019. No part of this publication may be reproduced in any material form (including by photocopy or storage in any medium by electronic means) or any copy or adaptation stored, published or distributed (by physical, electronic or other means) without the prior permission in writing of the Agriculture and Horticulture Development Board, other than by reproduction in an unmodified form for the sole purpose of use as an information resource when the Agriculture and Horticulture Development Board is clearly acknowledged as the source, or in accordance with the provisions of the Copyright, Designs and Patents Act 1988. All rights reserved.

AHDB is a registered trademark of the Agriculture and Horticulture Development Board.

All other trademarks, logos and brand names contained in this publication are the trademarks of their respective holders. No rights are granted without the prior written permission of the relevant owners.

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

Publications

AHDB Potatoes
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel: 02476 692051
E-mail: Potatoes.Publications@ahdb.org.uk

Our reports, and lists of publications, are also available at potatoes.ahdb.org.uk

CONTENTS

1. SUMMARY 4
   1.1. Aim 4
   1.2. Methodology 4
   1.3. Key findings 4
   1.4. Practical Outcomes 4
2. INTRODUCTION 5
3. MATERIALS AND METHODS 6
   3.1. WP1 Genetic basis of tuber dormancy in potato 6
      3.1.1. Phenotyping of 06H1 population 6
      3.1.2. QTL analysis and fine mapping 6
      3.1.3. Allele mining of candidate genes 6
   3.2. WP2: Identification of comparative expression markers of meristem dormancy in potato and onion 6
      3.2.1. Onion dormancy phenotyping 6
      3.2.2. Potato dormancy phenotyping 7
      3.2.3. RNA seq analysis for potato and onion 7
   3.3. WP3. Integrative biology of dormancy and sprouting, and development of predictive markers. 7
      3.3.1. Profiling of hormone and transcript markers and responses 7
      3.3.2. Transgenic tester lines 7
4. RESULTS 8
   4.1. WP1. Genetic basis of tuber dormancy in potato 8
      4.1.1. Phenotyping 8
      4.1.2. QTL analysis 9
      4.1.3. Fine mapping of large effect QTL 10
   4.2. WP2: Identification of comparative expression markers of meristem dormancy in potato and onion 12
      4.2.1. Potato dormancy phenotyping 12
      4.2.2. Onion gene expression 14
   4.3. WP3: Integrative biology of dormancy and sprouting, and development of predictive markers. 14
      4.3.1. Hormone profiling: Regulatory signals and/or potential markers 14
      4.3.2. Transgenic tester lines 16
5. DISCUSSION 18
6. CONCLUSIONS 19
7. REFERENCES 20
8. ACKNOWLEDGEMENTS 21

© Agriculture and Horticulture Development Board 2019

1. SUMMARY

1.1. Aim

This project is aimed at unravelling genetic and molecular processes underlying the very important traits, tuber and bulb dormancy in potato and onion respectively.

Potato and onion production relies on industrial-scale storage to ensure year round availability, for both fresh and processing sectors. Multiple strategies are used to extend dormancy and minimise sprouting, including low temperature storage and/or the application of sprout suppressant chemicals such as chlorpropham (CIPC) or ethylene. Such treatments are not fully effective, and CIPC is expected to be withdrawn under EU regulations. Currently, in the UK, 1.8 million tonnes of stored potatoes are treated with CIPC annually and so there is an urgent need to develop new storage strategies for potato and onion, with less reliance on CIPC.

1.2. Methodology

A number of approaches have been taken, exploiting the expertise and resources across the four collaborating institutions; James Hutton Institute, Cranfield University, Imperial Collage, University of Greenwich. These include:

- Phenotypic analysis of a population of potato lines derived from a cross between diploid potato parents, and the use of quantitative trait locus analysis to identify the locations on the genome that control length of dormancy.
- Identification of key controlling genes through sequencing of the gene transcription (RNAseq) at critical stages during dormancy and sprouting for potato lines with contrasting dormancy characteristics, and for onion bulbs with differing dormancy characteristics as influenced by ethylene treatment.
- Analysis of hormones and metabolites for both potato and onion tubers during dormancy and sprouting

1.3. Key findings

An updated unified model for hormonal dormancy control for potato (also relevant to onion).

- Genome locations (QTLs) identified associated with tuber dormancy and sprout growth. This could potentially lead to markers for tuber dormancy/sprouting for use in breeding within 5 year period
- Candidate genes involved in dormancy control have been identified. Transgenic lines for functional validation and further expression work have been produced for one gene (TERMINAL FLOWER 1/CENTRORADIALIS).
- Profiles of gene expression, hormone concentrations, key metabolites and respiration through progression of dormancy, dormancy break and sprout growth have been obtained for both potato and onion.

1.4. Practical Outcomes

We are currently initiating discussions with industrial collaborators on how to take the work forward into more practical applications to address a major industrial challenge. One way forward is to develop genetic markers for potato that can be used to breed for enhanced storage characteristics. A second possible outcomes is to identify molecular or biochemical markers that could be used to develop 'in store' assays or possibly sensor technology.

© Agriculture and Horticulture Development Board 2019

2. INTRODUCTION

Potato and onion production relies on industrial-scale storage to ensure year round availability, for both fresh and processing sectors. In the UK alone, 4.05 million tonnes of potato tubers and 0.60 million tonnes of onions are stored annually (Garthwaite et al. 2010). Dormancy properties of potato tubers and onion bulbs are key determinants of postharvest life. Premature sprouting of either organ during storage is accompanied by severe loss of quality (Fernie & Wilmitzer, 2001; Burton, 1989). Chemical inhibitors of sprouting (e.g. chlorpropham (CIPC) and maleic hydrazide (MH)) are the UK industry standards; yet they are under threat and it is expected that the loss of CIPC is imminent. For the potato crop stored in the UK, 44% is currently treated with CIPC (Garthwaite et al., 2010). Loss of CIPC without adequate alternatives would require the potato and onion industries to adopt strategies that would negatively impact on their environmental and economic sustainability such as increased energy use for long-term cold storage or increased imports. There is therefore an urgent need to develop new storage strategies for potato and onion.

Potato and onion are taxonomically distant crops, differing markedly in the morphology of their harvested organs (De Mason, 1990; Suttle, 2004), yet both have evolved to over-winter as storage organs with dormant axillary or primary meristems. There are moreover some clear commonalities in postharvest behaviour at the physiological, metabolite and transcript levels. Indeed, current storage technologies for onions and potato are similar (e.g. cold-storage, continuous ethylene supplementation to inhibit sprout growth or use of CIPC and MH). In both potato tubers and onion bulbs, onset and duration of dormancy and rate of subsequent sprout growth are governed by genetic factors, orchestrated by hormone signalling and influenced by environmental factors during crop growth and storage (Suttle, 2004; Chope et al., 2012; Simko et al., 1997; Downes et al., 2010; Turnbull & Hanke, 1985; Turnbull & Hanke, 1985; Hartmann et al., 2011; Suttle & Hultstrand, 1994). Furthermore, an emerging theme in dormancy research is that similar transcriptional modules are associated with specific dormancy states across species boundaries (Rohde and Bhalerao, 2007). However, there are still significant gaps in our knowledge which impact on the supply chain of these key crops. For potato and onion breeders there are no genetic markers that can be used to select for dormancy-related traits. For those involved in all sectors of the supply industries (seed, fresh and processed), predictive models that indicate how a crop may perform in storage are lacking. The availability of genomics tools, such as dense marker platforms, second generation sequencing, comprehensive microarrays and genome sequences for many crops has opened up new possibilities for studying this complex stage in the plant life-cycle.

In this project we sought to deploy genomic and other technologies to discover the underlying genetic, metabolic and physiological mechanisms that impact on dormancy-related traits, primarily in potato whilst simultaneously extending the tools and models available for dormancy research in onion.

© Agriculture and Horticulture Development Board 2019

2. MATERIALS AND METHODS

The project had a complex workplan over the 4 year project period that combined several different disciplines and which involved different partners for each activity. The methodologies employed in the project were summarised in the form of different workpackages at the outset of the project. The Materials and Methods for the three experimental workpackages are summarised here.

2.1. WP1 Genetic basis of tuber dormancy in potato

2.1.1. Phenotyping of 06H1 population

This work entailed the use of genetic resources held at JHI mainly in the form of a segregating diploid population (06H1) which had been previously characterised for several traits (including a preliminary assessment of tuber dormancy) in previous projects. The 06H1 population had been genotyped with an 8300 marker SNP panel and a genetic map produced.

In this project ~250 clones from the 06H1 population grown at JHI were phenotyped by University of Greenwich at their storage facility at East Malling. Over two seasons, tubers from ~250 clones of 06H1 grown at JHI were harvested and sent to University of Greenwich where they were stored in specialised facilities at 10^{o}C. in a carefully controlled environment. Days to >2mm sprout length for all eyes per tuber were recorded and sprout length of the apical bud was assessed at set intervals to determine sprout growth rate. Observations were made over a 3-5 month period, depending on the range of dormancy length observed. To provide statistical rigour 2 replicates of 10 tubers were assessed per clone.

2.1.2. QTL analysis and fine mapping

A 06H1 linkage map containing ~3000 markers (Prashar *et al*. 2014) was used to perform QTL analysis (using MapQTL5) of the phenotypes measured.

The data obtained by QTL analysis was strengthened using a novel Bulk Segregant Analysis (BSA) method. For this analysis, lines that were clearly high or low dormancy compared to the whole population (note dormancy was generally low compared to commercial tetraploid cultivars) were used to produce pools of DNA. Comparison of allele frequencies between the 'pools' allowed the identification of genomic regions responsible for the phenotypic differences between the phenotypic bulks (i.e. where the allele frequencies differed most).

2.1.3. Allele mining of candidate genes

Candidate genes mapping under target QTLs were resequenced from parents, bulks and representative individuals to identify polymorphisms that were consistent with phenotypic differences. Alleles were amplified by PCR and the cloned products analysed by Sanger sequencing. Capture libraries were subjected to Illumina sequencing using a barcoding approach to pool 24-96 genotypes in one lane of a HiSeq2000 run. Allele reconstruction was performed with the aid of Samtools or similar software for haplotype reconstruction.

2.2. WP2: Identification of comparative expression markers of meristem dormancy in potato and onion

2.2.1. Onion dormancy phenotyping

Commercial onion F_1 hybrid cultivar Sherpa was grown in replicated field trials. Bulbs were harvested at 100% 'fall down' (fully mature), cured and stored at 1^{o}C for 7 months as per standard commercial practice in controlled temperature rooms at CU. Bulbs were stored with and without ethylene supplementation but were not treated with Maleic Hydrazide before harvest. Bulb samples were assessed at 2 week intervals. Dormancy stage was classified using established physiological measurements developed at CU *viz*. sprouting as a degree of bulb height, rooting, and other changes in the basal plate, and meristematic tissue. Real-time changes in respiration rate were assessed using a bespoke 16 channel respirometer system at CU. Endogenous ethylene production was measured using a photoacoustic laser with resolution down to 0.3ppb as this has been recently shown to provide valuable data on judging

© Agriculture and Horticulture Development Board 2019

physiological state. Meristematic and lower basal plate tissue were isolated and stored at -80ºC in readiness for RNA seq. For air stored bulbs sampling was at 2, 8 and 12 weeks. For bulbs stored with ethylene, and therefore slower sprout growth, sampling was at 2, 8, 12, 16 and 20 weeks.

2.2.2. Potato dormancy phenotyping

Tubers representative of extreme phenotypes from 06H1 and varieties with divergent dormancy responses were stored at CU at 10ºC for 7 and 5 months, respectively, without CIPC or ethylene treatment. As for onion, fortnightly samples were removed from storage and phenotyped *viz*. respiration rate, ethylene production, degree of eye movement, incidence of sprouting (Foukaraki 2012).

2.2.3. RNA seq analysis for potato and onion

For potato, meristematic tissue from eyes in proximal regions of the tuber and peel were separated and stored at -80°C. Material from low and high dormancy lines were pooled for subsequent RNA seq analysis. For low dormancy lines samples were taken at 0 and 1 weeks of storage, whereas for high dormancy lines, samples were taken at 0, 1, 2, 4, 6, 8 weeks of storage.
RNA sequencing was carried out by the Genome Analysis Centre (Now called the Earlham Centre) using illumina sequencing, paired end reads.
Data analysis was carried out by CU. Blastx against nr database with threshold of E = $10e^{-3}$. Gene Ontology, Enzyme and KEGG pathway annotations performed

2.3. WP3. Integrative biology of dormancy and sprouting, and development of predictive markers.

2.3.1. Profiling of hormone and transcript markers and responses

Hormone analysis was carried out for both potato tubers and onion bulbs to explore the potential for using hormone profiles as predictive markers of dormancy exit. High sensitivity analytical protocols were applied to the classes of plant hormone already known to be associated with dormancy (especially cytokinins, strygolactones, and ethylene) and others that are implicated (auxin, ABA). Analysis was carried out at IC using LC-MS systems (Thermo Orbitrap and AB QTrap 6500), with multiple reaction monitoring (MRM) with inclusion of stable isotope labelled internal standards whenever possible (Foo et al 2007, Braun et al 2012).
Standard qRT-PCR was used to validate RNASeq data.

2.3.2. Transgenic tester lines

Transgenic tester lines were produced using the proven JHI potato transformation platform. Full details are given in Morris et al. 2018

© Agriculture and Horticulture Development Board 2019

3. RESULTS

3.1. WP1. Genetic basis of tuber dormancy in potato

3.1.1. Phenotyping

Within this project the O6H1 diploid potato population, was phenotyped to identify the locations on the genome, or Quantitative Trait Loci (QTLs), relating to length of dormancy/sprout growth. While most commercial potato varieties are tetraploid, this diploid population was selected for analysis to avoid the complications of analysing a tetraploid population. The parentage of 06H1 is shown in Figure 4.1.

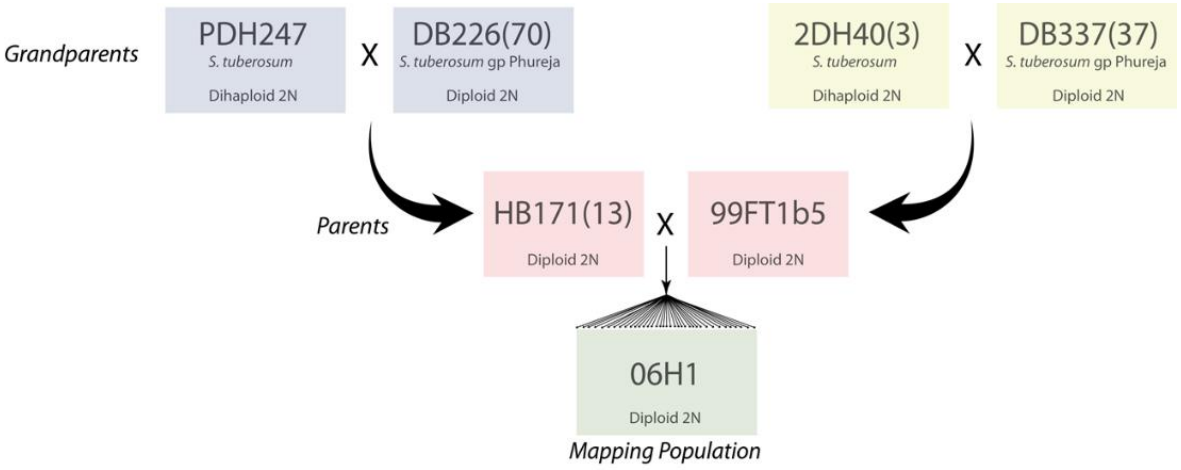


Figure 4.1 The parentage of the 06H1 mapping population used for this project.

Biologically the length of dormancy starts at tuber initiation and ends at sprout initiation. However practically it is very difficult to accurately monitor the point of tuber initiation during phenotyping trials. For the phenotyping within this project, sprout length measured at several time points was modelled using a "Gompertz" analysis, a standard method for modelling growth curves. This provided two parameters for each potato line phenotyped; *kappa*, which gives the time point of sprout growth initiation, and *beta* which gives the rate of sprout growth.
Phenotyping was carried out by University of Greenwich. There was wide variation within the population and good consistency between seasons as demonstrated in Figure 4.2.

© Agriculture and Horticulture Development Board 2019

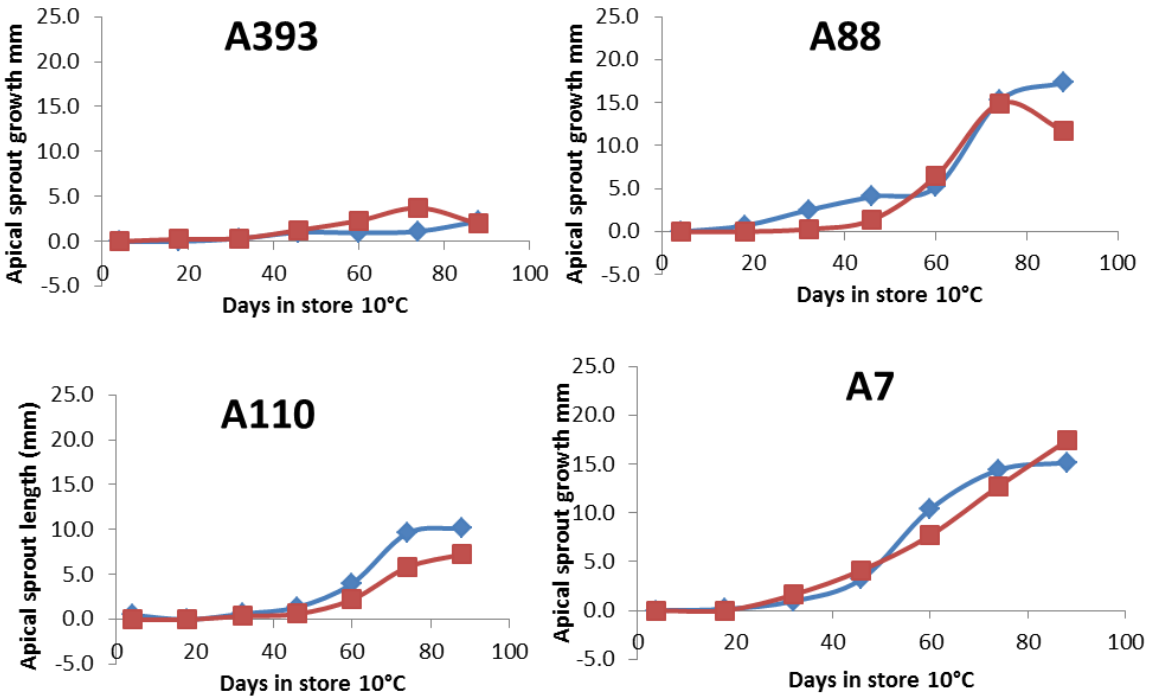


Figure 4.2 Length of apical sprout growth for four out of the 250 lines of the O6HI mapping population over 2 seasons. Each data point is the mean of 20 tubers (2 replicates of 10 tubers). Red season 1, Blue season 2.

3.1.2. QTL analysis

The phenotyping led to the identification of 5 QTLs on 4 chromosomes. The location of these QTLs which indicates the location of genes affecting the length of dormancy are shown in Figure 4.3.

Figure 4.3 Location of five QTLs identified through phenotyping of the O6HI mapping population undertaken within this project.

© Agriculture and Horticulture Development Board 2019

The locations on chromosomes 2, 3 and 5 agree exactly with putative QTLs for sprouting in air identified in a previous DEFRA-LINK project 'Reducing energy usage and wastage by improving ethylene control of potato sprouting' undertaken by a consortium including University of Greenwich and JHI.

3.1.3. Fine mapping of large effect QTL

The data obtained by QTL analysis was strengthened using a novel Bulk Segregant Analysis (BSA) method. For this analysis, lines that were clearly high or low dormancy compared to the whole population (identified by the length of the apical sprout on day 46) were used to produce pools of DNA. Comparison of frequencies of alleles (variants of individual genes) between the 'pools' allowed the identification of genomic regions that were responsible for the phenotypic differences between the phenotypic bulks (i.e. where the allele frequencies differed most). The regions identified using this analysis were close to the QTLs identified on chromosomes 2 and 3, thereby providing confirmation of the importance of these regions in control of dormancy length.

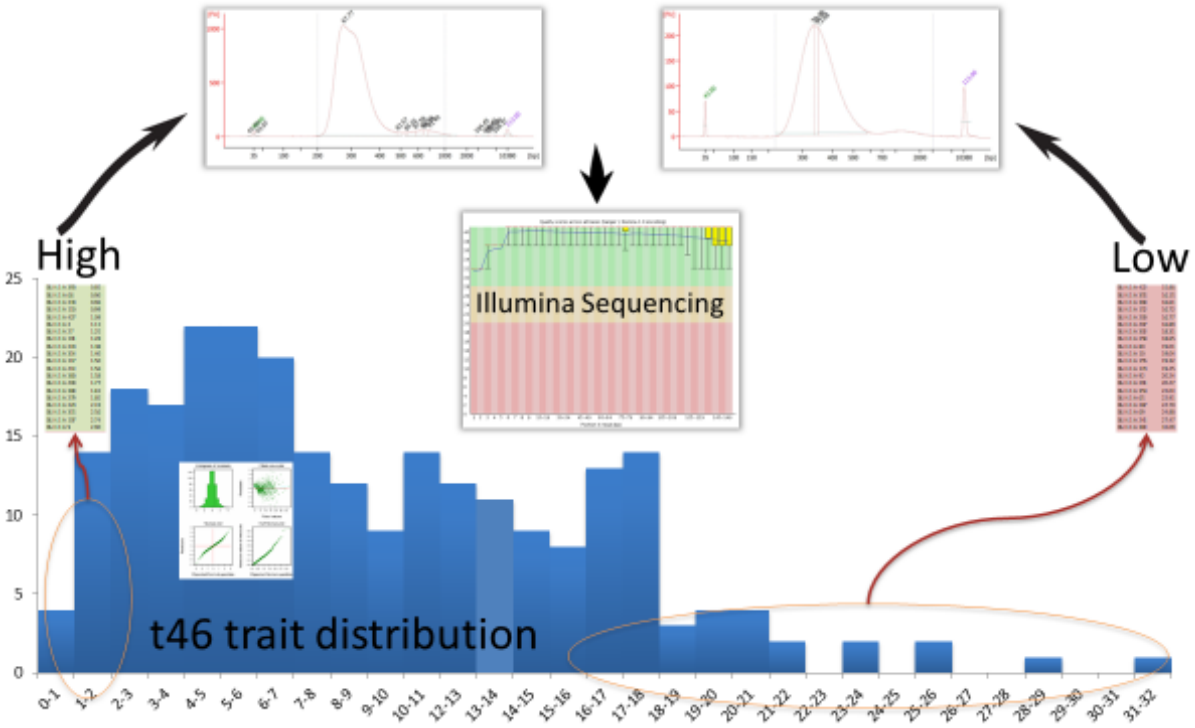
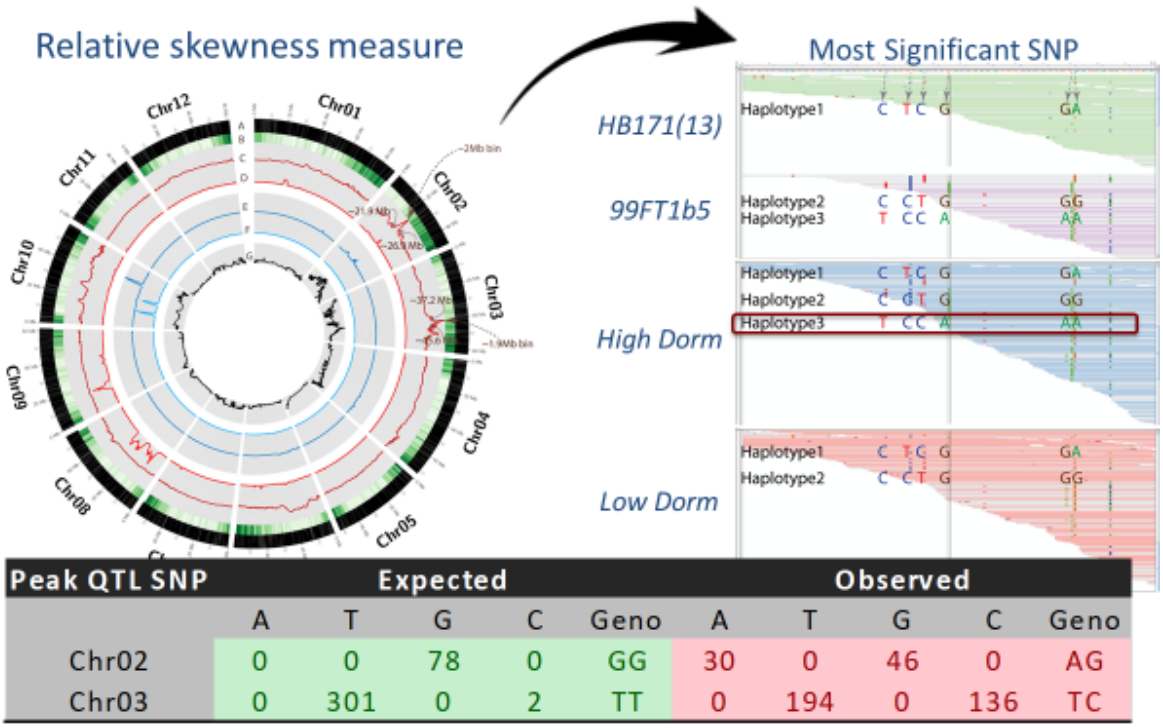


Figure 4.4 Illustration of the Bulk Segregant Analysis method; the graph shows the number of lines within each range of apical sprout length on day 46, the range selected for pooling each each of High and Low dormancy.

Single Nucleotide Polymorphism (SNP) markers have been identified that are associated with the QTLs (Figure 4.5). These SNPs could potentially be used for development of diagnostic assays for use in marker assisted breeding, however, as described in the Discussion it would be necessary to validate any markers identified using association mapping across a genome wide panel of commercial (tetraploid) potato cultivars.

© Agriculture and Horticulture Development Board 2019



Peak QTL SNP	Expected					Observed				
	A	T	G	C	Geno	A	T	G	C	Geno
Chr02	0	0	78	0	GG	30	0	46	0	AG
Chr03	0	301	0	2	TT	0	194	0	136	TC

Figure 4.5 Allelic variation was used to identify regions responsible for phenotypic differences and therefore to identify SNP (Single Nucloetide Polymorphisms) markers for length of dormancy.

© Agriculture and Horticulture Development Board 2019

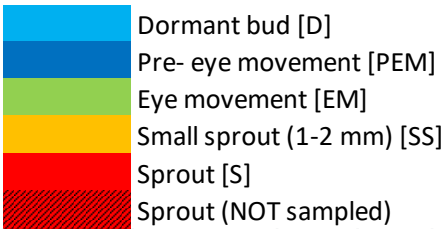
3.2. WP2: Identification of comparative expression markers of meristem dormancy in potato and onion

3.2.1. Potato dormancy phenotyping

In order to understand the biochemical and molecular control of dormancy, selected lines from the mapping population were phenotyped in detail in terms of metabolite and hormone concentration and gene expression using RNAseq. Samples were taken from buds at specific stages of sprout development and from peel at time points as described in Methods.

Season 2

University of Greenwich data	Cranfield University phenotyping				
Dormancy	Week 0	Week 1	Week 2	Week 4	Week 6
Low	EM S				
Low	PEM S	EM S			
Low					
Low	D	EM S			
Low					
Low	D EM				
Mid			EM S		
Mid			EM S		
High			PEM	EM	
High				PEM S	
High				D	EM
High				D	PEM
High		D EM		EM S	
High		D	PEM	EM S	



Season 3

University of Greenwich data	Cranfield University phenotyping					
Dormancy	Week 0	Week 1	Week 2	Week 4	Week 6	Week 8
Low	PEM	EM S*				
Low	EM	EM S*				
Low	EM	EM S*				
Low	PEM	EM S*				
Low	PEM	EM S*				
Low	PEM	EM S*				
Mid	EM	EM	EM			
Mid	EM	EM	EM	EM		
High	PEM	D PEM	PEM	missing line	missing line	missing line
High	D	D PEM	PEM	EM PEM	EM PEM	EM SS
High	D	PEM	PEM	PEM EM	EM	SS
High	D/PEM	D PEM	PEM	EM PEM	SS S**	S
High	D	PEM	PEM	EM PEM	EM	S
High	D	D	PEM	EM	EM	S

Figure 4.6 Dormancy break profiles for season 2 and season 3 for six low dormancy, six high dormancy lines and parents of the mapping population

The relative dormancy break profiles (Figure 4.6) were reasonably consistent between seasons. Lines defined as having low dormancy all sprouted in advance of lines defined as having high dormancy for both seasons. The parents; HB171(13) and FT1B5 had phenotypic behaviour between the low and high lines.

© Agriculture and Horticulture Development Board 2019

The information obtained from RNAseq on gene expression is very extensive and will provide a database that will be valuable for many years. Strong differences in gene expression profiles were observed between buds and peel and between low and high dormancy lines. The initial analysis set out as a 4 step process below, has focused on identifying the genes that behave differently between dormancy break and early sprouting, in particular those that were consistent between the two years of sampling. This led to the identification of 144 genes. (Figure 4.7).

Gene selection process

1. Differentially Expressed between w0 and w1 on Low dormancy
2. AND between w0 and w4 on High dormancy
3. AND between High and Low on week 1.
4. With an FDR<0.05 and logFC>1

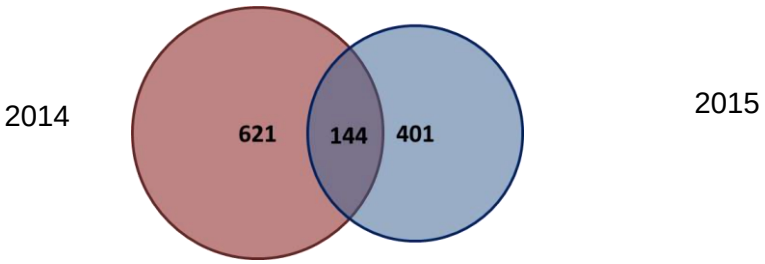


Figure 4.7 Venn diagram showing the genes differentially expressed between dormancy break and early sprouting in the two seaons of analysis.

The genes identified have a range of expression profiles. Some examples are shown in Figure 4.8. This includes genes showing a continuous rise or fall through dormancy, or those that show a step change prior to or at dormancy exit. qPCR was carried out to confirm expression profiles (data not shown).

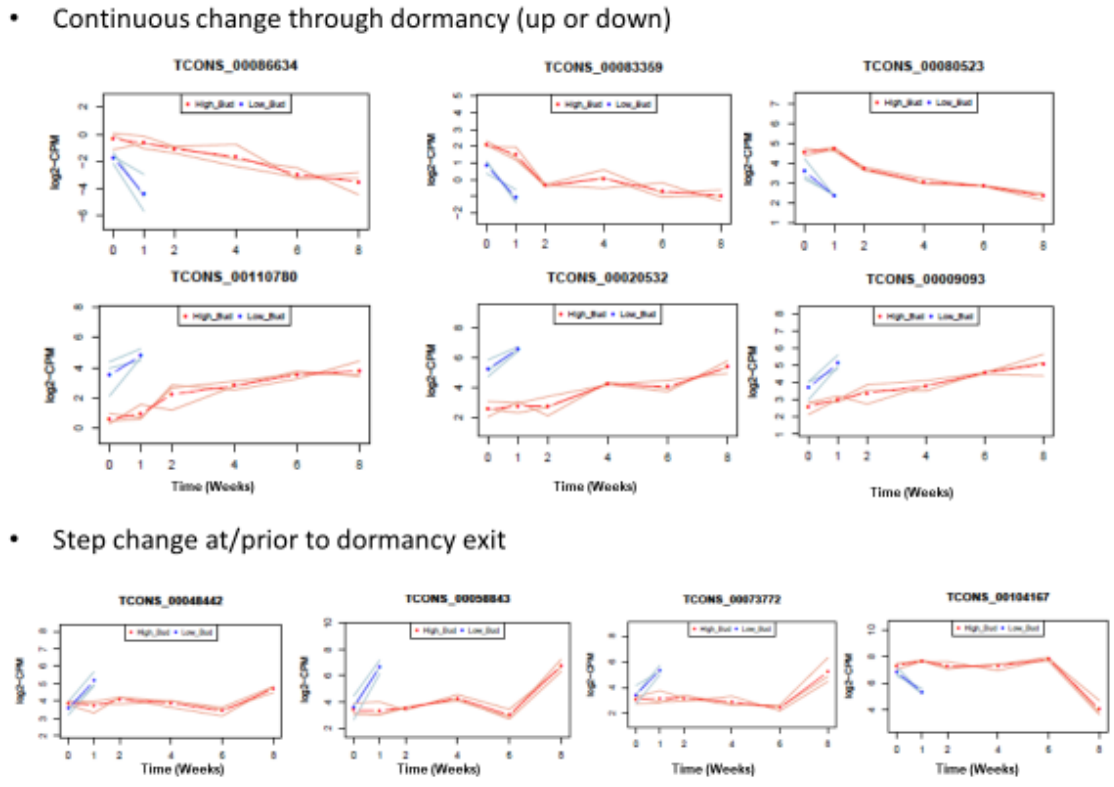


Figure 4.8 Expression profiles for selected genes through dormancy and exit of dormancy

© Agriculture and Horticulture Development Board 2019

3.2.2. Onion gene expression

The transcriptome of onion bulbs has also been sampled through dormancy and dormancy exit in the presence and absence of externally added ethylene, as described in Methods. The analysis of the data is still on-going and will be added to this report at a later date.

3.3. WP3: Integrative biology of dormancy and sprouting, and development of predictive markers.

3.3.1. Hormone profiling: Regulatory signals and/or potential markers

In addition to gene expression profiles, hormone concentration was investigated as a potential marker for dormancy exit. The figures below show examples of analytical results. (*The identity of the hormones is not given in this report for reasons of commercial confidentiality.*)
It was observed that potato buds and peel exhibit very different profiles (Figure 4.9 a, b), but that changes in hormone profiles are very consistent between years (Figure 4.10). The hormone profiles of potato and onion buds were compared in order to determine whether it is possible to develop a common model of sprout control. Some similarities in behaviour exist, notably hormone C (Figure 4.9 a, c). Likewise it was observed that hormone profiles were similar between the diploid lines and commercial tetraploid varieties.

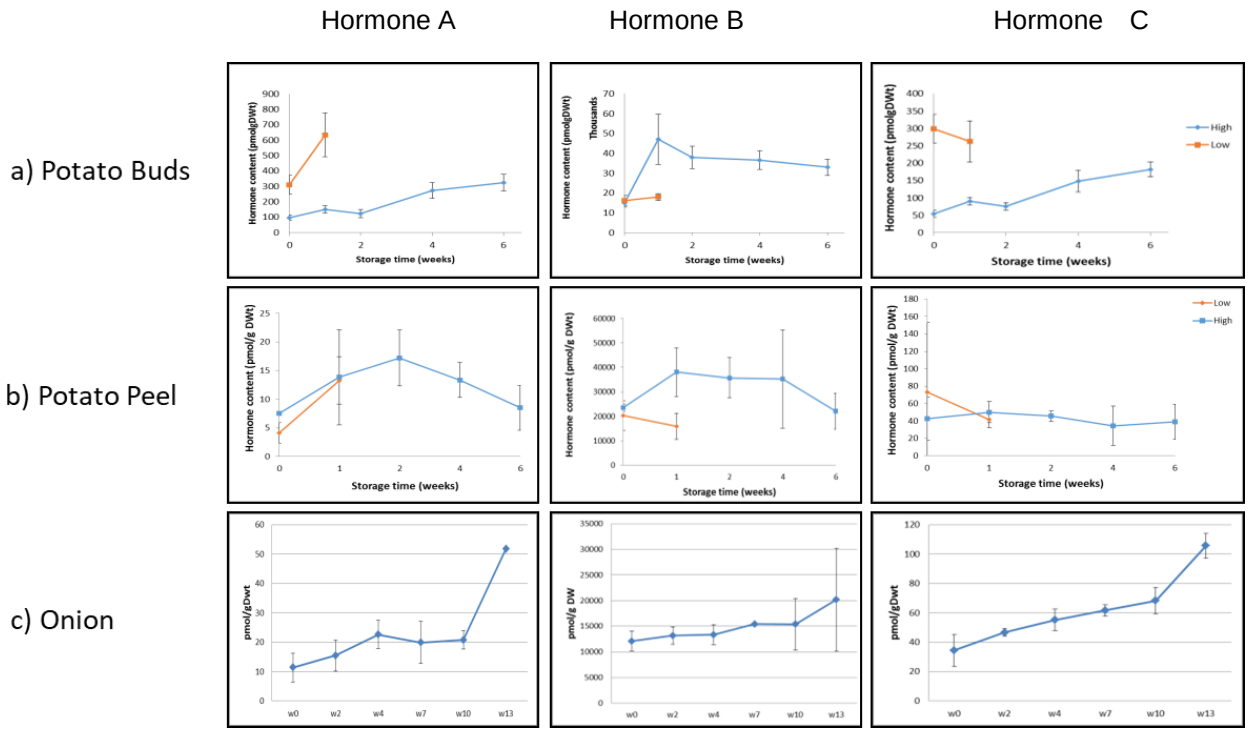
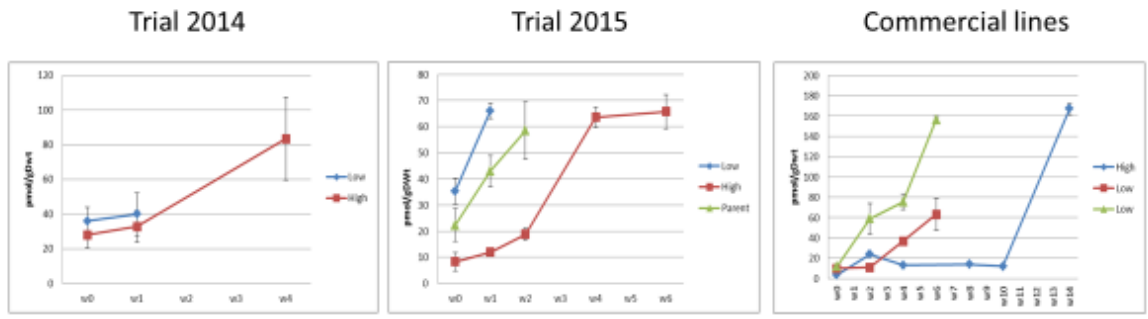


Figure 4.9 Example hormone profiles obtained for potato buds, peel and onion in season 3

© Agriculture and Horticulture Development Board 2019

a) Hormone D



b) Hormone E

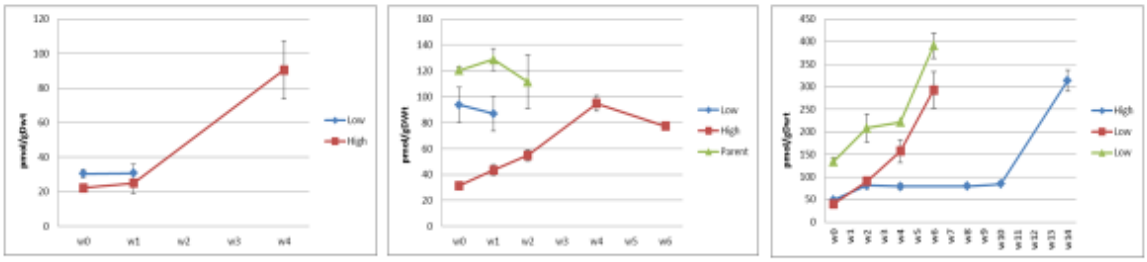


Figure 4.10 Comparison of hormone profiles (hormones D and E) for lines from the mapping population in 2014 and 2015 and three commercial tetraploid lines assessed in 2015.

© Agriculture and Horticulture Development Board 2019

3.3.2. Transgenic tester lines

Two candidate genes were identified during this project as being of interest and that were physically located 'under' QTL peaks; one of these, a *CENTRORADIALIS*/*TERMINAL FLOWER 1* orthologue (CEN1) was studied in more detail through transgenics.

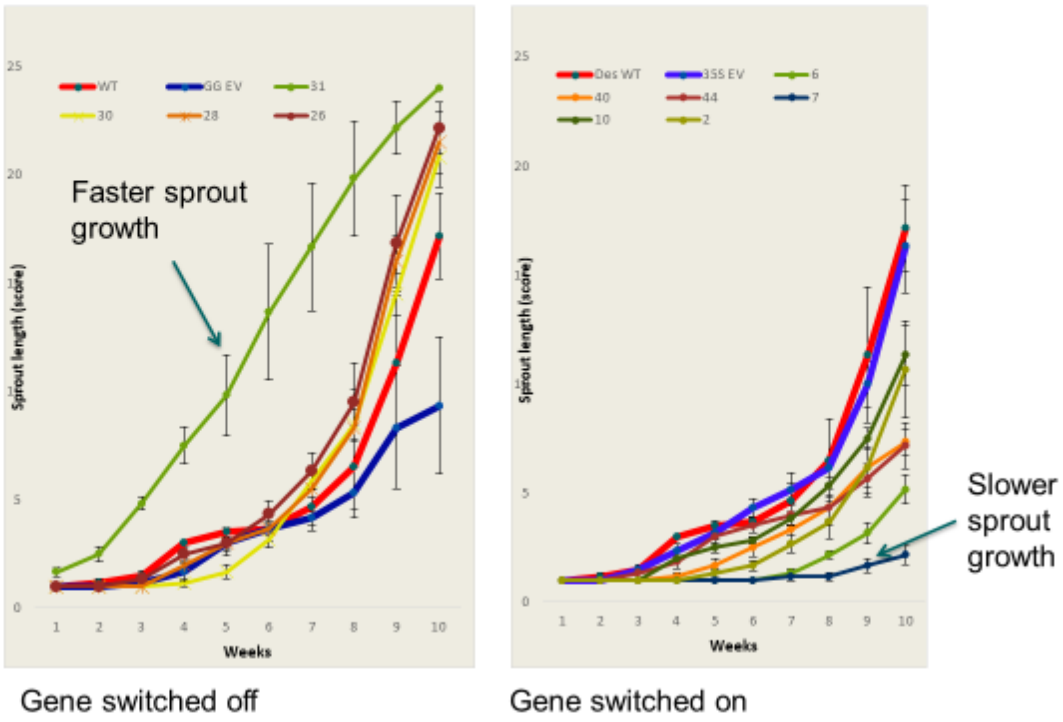


Figure 4.11 Comparison of sprout length of wild-type (WT) Désirée with transgenic plants either (A) down-regulating (RNAi) or (B) overexpressing (OEX) the StCEN gene. Empty vector (EV) transformed control is also included for comparison. Error bars represent the SE of six biological replicates.

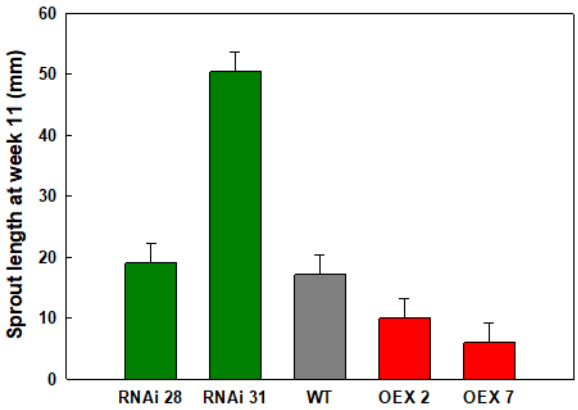


Fig. 4.12. Sprout length (mm) after 11 weeks of cold storage (10 °C) for wildtype (WT - Désirée) tubers and StCEN transgenic potatoes, where RNAi28 and RNAi31 are silenced lines and OEX2 and OEX7 are overexpressed lines. For each genotype, four tubers from three individual plants were studied. Histogram bars correspond to the mean of three replicates per line, where four tubers per replicate were assessed. LSD bars (P<0.05) are shown.

© Agriculture and Horticulture Development Board 2019

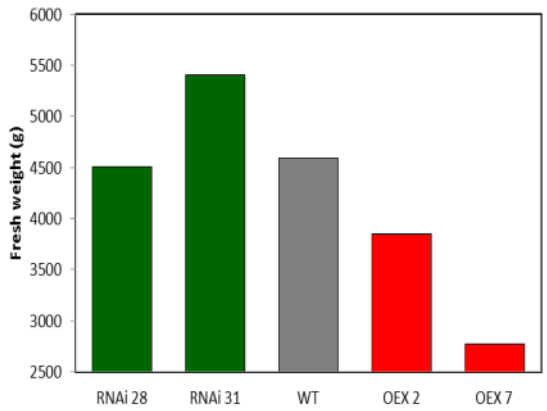


Figure 4.13 comparison of total tuber yield for the transgenic plants used for Figure 4.12

The effects of down regulating or up regulating this gene on sprout growth, relative sprout length and tuber yield are shown in Figures 4.11, 4.12, 4.13. CEN 1 appears to influence sprout vigour rather than dormancy break. RNAi lines with lower expression of the gene tuberise and initiate flowers earlier than controls; over-expressing lines show opposite responses. Overall down-regulation increases yield and accelerates tuber life-cycle. This discovery augments existing models of tuber life-cycle regulation. More information and a more in-depth discussion is given in Morris et al. 2018.

© Agriculture and Horticulture Development Board 2019

4. DISCUSSION

This project is aimed at unravelling genetic and molecular processes underlying the very important traits, tuber and bulb dormancy in potato and onion respectively. We have made significant progress in gaining a better understanding of the genetics of tuber dormancy in potato using a diploid cross that shows a high level of variation in the trait. We have detected significant genetic effects on four potato chromosomes and we believe we have identified a good candidate gene, CEN 1, for one of these. This has been confirmed using transgenic approaches. Other potato work is looking gene expression and other changes (plant growth regulators, hormones etc) in extreme lines from the crossing population. The onion work does not involve genetic analysis so is more focused on a transcriptomic and biochemical approach.

This project has identified 5 QTLs associated with potato tuber dormancy and sprout growth, and has identified genomic regions containing candidate genes/potential markers for tuber dormancy/sprouting. 3 of these QTLs correspond to QTLs identified previously for a tetraploid cross (DEFRA-LINK project 'Reducing energy usage and wastage by improving ethylene control of potato sprouting') which gives confidence that they will be validated with commercial lines, and can be potentially useful for both diploid and tetraploid breeding. Furthermore the findings are consistent with the results of a Genome Wide Association Study ((GWAS) using 300 cultivars and about 50,000 SNP markers (JHI and Sutton Bridge Crop Storage Research).

In the current study we characterised a candidate gene associated with one of the largest effect QTL for rapid tuber sprout growth on potato chromosome 3. Underlying this QTL is a gene encoding a *TERMINAL FLOWER 1/ CENTRORADIALIS* homologue (PGSC0003DMG400014322). Here we use a transgenic approach to manipulate the expression level of the *CEN* (termed *StCEN*) family member in a potato tetraploid genotype (cv. Désirée). We demonstrate a clear effect of *StCEN* expression manipulation, with decreased expression levels associated with an increased rate of sprout growth, and over-expressing lines showing a lower rate of sprout growth than controls. Associated with different levels of *StCEN* expression were different levels of ABA and cytokinins implying a role in controlling the levels of plant growth regulators in the apical meristem. The findings are discussed in more detail in Morris et al 2018.

A process of validation would be necessary before SNP markers identified within this project could translate to valid markers for a breeding programme for tetraploid cultivars, however, potentially this could lead to markers for tuber dormancy/sprouting for use in breeding within 5 year period.

The analysis of gene expression by RNAseq and profiles of hormone and metabolite concentration for both potato and onion through dormancy, exit of dormancy and initial sprout growth has resulted in the establishment of a database that will provide an invaluable resource for many years to come as models of the control of dormancy are further investigated and tested. Data on the changes in gene expression and in concentrations of PGRs and metabolites in potato tubers prior to dormancy break suggest there are many consistent patterns between cultivars and between the two commodities. This indicates that there may be potential in future to develop transcriptome or metabolite markers for dormancy.

© Agriculture and Horticulture Development Board 2019

5. CONCLUSIONS

In summary the main project outputs are:

- Updated unified model for dormancy control for potato (also relevant to onion).
- QTLs identified associated with tuber dormancy and sprout growth – potential markers for tuber dormancy/sprouting for use in breeding within 5 year period
- Candidate genes involved in dormancy control identified. Developed transgenic lines for functional validation and further expression work
- Profile of gene expression, hormone concentrations, key metabolites and respiration through progression of dormancy, dormancy break and sprout growth.

© Agriculture and Horticulture Development Board 2019

6. REFERENCES

Braun, N., de Saint Germain, A., Pillot, J-P., Boutet-Mercey, S., Dalmais, M., Antoniadi,I., Li, X., Maia-Grondard,A., Le Signor,C., Bouteiller, N., Luo, D., Bendahmane, A., Turnbull, C. and Rameau, C. (2012). The Pea TCP Transcription Factor PsBRC1 Acts Downstream of Strigolactones to Control Shoot Branching Plant Physiol 158, 225–238;

Burton, W. G. 1989. The Potato, 3rd ed.: Longman Scientific and Technical.

Chope, G., Cools, K., Hammond, J.P., Thompson, A.J. and Terry, L.A. (2012) Physiological, biochemical and transcriptional analysis of onion bulbs during storage Ann. Bot. 109, 819-831;

DEFRA (2006) Code of practice for using plant protection products (2006). Defra

De Mason (1990) orphology and anatomy of Allium. In: Rabinowitch et al. eds. Onions and allied crops, Vol. 1. Boca Raton, FL: CRC Press, 27–51;

Downes, K., Chope, G., and Terry, L.A.. (2010) Postharvest application of ethylene and 1-methylcyclopropene either before or after curing affects onion (Allium cepa L.) bulb quality during long term cold storage. Postharvest Biol. & Technol. 55, 36-44.

Fernie, A. R. and Willmitzer, L. 2001. Molecular and biochemical triggers of potato tuber development. Plant Physiology, 127(4), pp 1459-1465.

Foo, E., Morris, S.E., Parmenter, K., Young, N., Wang, H., Jones, A., Rameau, C., Turnbull, C.G.N. and Beveridge, C.A. 2007. Feedback Regulation of Xylem Cytokinin Content Is Conserved in Pea and Arabidopsis Plant Physiol. 143, 1418-1428;

Foukaraki,S. (2012). Storage of potatoes : effects of ethylene and 1-MCP on potato tuber quality and biochemistry. PhD thesis, Cranfield Univ.;

Freyre, R. Warnke, S., Sosinski, B., Douches, D.S. (1994) Quantitative trait locus analysis of tuber dormancy in diploid potato (Solanum spp.) Theor Appl Genet. 89, 474-480;

Garthwaite, D.G., Barker, I.,Parrish, G., Smith, L. (2010); Defra pesticide usage survey in potato stores 2009 Report no. 227 FERA York.

Hartmann, A., Senning, M., Hedden, P., Sonnewald, U. and Sonnewald, S. (2011) Reactivation of Meristem Activity and Sprout Growth in Potato Tubers Require Both Cytokinin and Gibberellin Plant Physiol. 155, 776-796;

Morris, WL, Carmen Alamar, M, Lopez-Cobollo, RM, Cañete, J Bennett, M. JVan der Kaay,J, Stevens, J Sharma, SK, McLean, K Thompson, AJ Terry, LA Turnbull, C Bryan, G Taylor M (2019) A member of the TERMINAL FLOWER 1/CENTRORADIALIS gene family controls sprout growth in potato tubers Journal of Experimental Botany, Volume 70, Issue 3, 30 January 2019, Pages 835–843, https://doi.org/10.1093/jxb/ery387

Prashar A, Hornyik C, Young V, McLean K, Sharma SK, Dale MF, Bryan GJ. **(2014)** Construction of a dense SNP map of a highly heterozygous diploid potato population and QTL analysis of tuber shape and eye depth. Theor Appl Genet. 2014 Oct;127(10):2159-71. doi: 10.1007/s00122-014-2369-9.

Rohde A. and Bhalerao R.P. (2007) Plant dormancy in the perennial context. Trends in Plant Sci., 12, 217-223;

Simko, I., McMurry, S., Yang, H.M., Manschot, A., Davies, P.J. and Ewing, E.E. (1997) Evidence from Polygene Mapping for a Causal Relationship between Potato Tuber Dormancy and Abscisic Acid Content. Plant Physiology 115, 1453-1459;

Suttle (2004) Involvement of endogenous gibberellins in potato tuber dormancy and early sprout growth: a critical assessment. J. Plant Physiol. 161, 157-164;

Suttle, J.C. and Hultstrand, J.F. (1994) Role of Endogenous Abscisic Acid in Potato Microtuber DormancyPlant Physiol. 105, 891-896;

Turnbull, C.G.N and Hanke, D.E. (1985) The control of bud dormancy in potato tubers

Evidence for the primary role of cytokinins and a seasonal pattern of changing sensitivity to cytokinin Planta 165, 359-365;

Turnbull, C.G.N and Hanke, D.E. (1985) The control of bud dormancy in potato tubers. Measurement of the seasonal pattern of changing concentrations of zeatin cytokinins,Planta 165, 366-376;

© Agriculture and Horticulture Development Board 2019

7. ACKNOWLEDGEMENTS

This work was funded by the BBSRC Biotechnology and Biological Sciences Research Council (2016) Horticulture and Potato Initiative (HAPI) (grant no. BB/K020889/1) and the Scottish Government Rural and Environment Science and Analytical Services Division as part of the Strategic Research Programme 2016–2021. Financial support was also provided by AHDB Potato, PepsiCo International and Albert Bartlett.

© Agriculture and Horticulture Development Board 2019