IDENTIFICATION OF GENES INVOLVED IN EARLY ENDODORMANCY BREAK IN POTATO TUBERS

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ABSTRACT

The growth and harvest of potato tubers for agricultural purposes is important both in Canada and worldwide, and the agronomic importance of this crop is steadily growing. A major issue affecting the storage and transport of raw potatoes is the precocious sprouting of tuber shoot apical meristems (SAMs), which is a result of tuber emergence from endodormancy. Current methods of potato storage that prolong endodormancy require either large cold storage facilities, or the use of growth suppressing chemicals. In this study, tuber SAM growth and the molecular mechanisms underlying endodormancy emergence were investigated to better understand the physiological processes involved. During emergence, SAM length increased and was paralleled by increased DNA synthesis as early as 7 weeks post-harvest, suggesting endodormancy release before or at this time. The genes *WUS* and *STM*, which are involved with meristem maintenance and growth, were expressed as early as 7 weeks post-harvest. By 11 weeks post-harvest, *WUS* was expressed at significantly higher levels (~5 fold) than in endodormant tuber meristems. *PKL* and *CUC1*, genes thought to be involved in the activation of *WUS* and *STM,* increased in expression at 11 weeks, then decreased to significantly lower levels at 15 weeks. Expression of other dormancy enforcing genes such as *ABI5* and *DHN* also significantly decreased during emergence. Suppressive subtractive hybridization identified other genes that were down-regulated in non-dormant tissues. These included *BPS1,* thought to be involved with synthesis of a mobile signal, whose mRNA levels were significantly lower at 15 and 19 weeks, and RCE1, a member of the SCF^{TIR1} complex involved with auxin regulated protein degradation, which had significantly lower mRNA levels after 19 weeks. Additionally,

the stress tolerance gene *DREB2a* showed a slight increase in expression (~20%) at 11 weeks post-harvest but then decreased, and at 19 weeks post-harvest it was significantly lower (~40%). Expression of the cell cycle dependent gene *cyc07* and a *WD-Repeat* gene were characterized as well. The molecular mechanisms investigated contribute to the understanding of tuber endodormancy, which may lead to better cultivation and storage practices.

LAY SUMMARY

In this study, the underlying molecular mechanisms that control plant dormancy were examined. Plant dormancy plays a large role in agriculture as it is seen in seeds and other crops such as potatoes. This phenomenon has an effect on sprouting times as well as the shelf life of some vegetables such as potatoes. A better understanding of the processes at work during dormancy can help to develop new and more efficient cultivation and storage practices. In this study it was found that potatoes can emerge from dormancy as early as 7 weeks after harvest (under typical storage conditions). Molecular mechanisms contributing to shoot apical meristem growth and organization were expressed early upon dormancy release. In contrast, genetic mechanisms contributing to dormancy maintenance include factors involved in stress tolerance. This study identified possible mechanisms involved in dormancy release that were not previously characterized in potato and that can be used as a basis for further studies.

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LIST OF ABBREVIATIONS

ABA	Abscisic Acid
AA	Amino Acid
ABI1,2,3,4,5	ABSCISIC ACID INSENSITIVE 1,2,3,4,5
ABRE	ABA-Responsive Element
ADK1	Adenosine Kinase 1
AGB1	ARABIDOPSIS G-PROTEIN BETA SUBUNIT
AP2	APETALA 2
ARF	Auxin Response Factor
ARR	*Arabidopsis* Response Regulator
AT	*Arabidopsis thaliana*
BARD1	BRCA1 Associated RING Domain 1
BPS1	BYPASS1
bZIP	Basic Leucine-Zipper
CAF-1	Chromatin Assembly Factor 1
CAK	CDK-Activating Kinase
CBL	Calcineurin B-Like
CDK	Cyclin Dependent Kinase
CDKB	CDK B-type
CHD	Chromatin Helicase DNA Binding Protein
CHD3	Chromatin Helicase DNA Binding Protein 3
CIPK	CBL-Interacting Protein Kinase
CLV	CLAVATA
CLV1	CLAVATA1
CLV3	CLAVATA3
CRT	C-Repeat
CUC1	CUP-SHAPED COTYLEDON 1
CUC2	CUP-SHAPED COTYLEDON 2
CUL1	Cullin 1
CYC	Cyclin
CYC07	Cell Cycle Dependent 7
CYCB	B-type Cyclin
DHN	DEHYDRIN
DRE	DEHYDRATION RESPONSIVE ELEMENT
DREB	DEHYDRATION RESPONSIVE ELEMENT BINDING
E2F	E2F Transcription Factor
ERF	Ethylene Response Factor
FAS1	Fas (TNF receptor superfamily, member 6)
FAS2	FASCIATA2
FT	FLOWERING LOCUS T
GA	Gibberellic Acid
H2A	Histone 2A
H2B	Histone 2B
Hsp90	Heat Shock Protein 90
HTP	Histidine Phospho-transferase
HUB	HISTONE MONOUBIQUITINATION
IAA	Indole-3-Acetic Acid

ICK1	Inhibitor 1 of Cdc2 kinase
KEG	KEEP ON GOING
KNOX	KNOTTED-like Homeobox
LEC1	Leafy Cotyledon 1 Transcription Factor
LUG	LEUNIG
MADS	MCM1 AGAMOUS DEFICIENS SRF
MAPK	Mitogen-Activated Protein Kinase
MS	Murashige and Skoog
NAC	NAM, ATAF1/2, CUC2
PCNA	Proliferating Cell Nuclear Antigen
PCR	Polymerase Chain Reaction
PGR	Plant Growth Regulator
PKL	PICKLE
PLC	PHOSPHOLIPAS E C
RB	Retinoblastoma
RCE1	RUB Conjugating Enzyme
RT PCR	Real Time Polymerase Chain Reaction
RUB1	RELATED TO UBIQUITIN 1
SAM	Shoot Apical Meristem
SCF	Skp, Cullin F-Box
SDR	Short-Chain Dehydrogenase/Reductase
SNF	Sucrose Non Fermentable
SPK1	Serine/Threonine-Protein Kinase 1
SRK	Serine Receptor Kinase
SSH	Suppression Subtractive Hybridization
STM	SHOOT MERISTEMLESS
SWI-SNF	Switch-Sucrose Non Fermentable
TF	Transcription Factor
TFL1	TERMINAL FLOWERING LOCUS 1
UAF	Upstream Activation Factor
Ub	Ubiquitin
UBC	UBIQUITIN CARRIER PROTEIN
WDR	WD-Repeat
WRKY	Tryptophan Arginine Lysine Tyrosine
WT	Wild Type
WUS	WUSCHEL

1.0 INTRODUCTION

1.1 Potato Physiology

The potato (*Solanum tuberosum* L.) (Figure 1) is a herbaceous perennial that is thought to have originated in the Andean mountain range somewhere in Peru (Spooner, 2005). The potato flowers each growing season and is insect-pollinated. Propagation by sexual reproduction is mainly via cross pollination, although self-pollination is possible; in either case green fruits containing seeds are formed. Potatoes can also propagate clonally by tubers. The part of the potato plant that most people are familiar with is the tuber, which is commonly just called a potato. The tuber contains shoot apical meristems (or eyes) on the surface that each have the ability to differentiate and grow into an entirely new clone of the parent plant (Vreugdenhil, *et al.*, 2007).

The potato tuber develops underground from modified shoots called stolons that are derived from buds located underground, at the base of the stem. Stolons grow outward and form tubers at their tips. Tuberization is triggered by short days, cool temperatures, and low nitrogen fertilization (Fernie and Willmitzer, 2001; Jackson, 1999). Additionally, plant growth regulators influence the tuberization response. For example, gibberellic acid (GA) levels decline during tuber formation and the exogenous application of GA can inhibit tuber formation. Conversely, cytokinins may accelerate tuber induction (Galis, *et al.*, 1995). A recently identified plant growth regulator (PGR), jasmonic acid, and its derivative tuberonic acid also possess tuberizing properties (Jackson, 1999).

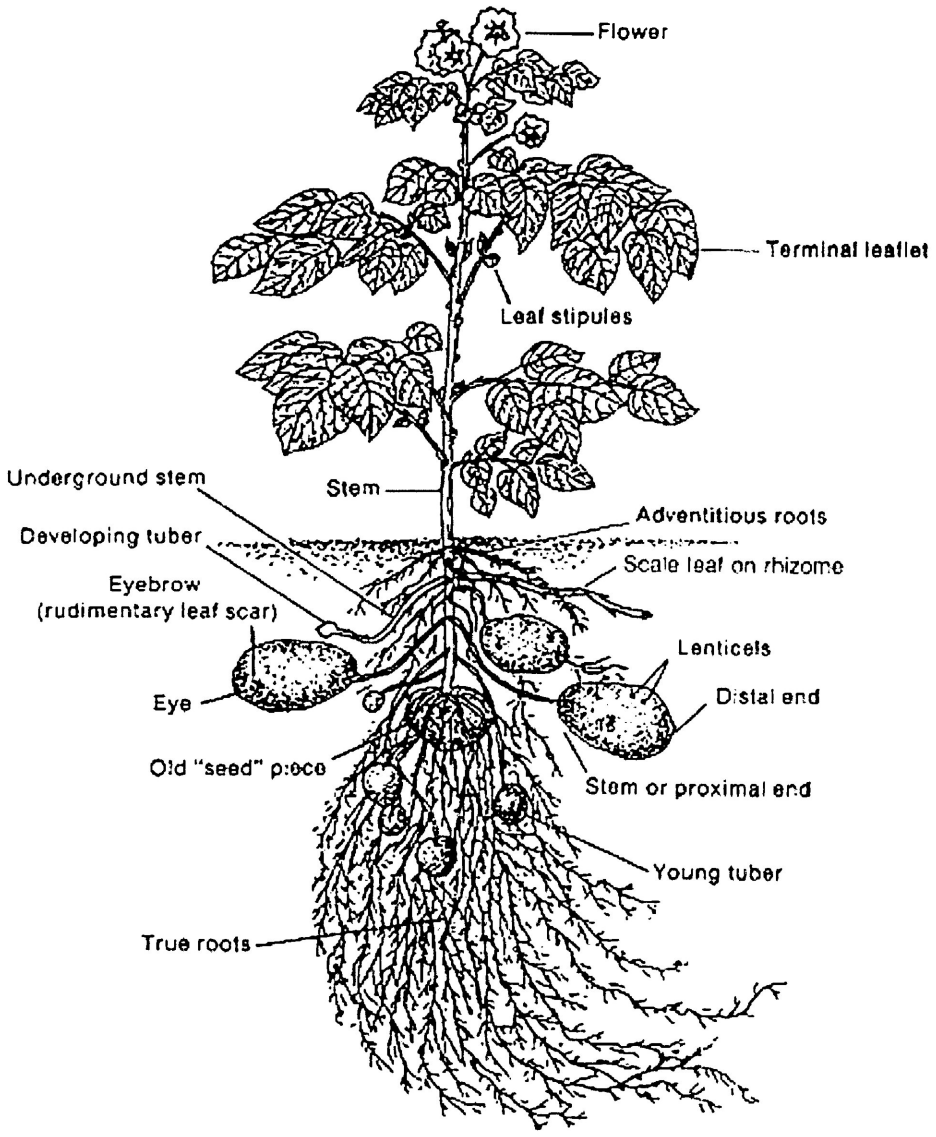

Figure 1: Structure of the potato plant (*Solanum tuberosum* L.) (Bains, *et al.*, 2011).

Once environmental conditions cause the plant to start producing tubers, the end of the stolon stops growing and becomes a carbohydrate sink that stores large amounts of starch. The tuber also greatly reduces its metabolism to allow for the accumulation of these carbohydrates (Fernie and Willmitzer, 2001).

1.2 Nutritional Value

A diet consisting of only tubers and whole milk is enough to supply a person with their daily requirements of protein, vitamins and minerals. In a 150 g serving of baked potato there is 0 g of fat, cholesterol or salt and only 100 kcal; it also contains 45%, 21%, 12% and 10% of daily vitamin C, potassium, fiber and vitamin B6 requirements, respectively. Potatoes are also a source of all essential amino acids (McLaughlin, 2005). While tubers are primarily composed of starch, only about 2% by dry weight is protein (Fernie and Willmitzer, 2001).

1.3 Economic Importance

The potato is an agronomically important crop in much of the world and its popularity is growing in developing countries. With over 320 million metric tonnes produced in 2010, it is the fifth most produced crop in the world behind sugarcane, maize, wheat and rice (Food and Agricultural Organization of the United Nations, 2012). The potato is a good candidate crop in developing regions, because of its ease of propagation and growth, high yield and high nutritional value. In Europe and North America, the potato tuber is primarily consumed after processing into frozen or dehydrated foods. In developing countries, the potato is an important staple food and is grown by many subsistence-level farmers. Additionally, tubers are not only edible but are propagules that are able to grow another generation of plants with identical agronomic traits (Fernie and Willmitzer, 2001).

In 2010, Canada produced over 4.4 million tonnes of potatoes. While this is a large amount, it is dwarfed by cultivation in other areas of the globe. Currently, China is the largest producer of potatoes and its production has increased by ~15% over the past 5

years. In 2010, China produced over 74 million tonnes of potatoes. Other countries that boast high potato production numbers include India, the United States, Russia, Belarus, Netherlands, Germany, Ukraine, France, and Poland (Food and Agricultural Organization of the United Nations, 2012). Most of these countries are located in the northern hemisphere where ideal potato growing conditions exist. In Canada, the potato is grown in the summer. Subtropical areas such as India and parts of China have a potato growing season over their colder months, during fall, winter or spring. Potatoes can be grown in cool, high elevations of tropical areas as well (Spooner, 2005).

Potato products exported from Canada are primarily frozen and dehydrated. This requires a larger production of potatoes considering that it requires 2 kg of fresh potato to produce 1 kg of frozen potato product, such as frozen French fries, and up to 9 kg fresh potatoes to produce 1 kg dehydrated potato product, such as potato chips. While fresh potatoes are exported by Canada, most exports are within North America. This is due to the high cost of transporting potatoes in a manner that will keep them in a dormant and edible state (McLaughlin, 2005).

1.4 Potato Storage

One of the major limiting factors to the export of fresh potatoes is their storage. Potatoes will often sprout during storage, making the potato undesirable for both, fresh consumption and further processing which reduces the marketability of tubers by producers. The premature sprouting of potatoes causes the starch in the tuber to degrade into reducing sugars such as glucose and fructose that are used to provide energy to the growing shoot. These reducing sugars can give the potatoes a sweet taste and a mushy texture that makes them undesirable for processing because reducing sugars can react in

the production process, causing the finished product to have a brownish colour and nutritional changes (Wiltshire and Cobb, 1996).

The suppression of potato shoot apical meristem sprouting is an important aspect of their storage. The current methods of storing potatoes include the use of large cold storage facilities and bud growth suppressing chemicals. Neither of these methods is ideal. The use of large cold storage facilities requires a large amount of energy, which is not only costly for producers or processors, but the carbon footprint of this storage practice is very high. On top of this, cold storage is not always effective. Eventually, after about 4-5 months, the potatoes stored in the cold will begin to form reducing sugars as dormancy ends, at which point these potatoes are no longer desirable and will be discarded. The use of growth suppressing chemicals such as chlorpropham, propham, tecnazene, and methylnaphthalenes is always under scrutiny. These chemicals may have an adverse effect on the environment and many of them contain chlorinated benzene which may cause kidney or liver damage if consumed (Wiltshire and Cobb, 1996).

1.5 Types and Structure of Plant Meristems

The plant meristem is the place where active growth occurs and it is also able to develop into new plant organs. It is usually present in all seeds allowing them to grow and develop into an entirely new plant. Each meristem contains a small amount of stem cells that are able to continuously divide and be differentiated into new cell types (Medford, 1992).

Active meristems exist in plants, including the shoot apical meristem (SAM), root apical meristems (RAM), as well as lateral meristems and axial meristems from where

branching can arise (Stern, 2006) (Figure 2A). The SAM is responsible for all above ground growth in the plant, whereas the RAM is responsible for growth and development underground (Medford, 1992). These apical meristems are involved with new growth and the formation of plant organs including leaves, fruit, or flowers. Lateral meristems, including the vascular and cork cambium, are responsible for plant structural integrity by increasing the stem and root girth. Axial meristems are located along the stem and are undifferentiated vegetative meristems (Stern, 2006) that have the potential to develop into any organ required (Medford, 1992; Barton, 2010). Axial meristems are also present in perennials to act as back up meristems if the actively growing SAM is damaged and unable to continue growing (Horvath, *et al.*, 2003). The structure of the SAM has been studied more then the RAM and is therefore better characterized (Barton, 2010).

The SAM is organized into both layers and zones (Figure 2B). There are 3 layers in the SAM. The outermost layer is designated L1, and contains cells that divide in the anticlinal plane (perpendicular to the surface), later becoming the epidermis to parts of the plant that have been differentiated. The next layer, located beneath L1, is designated L2. This layer exhibits cell division in the anticlinal plane, as well as in the periclinal plane (parallel to the surface) when organs are being formed. Finally the L3 layer is the innermost section of the meristem. These cells divide in both planes and differentiate into the inner pith tissue in organs and stems (Medford, 1992) (Figure 2B). The SAM is also characterized by zones used to describe meristematic cell functions. The central zone (CZ) is located at the tip of the SAM (Figure 2B). While this zone contains tissue from all 3 layer types, its cells do not divide as frequently as those in the rest of the meristem. The CZ cells have more prominent nuclei and are thought to function as stem cells. These

cells act as source cells for the rest of SAM and remain undifferentiated. The rest of the meristem is designated the peripheral zone (PZ). The cells here are used to form lateral organs such as leaves. The final zone is known as the rib zone (RZ) which form a border between the meristematic cells and the rest of the shoot (Medford, 1992) (Figure 2B).

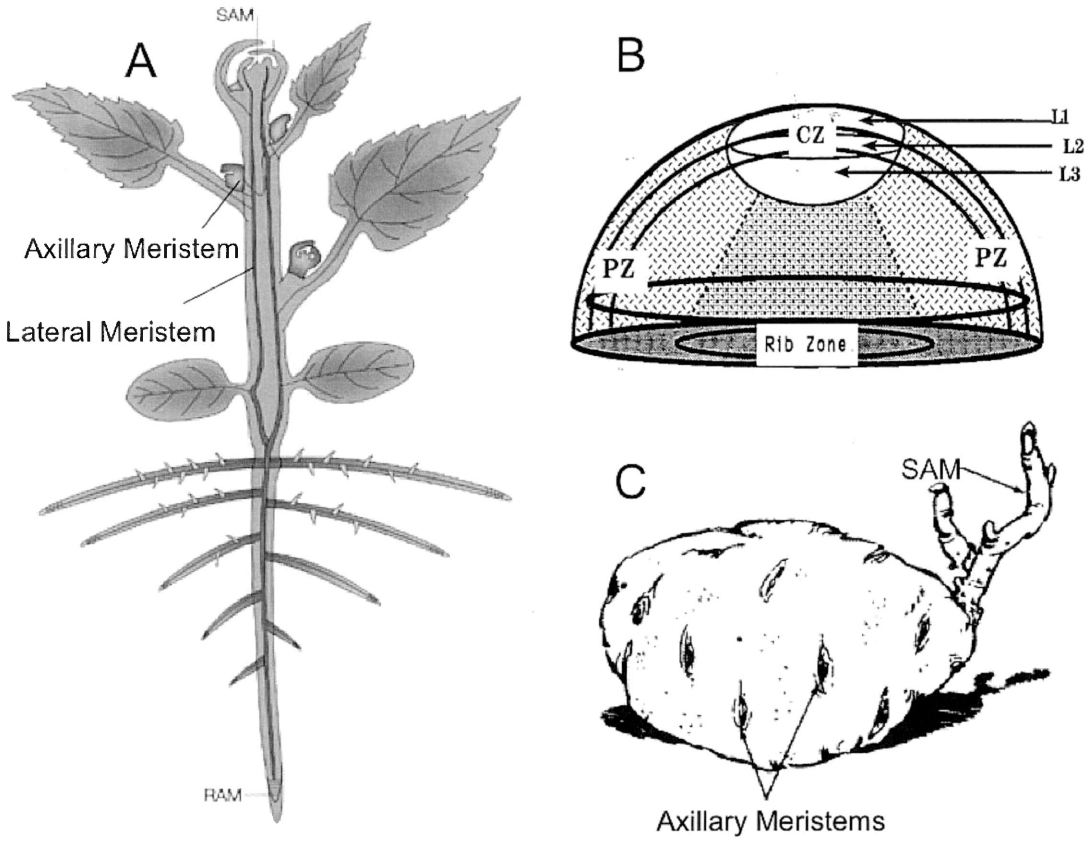

Figure 2: Plant meristems. A: Diagram showing the various meristems present in plants (Tsiantis and Hay, 2003). B: Diagram of the layers and zones within the shoot apical meristem (Medford, 1992). C: Diagram describing the location of growing SAMs and dormant Axillary meristems within the sprouting tuber (VanCleave, 2012).

Emergence from endodormancy is described as the new or continued growth of SAMs, and this is what occurs in sprouting potatoes (Rohde and Bhalerao, 2007) (Figure 2C). In seeds, trees and tubers, the SAM arrests its growth in order to survive during unfavourable growing conditions (Horvath, *et al.*, 2003). Perennial plants are characterized by this ability to suspend growth and resume it in the following season. In

principle, perennials require only one SAM to resume vegetative growth (Rohde and Bhalerao, 2007).

1.6 Plant Growth Regulators:

Plant growth regulators (PGRs) are mobile biochemical compounds produced in plants that act as signaling molecules affecting growth and development. PGRs that influence the dormant state of SAMs include auxin, cytokinin (CK), gibberellic acid (GA) and absicsic acid (ABA). Auxin, CK and GA promote plant growth. ABA prolongs dormancy or inhibits growth in potato tuber meristems (Wiltshire and Cobb, 1996).

Auxin is mainly produced in the SAM, young leaves and other new growth occurring in the plant. As with other PGRs, the effects of auxin vary with differing concentrations. At normal cellular concentrations, auxin promotes enlargement of cells by increasing plasticity of cell walls. Auxins may also be involved with producing other PGRs such as ethylene, controlling some aspects of respiration, and influencing growth and development. They promote cell growth and division, as well as differentiation of cell types. Through promotion of growth they may act to enforce dormancy emergence (Stern, 2006).

Gibberellic acid is involved with cell elongation required for normal cell growth. It has also been found to cause seeds to germinate and break meristem dormancy (Stern, 2006; Hartmann, *et al.*, 2011).

Cytokinins are formed in root tips and germinating seeds. In the presence of auxin, CK promotes cell division by helping progression from the G2 to the M phase of the cell cycle. This PGR also plays a role in cell enlargement, cell differentiation, and

chloroplast development (Hartmann, *et al.*, 2011; Stern, 2006). Cytokinin is thought to promote emergence from dormancy by promoting proper cell cycle function and cell growth.

Abscisic acid (ABA) is found in different plant materials, but primarily in fruits where it prevents seeds from germinating while still attached to the plant. It is also involved with drought stress response. Accumulation of ABA causes guard cells to close, and also roots to grow and increase water uptake (Destefano-Beltran, *et al.*, 2006; Stern, 2006). ABA is involved with enforcing the dormant state of the meristem.

1.7 Plant Dormancy

Since plant dormancy can be described as absence of visible growth to any plant structure that contains a meristem (Lang, 1987; Rohde and Bhalerao, 2007), the biochemical mechanisms at work in the meristem are what drive the progression, establishment and breakage of dormancy. Dormancy can occur in the buds on trees, the embryo in seeds and the eyes of potatoes. The meristems in these structures are able to grow and differentiate into new plant organs depending on where the plant is in its life cycle. For example, the transition between vegetative growth and dormancy onset is signalled by an environmental stimulus such as a change in day length or chilling temperatures (Fernie and Willmitzer, 2001). The system of internal signalling that occurs to maintain the SAM and guide its growth and development is thought to be conserved in different plants and their organs. The function of the SAM is to alternate growth and differentiation into new organs with periods of dormancy. This process and the continued longevity of the shoot meristem is achieved in two ways: while the stem cell pool within

the meristem must be maintained, the rapidly dividing cells in the outer layers of the meristem must be differentiated to produce new organs (Schoof, *et al.*, 2000).

Specifically, the meristems in the potato tuber progressively become dormant during tuber development, with the apical SAM being the last to arrest its growth (Figure 2C) (Xu, *et al.*, 1998; Fernie and Willmitzer, 2001). The tuberization process may thus play a pivotal role in initiating SAM dormancy in potatoes. Lower temperatures during tuber development lead to longer periods of dormancy, and the length of the dormancy period may be related to the history of the plant, including the genotype as well as the historical growing conditions in which it evolved (Claassens and Vreugdenhil, 2000; Fernie and Willmitzer, 2001). Application of exogenous GA is known to terminate dormancy in plant tissues such as shoot apical and axial meristems (Hartmann, *et al.*, 2011). Cytokinins are also able to break SAM dormancy but the time frame where they are able to do this seems to be very specific (Galis, *et al.*, 1995; Fernie and Willmitzer, 2001). By contrast, ethylene is thought to enforce the dormant state of potato tubers (Claassens and Vreugdenhil, 2000; Fernie and Willmitzer, 2001).

1.8 Initiating Dormancy

The initial onset of dormancy is triggered by environmental stimuli. Photoperiod is one of the major environmental cues that the plant perceives to initiate the dormant state. In model perennials, photoperiod is perceived by the leaf which then sends signals to the growing SAMs in order to terminate their growth (Wareing, 1956; Hemberg, 1949; Rohde and Bhalerao, 2007). One of the more recent discoveries about this signal transduction cascade leading to bud growth is the identification of the *FLOWERING LOCUS T* (*FT*) gene, which is influential in growth cessation. Studies with this gene in

transgenic aspen have shown that its down-regulation instills growth arrest in buds (Bohlenius, *et al.*, 2006). The *FT* gene has been previously shown to be involved in the flowering response of other plants and also in tuberization in potato (*Solanum tuberosum)* (Hayama and Coupland, 2004; Rodriguez-Falcon, *et al.*, 2006). Expression of *FT* correlates with the induction of tuberization. An antagonist to the *FT* gene is *CONSTANS* (*CO*) whose over-expression causes delayed tuberization in potatoes (Gonzalez-Schain, *et al.*, 2012). Other studies into the *FT* gene gamily have identified an *FT* like gene called *TERMINAL FLOWER 1* (*TFL1*) that has been shown to have antagonistic effects to that of *FT* in *Arabidopsis thaliana* (Ratcliffe, *et al.*, 1998).

The identification of FT's involvement in dormancy indicates potential commonalities between dormancy and flowering. Furthermore, the involvement of FT in tuberization enforces the idea that dormancy is initiated during tuberization. Knowledge of the underlying mechanisms initiating dormancy provides information about how dormancy is maintained and the cellular mechanisms necessary to break it.

1.9 Types of Dormancy

Dormancy maintenance mechanisms are hypothesized to act via morphological changes and hormonal suppression. ABA is thought to play a role in the maintenance of dormancy (Destefano-Beltran, *et al.*, 2006). There have also been studies showing that the SAM is physically isolated from receiving exogenous growth promoting signals. This is done by blocking the plasmodesmatal channels with callose, which prevents GA and auxin from entering the meristem (Rinne and van der Schoot, 1998; Rinne, *et al.*, 2001). Other studies suggest that molecular mechanisms may provide dormancy maintenance, such as altered epigenetic patterns between dormant and non-dormant meristems,

including histone acetylation and DNA methylation (Law and Suttle, 2005; Law and Suttle, 2003). This conformational change in the DNA structure can greatly alter the transcriptome of the dormant meristem, repressing certain growth promoting genes (Rohde and Bhalerao, 2007).

The dormant state of the meristem can be sub-classified as paradormant, ecodormant, and endodormant. While each of these states suppress SAM growth, they do so using different mechanisms.

1.9.1 Paradormancy

Paradormancy is defined as growth arrest promoted by signals originating in different areas of the plant and is accomplished by PGR signalling within the plant. A common example of paradormancy is the phenomenon known as apical dominance, a process that allows the plant to allocate energy and model its development to maximize light harvesting efficiency. Also, by keeping axial meristems dormant, the plant has "back up" growth potential pending destruction of the apical meristem. The process of apical dominance is attributed to transport of the PGR auxin throughout the plant. It is important to note that auxin produced in distal regions can have different effects than auxin produced in the SAM (Cline, 1991). The continuously growing SAM produces auxin, which promotes SAM growth, but suppresses that of more distal axial meristems (Horvath, *et al.*, 2003). The axial meristems will only begin to grow once the apical meristem stops growing/producing auxins, which will stop the expression of ABA sensitive genes and allow axial meristem growth (Campbell, 2006).

The action of the PGRs at work during paradormancy has been studied and a few key mechanisms have been identified. Auxin is able to inhibit the sensing or production

of cytokinin. Cytokinin has been found to express CYC type genes required in resumption of growth by promoting the G1-S phase transition (Francis and Sorrell, 2001). The dormant state of plant meristems can be attributed to balances in different PGRs, namely of auxin-cytokinin as described above, and gibberellic acid-abcisic acid balance. GA has been found to induce S-phase progression or cell division and growth (Horvath, *et al.*, 2002). On the other hand, ABA can inhibit the G1-S phase transition by inducing the expression of $p27^{CIP/KIP}$, an ICK1 ortholog that is a cell cycle inhibitor (Wang, *et al.*, 1997). From this information, it is apparent that PGRs produced by the plant affect gene expression, resulting in arrest of cell cycle progression. Pausing this phase of the cell cycle to stop SAM growth is a common mechanism utilized in other types of dormancy as well.

A component of auxin signalling in paradormant meristems is due to enzymatic degradation of specific proteins responsible for cytokinin production (Shimizu-Sato and Mori, 2001). This is localized to the area around lateral meristems, and is one of the ways that auxin produced in the apical meristem is able to prevent the outgrowth of the lateral meristems (Horvath, *et al.*, 2003). Other examples of protein degradation in relation to PGR-mediated cell cycle arrest exist in the roots of *Arabidopsis*. Here, a protein involved with blocking GA signalling is degraded by an auxin regulated ubiquitin-proteasome pathway (Fu and Harberd, 2003), which implicates protein degradation in SAM growth regulation.

Paradormancy in potato tubers begins with tuberization and is the first form of cell cycle arrest that is required to maintain metabolically inactive SAMs until favourable growing conditions present themselves.

1.9.2 Ecodormancy

In contrast to paradormancy, where growth arrest is enforced by PGRs, ecodormancy is influenced by external stimuli such as cold or drought and is more of an environmental stress response. The environmental signals that induce ecodormancy are also thought to be responsible for the breaking of endodormancy. ABA is a well-characterized signalling molecule in plant response to cold and drought (Gilmour and Thomashow, 1991). As discussed earlier, it is thought that ABA regulates *ICK1* and that ABA production in response to drought may thus activate ICK1, blocking cell cycle progression and SAM growth (Horvath, *et al.*, 2003).

1.9.3 Endodormancy

Endodormancy occurs during the transition from paradormancy to ecodormancy and is important in maintaining growth arrest in the absence of hormonal or environmental stimulators. Endodormancy occurs during transition seasons to prevent premature sprouting of the meristem. This type of dormancy is thought to be enforced by physiological changes within the meristem itself. Endodormancy is important in protecting the meristem from premature sprouting (Rohde and Bhalerao, 2007) long enough for winter to begin and ecodormancy to take over (Vreugdenhil, *et al.*, 2007). In grape and poplar, internal signals affect the axillary meristems and prevent their growth even under ideal environmental growing conditions and without the influence of PGRs such as auxin. In potato SAMs, developmental cues create an internal meristematic signalling network that controls the meristem growth (Rohde and Bhalerao, 2007).

Endodormancy is broken by an extended period of cold temperature. Hydrogen cyanide (HCN) can break endodormancy as well (Henzell, *et al.*, 1991). While little is

known about the mechanism by which HCN breaks dormancy, it is thought that it requires the action of an SNF-like protein kinase (Or, *et al.*, 2000). The SNF-like protein kinase is thought to be similar to a component of the protein complex SWI-SNF that is involved with epigenetic DNA modification in animals and yeast (Fan, *et al.*, 2003). Other proteins in this complex have been identified in RB-E2F interactions as well. The RB-E2F complex is involved in release of transcription factors involved in DNA synthesis and has also been suggested to be involved in chromatin remodelling, affecting expression of cell cycle genes (Shen, 2002; Horvath, *et al.*, 2003).

Ethylene can induce endodormancy in potato microtubers (Suttle, 1998). ABA is another PGR that has been shown to play a role in endodormancy (Weatherwax, *et al.*, 1998). Since ethylene and ABA are involved in plant senescence, senescence may be involved in the induction of endodormancy (Horvath, *et al.*, 2003).

Recent molecular studies have characterized a few potential genes involved in the breaking of endodormancy including genes coding for KNOTTED-like homeodomain proteins. Other known members of this family of proteins include CLAVATA and WUSCHEL, which are known to play a role in the maintenance and growth of the stem cell pool found in the meristem (Clark, *et al.*, 1996). Other studies found that the over expression of the KNOTTED-like protein in potato reduced levels of GA in the plant (Rosin, *et al.*, 2003). This is an interesting result, since GA promotes growth, and GA levels should thus increase with dormancy breakage.

Epigenetic alterations have also been implicated in endodormancy release. DNA methylation patterns change during the break of endodormancy (Law and Suttle, 2003).

Increases in DNA methylation after the onset of dormancy suggest that chromatin remodelling may play a role in endodormancy maintenance. It is also interesting to note that epigenetic factors and chromatin remodelling as well as changes in temperature and light are involved in the flowering process (Horvath, *et al.*, 2003). The FAS1 protein is a chromatin remodelling protein involved in flowering that is also regulated in a cell cycle dependent manner in *Arabidopsis* cell cultures (Horvath, *et al.*, 2003). It would not be surprising to find that many of the genes involved with the flowering response are also utilized in the termination of endodormancy.

1.10 Post Endodormancy Growth Resumption

During the onset of dormancy, SAM cells arrest their division during the G1/S transition (Horvath, *et al.*, 2003). The continued growth of the meristem after the period of endodormancy is caused by the re-activation of the cell cycle. As stated earlier, dormancy is growth arrest and the cells are suspended in the G1 phase of the cell cycle. The continuation of the cell cycle from this arrested growth phase occurs when the cells begin to expand and prepare for DNA replication taking place in the subsequent S phase. Once this occurs, the cells now contain two sets of molecular information and enter G2, where they continue to enlarge. In the subsequent M phase, cell division occurs during mitosis.

After endodormancy emergence, genes expressed include many cell cycle genes such as D-type cyclins (CYCD) and histones (Devitt and Stafstrom, 1995). In normal cell cycle progression, the G1/S transition is well understood. PGRs and growth factors act to trigger the transcription of CYCD genes. They do this by post-translationally modifying transcription factors responsible for the expression of the CYCD genes (Sherr, 1994).

PGR classes that are able to promote transcription of these cell cycle genes include cytokinins, brassinosteriods, and GA (Horvath, *et al.*, 2003). Once CYCD is eventually translated and activated, it binds to a cyclin-dependent kinase (CDK). However, before this occurs, CDK must be activated by a CDK activating kinase (CAK) (Fabian-Marwedel, *et al.*, 2002). The final CYCD-CDK complex is able to phosphorylate a retinoblastoma protein (RB) (Healy, *et al.*, 2001). RB is a protein in both plants and animals that controls progression to the S phase of the cell cycle. It is important to note the many regulatory steps necessary for the activation of just one component of the G1/S phase transition. Ultimately, the activation of the RB gene is controlled by a phosphorylation cascade. Once the RB is activated via phosphorylation, it releases bound transcription factors including E2F which allows expression of genes involved in DNA synthesis. There is also evidence suggesting that an RB-E2F complex acts as a chromatin remodelling protein that can actively regulate expression of the genes required in this stage (Shen, 2002).

The next stage of the cell cycle after the S-phase of DNA replication is G2, which is another rest phase in preparation for mitosis. The G2/M transition is also thought to be a stopping point for cell growth when transitioning into dormancy. Exiting the G2-M phase requires proteins similar to those found during the G1-S phase transition. They include a B-type cyclin (CYCB) and CDKB. These too can be induced by PGRs, including auxin, cytokinin and GA (Francis and Sorrell, 2001). Again, while the two B-type CYC and CDK proteins can activate other proteins via phosphorylation, at this point they are promoting cytokinesis rather than DNA replication (Mironov, *et al.*, 1999).

One of the identified M-phase repression mechanisms is phosphorylation of a tyrosine residue within CDK that can prevent its activity (Kumagai and Dunphy, 1991). It was found that cytokinin can dephosphorylate this residue, allowing normal activity of the protein. After cellular division, differentiation occurs once RB is activated again. Studies by Umeda *et al.* (2000) showed that in *Arabidopsis*, a decrease in CAK activity results in decreased CDK activity and ultimately in ceased cell differentiation (Umeda, *et al.*, 2000). This suggested a role for CAK in both cell division and differentiation. Upon completion of cell division, the RB protein is deactivated by hypophosphorylation. This allows the cells to expand and differentiate (Horvath, *et al.*, 2003).

The association of cell cycle arrest with dormancy make it a good area of exploration when trying to better understand endodormancy mechanisms. Identification of molecular mechanisms acting prior to regulation of cell cycle protein activity will provide further insight into plant endodormancy.

1.11 Molecular Mechanisms Involved in Dormancy

Molecular mechanisms controlling the growth and proliferation of the SAM have been identified. Research into this field has shown that different areas of the SAM may have distinct gene expression patterns (Yadav, *et al.*, 2009). For example, the central zone (CZ) of the meristem (Figure 2B) expresses high levels of mRNAs encoding proteins involved in chromatin modification and DNA repair. This may be indicative of these processes playing roles in plant stem cell function, as high levels of DNA repair proteins and chromatin remodelling proteins exist in the SAM of maize (Ohtsu, *et al.*, 2007). SAMs are also rich in mRNAs coding for proteins involved in transcriptional regulation, RNA binding, RNA processing and gene silencing (Barton, 2010). A number

of genes have been implicated in stem cell differentiation and endodormancy maintenance in plant species such as *WUS* and *STM* (Barton, 2010), *PKL* (Ogas, *et al.*, 1999), *CUC1* (Takada, *et al.*, 2001), *ABI5* (Finkelstein, 1994), and *DHN* (Campbell and Close, 1997). This study investigates their expression, therefore a review of their cellular function is warranted.

1.11.1 Chromatin Remodelling

Chromatin remodelling is a form of gene expression control that is based on conformational changes occurring in the chromatin. Chromatin remodelling complexes use energy from ATP hydrolysis to interfere with contacts between histones and DNA. This results in changes to the nucleosome and chromatin that either allows transcription factors to physical access the DNA or isolates the DNA from being accessed ultimately upregulating or silencing gene expression respectively (Sang, *et al.*, 2009). This epigenetic mechanism, as well as others such as DNA methylation have been previously implicated in potato endodormancy emergence (Barton, 2010; Law and Suttle, 2003).

1.11.1.1 WUSCHEL and SHOOTMERISTEMLESS:

WUSCHEL is a meristem specific gene that promotes stem cell activity in the meristem (Barton, 2010). The cells in the CZ (Figure 2B) expressing WUS are thought to function in meristem organization signalling the neighbouring cells to specify them as pluripotent stem cells able to differentiate into other cell types (Schoof, *et al.*, 2000). *WUS* has an antagonistic gene called *CLAVATA3* whose protein is thought to suppress stem cell activity (Barton, 2010). The expression of *CLV3* is localized to the tip of the meristem above the stem cell pool (Schoof, *et al.*, 2000). An increase in *CLAVATA3* expression results in a decrease in WUS activity. Since low levels of WUS cause down

regulation of *CLAVATA3* and high levels cause up-regulation, these two proteins likely interact in a feedback loop mechanism (Schoof, *et al.*, 2000; Muller, *et al.*, 2006; Fletcher, *et al.*, 1999; Brand, *et al.*, 2001). The CLAVATA3 signal originates in the L1 (Figure 2B) layer of the meristem and controls the inner layer expression of WUS through CLV1 membrane bound receptors that propagate the signal to the CZ. The feedback loop between these two gene products likely permits maintenance of a certain number of stem cells in the CZ stem cell pool (Barton, 2010). WUS is essential in maintaining stem cell identity and CLV3 is responsible for containing the growth of the meristem by restricting its size (Schoof, *et al.*, 2000). There have been transcriptional regulators of WUS identified including STIMPY, SPLAYED, BARD1, OBERON1 and OBERON2 whose expression and/or activity may be controlled by CLV3 signalling. *SPLAYED* encodes an SNF2 chromatin remodelling ATPase which binds upstream of the transcriptional start site and is required for proper transcription of *WUS* (Kwon, *et al.*, 2005). BARD1 also binds to the *WUS* promoter and has been shown in interact with SPLAYED (Han, *et al.*, 2008). However, studies have shown that in *bard1* mutants, WUS is upregulated, thus demonstrating an inhibitory function of BARD1. Interestingly, *BARD1* encodes a protein similar to BREAST CANCER ASSOCIATED GENE 1, which in humans is implicated in DNA repair, transcriptional regulation, recombination, and cell cycle control (Irminger-Finger and Jefford, 2006). The antagonistic nature of SPLAYED and BARD1 and where they interact on the *WUS* promoter suggests that they act opposite each other to affect the chromatin state around the *WUS* gene (Barton, 2010).

WUS encodes a transcription factor that possesses both an activation domain and a transcription repressing domain (Kieffer, *et al.*, 2006). Therefore WUS may be a

transcriptional activator and/or a repressor depending on its active state. Long *et al.* (2006) discovered corepressor proteins, belonging to the Groucho/Tup1 corepressor family, that interact with the carboxyterminal domain of WUS (Long, *et al.*, 2006). This type of corepressor downregulates transcription through altering nucleosome positioning by recruiting histone deacetylases. Therefore, WUS may influence transcription through chromatin remodelling (Barton, 2010). WUS is able to repress the expression of a few A-type response regulators, including ARR5, ARR6 and ARR7, by binding upstream from their genes (Gordon, *et al.*, 2009). The ARR proteins are thought to have a role in the cell's perception to cytokinin by inhibiting cytokinin signalling. Therefore, WUS may have a role in re-establishing plant cells' connection to cytokinin signals, which can result in growth (Barton, 2010).

There are a type of class 1 KNOX homeodomain containing transcription factors that function in meristem maintenance, and are also involved in the synthesis of cytokinin in the meristem. One of these genes is *SHOOTMERISTEMLESS* (*STM*) in *Arabidopsis*. Cytokinin levels are increased in plants that are overexpressing these KNOX homeodomain TFs (Barton, 2010). Therefore, STM and other KNOX proteins control meristem function through their effects on cytokinin biosynthesis (Barton, 2010). STM is thought to antagonize the function of CLV (Clark, *et al.*, 1996) and in this way affect the meristem by promoting WUS activity (Schoof, *et al.*, 2000). It has also been found that increased cytokinin levels can lead to increase in KNOX class gene expression as well, suggesting a positive feedback loop (Rupp, *et al.*, 1999). WUS is also up-regulated by cytokinins in another positive feedback loop. Since KNOX gene expression increases cytokinin levels in the meristem, it is possible that KNOX genes have an indirect positive

effect on the expression of WUS (Barton, 2010). However, since STM is expressed throughout the entire meristem and not in the stem cells specifically, it may be a general regulator of meristem development (Schoof, *et al.*, 2000). This make sense, given the indirect effects that STM has on WUS via cytokinin biosynthesis. STM appears to negatively affect the ability of cells to initiate organogenesis (Mayer, *et al.*, 1998). Therefore the system of cells transitioning from the stem cell pool seems to be regulated by both WUS and STM. WUS is needed to specify stem cells and STM allows the cells to grow before being specified to their final cell types (Mayer, *et al.*, 1998).

1.11.1.2 PICKLE:

PICKLE (*PKL*) encodes a CHD3 chromatin remodelling protein that is conserved in eukaryotes. This epigenetic transcription factor is thought to negatively regulate transcription (Woodage, *et al.*, 1997) and act in the GA signalling pathway that leads to the suppression of embryonic traits during germination. Its role in this system was found when the roots of *pkl* mutants were observed to retain embryonic traits and gene expression patterns, including seed storage proteins, and accumulation of large amounts of lipids. The resulting phenotype was a root that was swollen and greenish (Ogas, *et al.*, 1997). Since PKL was identified as a type of CHD3 protein, it was thought to have a role in development through remodelling chromatin architecture (Ogas, *et al.*, 1999).

PKL belongs to the CHD class of proteins that have been identified in many different eukaryotes. The "CHD" designation comes from three domains recognized in the conserved sequence: a chromodomain, a SNF2-related helicase/ATPase domain, and a DNA binding domain (Woodage, *et al.*, 1997). Uniquely, the CHD3 type contains a zinc finger motif while the other CHD proteins do not (Woodage, *et al.*, 1997). Previous

experiments surrounding the CHD protein have suggested that it has a role in transcriptional inactivation. CHD3 proteins in other eukaryotes (*Xenopus* and *Homo sapiens*) have been identified as members of a multiprotein complex that contains histone deacetylase activity (Tong, *et al.*, 1998). Since histone deacetylation is correlated with inhibition of transcription, the CHD3 protein is thus likely involved in transcriptional deactivation (Ogas, *et al.*, 1999).

Observations surrounding the *pkl* mutant include the increased mutant phenotype when GA is removed (Ogas, *et al.*, 1999). Therefore, it is thought that both PKL and GA are necessary for embryonic gene repression in seeds after their germination.

Another member of the PKL family was identified and thought to be involved in repression of genes involved in promoting meristematic activities. Eshed *et al.* found this experimentally because the *pkl* mutant enhanced the mutant phenotype of the *crab claw* (*crc*) mutant (Eshed, *et al.*, 1999). This transcriptional repression is consistent with the function of PKL in embryonic gene expression suppression (Ogas, *et al.*, 1999).

Many genes have been identified that appear to function in chromatin remodelling and affect seed dormancy. PKL is a chromatin remodelling factor but does not seem to influence the dormancy state of seeds when it is mutated (Holdsworth, *et al.*, 2008). Therefore, the mode of transcriptional deactivation by PKL itself may be redundant. It is also possible that since it is part of a larger complex, it may not be active in the endodormancy process. The PKL domain of the multimeric protein may be involved in other aspects of plant development, where it would be active. PKL mutations can also cause ectopic meristem formation due to KNOX gene expression which would be

otherwise repressed (Sang, *et al.*, 2009). Therefore, one or more of the genes that are being repressed by PKL chromatin remodelling are likely targets of KNOX genes such as *STM* (Ori, *et al.*, 2000).

1.11.2 DNA binding Transcription Factors

1.11.2.1 CUP-SHAPED COTYLEDON 1:

Previous studies by Aida *et al.* (1997) surrounding the functions of CUC1 and CUC2 show that they are redundant genes. In *cuc1 – cuc2* double mutants, there was a complete lack of shoot apical meristem, while in single mutants the plant was able to develop normally. Another observation in the double mutant was the fusing of the two cotyledons and other floral organs. This identifies CUC's function in both SAM development and organ separation (Aida, *et al.*, 1997). These two *CUC* genes are thought to promote SAM formation through transcriptional activation of the STM gene. This is due to a noted reduced accumulation of STM transcripts in the double mutant (Aida, *et al.*, 1997; Takada, *et al.*, 2001).

Both of the CUC proteins have an NAC domain. The NAC domain transcription factors are specific to plants (Takada, *et al.*, 2001) and are DNA binding proteins, although the mode of DNA site recognition is still unknown (Olsen, *et al.*, 2005). While little is known about the transcription factors acting upstream of *NAC* genes, there is some information about the control of the *NAC* genes themselves. Studies have shown post-transcriptional control of the *NAC*s by interfering micro-RNAs (Bartel, 2004). In *Arabidopsis*, the miRNA gene miR164 was shown to cleave both CUC1 and CUC2 mRNA (Kasschau, *et al.*, 2003). Post-translational control of the NAC protein has also

been observed with ubiquitin mediated protein degradation as the mechanism (Greve, *et al.*, 2003; Olsen, *et al.*, 2005). This mechanism is described in further detail below.

CUC1 is widely distributed throughout the plant while CUC2 is more meristem specific (Takada, *et al.*, 2001). CUC1 mRNA has been identified in cells thought to become the SAM during embryogenesis. Therefore, it is thought that it functions in SAM development. However, it is not found in the CZ of SAM but is instead located at the boundary region between meristems newly-forming organs. With the location of CUC1 expression in the meristem as well as the phyenotype of the double mutant, the CUC1 protein may function to inhibit growth of cells at the boundaries between organs to prevent their fusing. Other studies showed over expression of CUC1 lead to ectopic shoot formation suggesting a role in promoting early SAM development (Takada, *et al.*, 2001). It is also interesting to note that the ectopic shoots formed on the adaxial surface of cotyledons and leaves however none formed on the abaxial side. This may also indicate a role in SAM development due to the past hypothesis and observations suggesting that the adaxial surface is involved in promoting SAM development (Takada, *et al.*, 2001).

CUC1 and 2 act upstream of STM and are required for STM expression. Over-expression of CUC1 can lead to ectopic STM expression as well as ectopic shoot formation (Takada, *et al.*, 2001). Therefore, it is thought that CUC1 is a positive regulator of STM but the mechanism is unknown.

1.11.2.2 ABSCISIC ACID INSENSITIVE 5:

One family among the many genes that are sensitive to ABA is the abscisic acid insensitive (ABI) transcription factors. These genes were identified by looking for

mutations in *Arabidopsis thaliana* seeds that allowed germination in the presence of ABA (Leung and Giraudat, 1998). The different *ABI* genes have different properties; ABI1 and ABI2 were found to be phosphatases acting in the ABA signal transduction pathway. However, ABI3, ABI4, and ABI5 were found to be transcription factors (Holdsworth, *et al.*, 2008).

ABI5 is a basic domain/leucine zipper (bZIP) transcription factor (Holdsworth, *et al.*, 2008). The basic leucine zipper differs from a normal leucine zipper in that is contains a region that possesses sequence specific binding properties. The leucine zipper is a DNA binding motif that is rich in the amino acid leucine and is able to dimerize with its target DNA (Liu and Stone, 2010).

ABI5 is essential to ABA-dependent post-germination growth arrest (Finkelstein, 1994). The growth arrest that the gene is responsible for is attributed to accumulation of ABI5 via transcriptional activation and enhanced protein stability (Brocard, *et al.*, 2002; Liu and Stone, 2010). Plants' ability to arrest in growth following germination is an evolutionary adaptation in order to survive drought conditions. Once the seed germinates, the ABA responsive pathway relays the existence of stress conditions to the SAM, resulting in ABI5 activation and growth arrest until more favourable conditions present themselves (Lopez-Molina, *et al.*, 2002).

The ABI5 bZIP transcription factor binds to a specific promoter sequence that is known as the ABA-responsive element (ABRE). This binding domain has been found to be involved with the activation of seed storage protein genes that are important during seed development. It has also been shown that ABI3 interacts with ABI5 and the two are

thought to act synergistically in the activation of gene expression (Holdsworth, *et al.*, 2008).

1.11.2.3 DEHYDRATION RESPONSIVE ELEMENT BINDING 2:

Another type of gene that responds to abiotic stressors in plants is the dehydration-responsive element-binding protein (DREB) family. These are a type of ABA independent transcription factor meaning that their activation does not require signalling from ABA. DREBs bind specifically to the dehydration responsive element or DRE in DNA. The abiotic stresses that are mitigated through DREB gene expression include cold temperatures, salinity and drought (Yamaguchi-Shinozaki and Shinozaki, 1994).

The DREBs have binding domains that are homologous to those seen in previously isolated transcription factors including ethylene response factor (ERF) and AP2. These, as stated above, bind to the conserved DRE promoter sequence. Downstream genes from the DREBs include those coding other for transcription factors such as zinc-finger TFs and other AP2/ERF type proteins. This suggests that there is further regulation of expression downstream of DREBs as well and that these elements act in a cascade to ultimately elicit responses to the environmental stressors (Maruyama, *et al.*, 2004).

DREB2A is a more specific type of DREB that has been found to be involved in drought responses in plants. Previous studies have shown that overexpression of *DREB2A* does not directly influence the expression of downstream drought mitigating genes (Liu, *et al.*, 1998). This suggests that DREB2A is post-transcriptionally modified. Further exploration showed that removal of the predicted modification zone of the protein

resulted in activation of the protein. Therefore, modification of the TF acts to restrict its activity rather than promote it (Sakuma, *et al.*, 2006). Later studies by Agarwal *et al.* showed that DREB2A is a phosphoprotein and its activity is negatively influenced by phosphorylation (Agarwal, *et al.*, 2007). Therefore, the basic translated form of DREB2A is inactive and is activated via post-translational modification under stress conditions.

Along with the dehydrative responses, DREB2A expression was also regulated by heat shock, and that thermotolerance is significantly increased in plants expressing DREB2A. Heat shock proteins and TFs were upregulated with DREB2A activation. These included the expected stress tolerance genes but also molecular chaperones and enzymes involved in toxin catabolism. The same study looked at DREB1A activated genes as well, which also included stress tolerance genes, mostly for cold tolerance, but also genes coding proteins involved in carbohydrate metabolism (Sakuma, *et al.*, 2006). Many of the genes found coded for starch degrading enzymes. It may be that the accumulation of certain metabolites or carbohydrates helps in freezing tolerance (Maruyama, *et al.*, 2009).

1.11.3 Downstream Regulatory Proteins Involved in Dormancy

1.11.3.1 DEHYDRIN:

Dehydrins (DHN) are a type of plant specific protein that accumulate during the late stages of embryogenesis and also in response to ABA, low temperature, drought, freezing, or salinity and are thus late embryogenesis abundant proteins. They have a conserved amino-acid sequence that is unlike any enzyme found in either protein or DNA databases. They are highly concentrated in the cell, making it unlikely that they posses any enzymatic function (Campbell and Close, 1997).

Dehydrins possess a conserved sequence designated the Y-segment which is similar to the nucleotide binding site motif of other molecular chaperonins from plants and bacteria. It also possesses a conserved segment designated the S-segment which is able to be phosphorylated, allowing regulation of protein activity (Martin, *et al.*, 1993).

The reaction of DHN expression to drought is mediated by ABA (Campbell and Close, 1997). DHN proteins might act to stabilize proteins or membranes under stress conditions such as drought. It seems that dehydrins likely interact with a type of surface, assumed to by hydrophobic, rather than any certain type of protein.

Along with the response to drought and other stressors, DHN has also been found to be expressed during flowering and vernalization (Campbell and Close, 1997). Since the process of dormancy break in potatoes is influenced by chilling temperatures and drought, it is possible that the DHN protein may mediate the transition from growth arrest to resumption of growth.

1.11.3.2 BYPASS1:

More recently, a new mobile signalling protein, BYPASS1, has been identified in plant species that is not a currently identified PGR. It is a root derived signal that is able to travel through the xylem and affect normal shoot and meristem development (Van Norman, *et al.*, 2011). *BYPASS1* encodes a 349 amino acid protein with no known functional homologs. BYPASS1 negatively regulates the production of a root derived signal that has been designated the BPS1 signal. This has been discovered through experimentation where *bps1* mutants show constitutive signal production. The signal has been found to cause defects in root and shoot development (Van Norman, *et al.*, 2004).

There are some pieces of evidence that suggest that the signal may be a carotenoid derivative, however nothing has yet been confirmed (Van Norman and Sieburth, 2007).

Nonetheless, a few characteristics of the *bps1* mutants and therefore the action of the BPS1 signal (Van Norman, *et al.*, 2004) have become apparent. For example, *bps1* mutants have a loss of shoot apical meristem function and root meristem activity leading to improper root development. Overall the regular expression of BYPASS1 seems to be necessary for normal plant growth and development. While BPS1 is expressed in all root cell types, there are differences in expression within differentiating cells, where there is twice the amount of BPS1 present here than in other cell types. A look at the *BPS1* expression profile clustered *BPS1* with genes coding for transcription factors as well as kinases, suggesting a possible role for BPS1 in a signalling network. Meristem arrest was only maintained as long as the BPS1 signal was being supplied, and when the signal was lost, normal growth resumed. An unexplained observation in the experiment was the *bps1* mutant phenotype's sensitivity to temperature. This may be due to an enzyme being over expressed or activity being more efficient at higher temperatures.

A largely unexplained observation is how the BPS1 signal is able to regulate biological activity and arrest growth. The two theories previously presented are: 1) BPS1 is involved in a root-specific carotenoid processing event, so BPS1 mutation leads to accumulation of the precursor. This precursor may be a mobile, active signalling molecule. 2) BPS1 may be required to negatively regulate a carotenoid-processing event. The loss of function in BPS1 would allow the signal to be produced (Van Norman, *et al.*, 2004).

1.11.3.3 WD-Repeat:

WD-repeat (WDR) proteins are a family of regulatory proteins that are expressed to regulate signal transduction, cytoskeletal dynamics, protein trafficking, nuclear export, RNA processing, chromatin modification and transcriptional regulation. The plant processes that utilize WDR proteins include cell division, apoptosis, light signalling, flowering and floral development, and meristem organization (van Nocker and Ludwig, 2003).

It is known that the WDR domain of a protein functions as a site to interact with other proteins. The general role of the WDR gives it a several different functions. First, it is able to bind to other proteins, forming protein complexes. Many of the WDR containing proteins have more than one WDR site and are able to bind to multiple proteins forming multimeric structures. In this sense the WDR protein acts as a scaffolding protein holding other units together. Second, WDR proteins are integral components of protein complexes. This differs from the first function as this implies WDRs may possess an active site as well as protein binding properties. Third, WDRs may contain modular interaction domains for larger proteins, functioning as a guide to position active sites on the protein correctly on the substrate it acts upon (van Nocker and Ludwig, 2003).

Studies on different WDR proteins in different organisms found a level of sequence conservation that implies some WDR proteins are likely involved in basic cellular mechanisms. More specifically, in *Arabidopsis* there are WDR proteins that are orthologs with cell cycle regulatory proteins in yeast that activate a ubiquitin ligase that targets cell cycle regulators for degradation (Schwab, *et al.*, 2001). Other WDR

containing proteins found in *Arabidopsis* include the developmental regulator LEUNIG (LUG). This protein contains several WD repeats along with other functional domains including a single stranded DNA binding domain (Conner and Liu, 2000). Studies have shown that LUG is likely part of a larger transcriptional regulatory complex where the WDR region binds to another protein to create an active co-repressor (van Nocker and Ludwig, 2003). The LUG protein functions in floral development.

The FASCIATA2 (FAS2) protein is a highly conserved WDR protein involved in plant specific processes. FAS2 is homologous to a subunit of the chromatin assembly factor 1 (CAF-1) which is involved in chromatin assembly post DNA replication and repair (Mello and Almouzni, 2001). Interestingly, mutations in the FAS2 gene lead to meristem disorganization in the shoot and root. The disorganization seems to be due to improper expression patterns of WUS in the shoot and SCARECROW in the root (Kaya, *et al.*, 2001). It is also worth noting that certain CAF-1 subunits have also been found in chromatin remodelling complexes (Wolffe, *et al.*, 2000). It has been suggested that these proteins are involved with the formation of heterochromatin that would be necessary both with initial chromatin assembly but also gene silencing via chromatin remodelling (van Nocker and Ludwig, 2003).

Other WDR proteins are utilized in gene silencing as they are components of heterogeneous protein complexes possessing zinc-finger proteins or histone deacetylases. These complexes have been shown to affect gene expression by interacting with the DNA or affecting DNA architecture via modifying histone acetylation patterns (van Nocker and Ludwig, 2003).

1.11.3.4 Related to Ubiquitin 1-Conjugating Enzyme:

Modifications to the ubiquitin-proteasome pathway are used to regulate the activity of this type of protein degradation. One way that this is achieved is through modification of the SCF complex, which is integral in the ubiquitin degradation pathway, by ubiquitin related protein or RUB. The activity of the SCF complex relies on the conjugation of an RUB protein (RUB1) via the RUB1-conjugating enzyme. Therefore, the RUB1-conjugating enzyme (RCE1) is a potential point of regulation in the activity of the SCF and the ubiquitin proteolysis pathway (Moon, *et al.*, 2004).

The action of the ubiquitination pathway is well understood and is divided into three parts. First the ubiquitin protein is activated by a ubiquitin activating enzyme designated E1, next the Ub is transferred to a ubiquitin conjugating enzyme designated E2 which transfers the Ub to the final E3 enzyme which is responsible for substrate recognition and transfer of the ubiquitin to the substrate to be degraded. This is repeated multiple times until there are multiple ubiquitin molecules attached to the target protein. The ubiquitinated protein is then targeted by the 26S proteasome which degrades the protein and recycles its amino acids (Wilkinson, 2000).

The SCF mentioned previously is a type of E3 complex acting in this pathway. It is named the SCF due to four subunits of the protein including SPK1, CDC53 (or cullin) and the F-box protein. The cullin provides a scaffold like binding site for the other subunits and the F-box protein is substrate specific in this complex and it is where the SCF gets its specific binding properties (Moon, *et al.*, 2004).

The SCF has many known target substrates, including transcription factors, cell cycle regulators, and factors involved in signal transduction and development (Moon, *et al.*, 2004). In plants, SCFs can affect processes such as hormone response, photomorphogenesis, circadian rhythms, floral development and senescence. In the auxin signalling pathway, the SCF targets repressors of hormone response, allowing proper perception of the auxin signal (Moon, *et al.*, 2004).

Mutations in *RCE1* affect SCF function and leads to phenotypes characteristic of loss of auxin perception (Moon, *et al.*, 2004). While a lack of RUB1 binding leads to these effects, the function of RUB1 in this complex is not yet known. The conjugation and deconjugation of RUB1 to the complex thus appears necessary for proper activity of the SCF complex (Moon, *et al.*, 2004).

Another multisubunit E3 complex has been reported in the control of ABI5 levels. ABI5 is targeted by a complex, that contains the KEEP ON GOING (KEG) protein, and is degraded. It has been shown that ABA actually promotes autoubiquidation of the KEG protein, which targets itself for degradation and allows higher levels of ABI5 to accumulate (Liu and Stone, 2010).

It has also been observed that while multiubiquitination can target a protein for degradation, monoubiquitination can activate certain proteins (Zhang, 2003). One class of the proteins susceptible to activation via ubquitination is the histones, specifically H2A and H2B (Zhang, 2003). In animals, monoubiquitination of H2A is necessary for silencing of homeobox genes (Wang, *et al.*, 2004). Monoubiquitination of H2B controls the binding of histone H3 lysine methylases which is required for methylation of the

chromatin (Cao, *et al.*, 2008). In yeast, monoubiquitination is accomplished by E3 ligase and an E2 conjugase. Homologs of these genes were found in *Arabidopsis* and are called HISTONE MONOUBIQUITINATION (HUB) and UBIQUITIN CARRIER PROTEIN1 (UBC). Mutations in the HUB proteins lead to defects in seed dormancy, suggesting that HUB may control seed dormancy (Liu, *et al.*, 2007).

1.11.3.5 Cell Cycle-dependent Gene:

Other potential genes involved in control of dormancy include cell cycle dependent genes. Since endodormancy is characterized by growth arrest either at the S or G2 phase, it is possible that the gene being controlled through the signal cascade may be a cell cycle gene.

Cell cycle dependent genes are those that have varying levels of expression through cell cycle progression (McKinney and Heintz, 1991). While other genes involved in the cell cycle may be continuously expressed, the cycle dependent genes are only expressed or active at certain points. An example of these is *cyc07*, which is expressed at the S phase of the cell cycle (Ito, *et al.*, 1994).

Cyc07 is a plant specific gene that has been shown to accumulate in actively cycling cells (Ito, *et al.*, 1991). This gene also has homologues in other species. The *PLC* gene family in yeast possesses a similar amino acid sequence, suggesting that these genes have a conserved function in cell proliferation. Other studies surrounding the activity of cyc07 have shown a distinct expression pattern of the gene where mRNA is found to be highest in meristematic tissue that is undergoing cell proliferation (Ito, *et al.*, 1991). With

this as well as other experimental evidence, cyc07 is likely a protein involved in DNA synthesis.

Other S phase specific genes found in higher plants include those encoding histone proteins and PCNAs (proliferating cell nuclear antigen) (Lepetit, *et al.*, 1992; Kodama, *et al.*, 1991). Both of these genes are expressed in actively cycling cells and appear to be meristem specific. However, histone genes are dependent on the occurrence of DNA replication where *PCNA* is not. Expression patterns during DNA replication is similar between cyc07 and histones. This observation and similar protein sizes between cyc07 and histones suggest that *cyc07* may code for a histone. Additionally, the transcription of the H4 gene is dependent on DNA replication (Lepetit, *et al.*, 1992). No promoter of the *cyc07* gene has been identified, and while the conserved elements of other histone promoters were probed in the sequence upstream of the cyc07 protein, none were identified (Ito, *et al.*, 1994).

1.12 Studying Gene Expression

1.12.1 Real Time PCR

The transcriptome is able to tell us a lot of information about the metabolic processes taking place at a given time in any tissue. This holds true for SAMs, and the transcriptome may be examined to elucidate the mechanisms responsible for dormancy emergence in potato SAMs. One way that this can be done is through real-time quantitative PCR (qPCR), which analyzes the abundance of a transcript in a tissue sample. Comparing the measurement of mRNA concentration for a certain gene product between treatment and control samples can provide information about when the genes are active in the system of interest.

1.12.2 Suppression Subtractive Hybridization

While qPCR allows us to measure gene expression in real time, how do we know what genes to investigate? The classical way is to look at mutants and try to identify the gene that is responsible for that mutation. Once mutants are characterized and genes responsible for certain processes are identified, they may be studied in organisms outside the original species where the mutated gene was witnessed. More recently, new techniques have been developed such as microarrays, where a standard template of mRNA probes is tested with cDNA from the sample and fluorescence is measured. The more sample template to bind the probe, the more the probe fluoresces giving a semi-quantitative measure of the gene abundance in the sample. Another method for identifying genes upregulated in a certain processes is subtractive hybridization. This can provide the sequences of genes that are being upregulated in the treatment tissue, which can be further identified by looking at homology with known genes. More recently, a new method of subtractive hybridization has been developed called suppressive subtractive hybridization (SSH). SSH is a way of looking at differentially expressed genes using a PCR based method called suppression PCR. Suppression PCR attaches specific primer receptor sequences to the cDNA that is used in the process and amplifies only specific cDNA sequences. This method has been successfully utilized in experiments surrounding *Euphorbia esula* meristem dormancy (Jia, *et al.*, 2006; Campbell, *et al.*, 2008) and is promising in other studies involving plant metabolism, growth and development.

The suppressive subtractive hybridization process itself is based on suppression PCR. It uses specific primer sets to either amplify or suppress the amplification of certain gene products (Diatchenko, *et al.*, 1996). The process requires two sets of cDNA, a control or

"driver" sample which acts as a reference of genes to be removed, and the sample of interest or "tester" sample which contains the genes you are interested in. First, cDNA from the tester sample is divided into two pools and different adapter sequences are ligated to the 5' ends of the cDNA sequences. Next, each type of adapter ligated tester cDNA and the driver cDNA in excess are mixed together in separate hybridization reactions. The cDNA in this reaction is denatur ed at ~98°C for ~1.5 min and then it is allowed to hybridize by annealing with one another for ~10 h at ~68°C. After this initial tester separate hybridization step, another is done mixing the two types of tester together, along with more driver cDNA. The same cycle is performed as described above. After this hybridization step, any double stranded fragment containing an adapter will be ligated with the same adapter to the complementary strand. The next step includes the suppression PCR step, as only double stranded fragments containing differing adapters on each end will be exponentially amplified in the PCR. The PCR uses primers based on the sequence of the adapters used. If any fragment does not contain an adapter (driver) it will not be amplified, if it has the same adapter on the 5' and 3' end, it will fold over on itself and amplification is not possible (Diatchenko, *et al.*, 1996). Therefore, only genes present in the tester samples will be amplified. Figure 3 provides a graphical representation of this process.

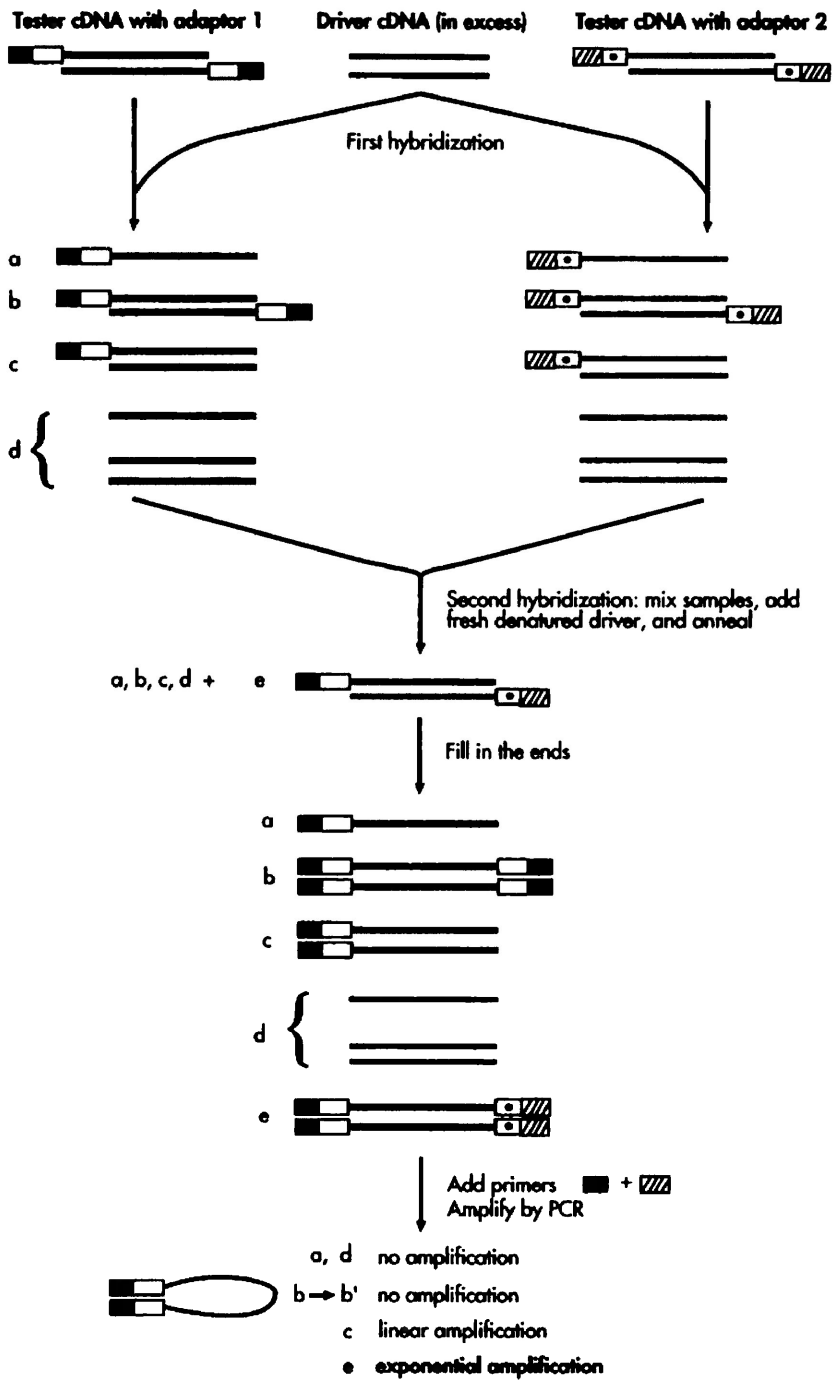

Figure 3: Suppressive subtractive hybridization process (Diatchenko, *et al.*, 1996).

1.13 Summary

The potato is a nutritional, high yield crop that can be grown in most areas of the world (Spooner, 2005). The exports of potato products is largely reduced to frozen and

dehydrated foods due to the poor storing properties of tubers (McLaughlin, 2005). The reason for tubers sprouting in storage is their meristems emergence from dormancy, which leads to meristem growth. There are three types of dormancy associated with potato tubers (para-, endo-, and ecodormancy) all of which play a role in maintaining tuber meristems. The most important and least understood of these is endodormancy (Sonnewald, 2001). Endodormancy is enforced by internal cues in the meristem rather than PGRs or environmental stimuli (Rohde and Bhalerao, 2007). Investigating the molecular mechanisms at work during this time can help better understand how the meristem is able to remain dormant without external influences. Gene expression data can reveal information about how the meristem is maintaining dormancy through investigation about which genes are being silenced/expressed. Having a better understanding about what is happening at this level, we are able to propose possible mechanisms as to how dormancy is maintained. Further investigation into these mechanisms can lead to the development of better tuber growth or storage practices, which may ultimately extend their state of dormancy and shelf life.

2.0 MATERIALS AND METHODS

2.1 Potato Explant Medium and Growth

The potato explant medium was designed under the guidelines of Murashige and Skoog plant growth medium (Murashige and Skoog, 1962), and contains 10% v/v 10X MS macronutrients (Sigma, M0654), 10% v/v 10X MS micronutrients (Sigma, M0529), 0.1% v/v 1000X MS vitamins (Sigma, M3900) and 3% w/v sucrose, pH adjusted to 5.8 with 1 M KOH. Medium is solidified with 0.24% w/v of plant grade phytagel. Phytagel was dissolved into medium in a microwave and then medium was aliquoted into 16x125 mm glass tubes at 5 ml per tube. Medium in tubes was capped then autoclaved at 121°C for 20 min and left in a sterile environment to solidify.

Potato explants were planted in the tubes under sterile condition in a bio-safety cabinet. Explants were derived from existing potato shoots that were cut just above each leaf node. Each cutting from the parent plant was positioned basipetally in a new tube containing medium. The new daughter plants were left in growth chamber at 20°C with a 16 h light / 8 h dark cycle. The cuttings were left to grow for 4-5 weeks and then new cuttings were taken and replanted, this process was repeated to maintain growing potato explants. The plants cannot be left for more than 9 weeks without being re-cultured.

2.2 Microtuber Medium and Growth

Medium consisted of 10% v/v MS macronutrients (Sigma, M0654), 10% v/v MS micronutrients (Sigma, M0529), 0.1% v/v MS vitamins (Sigma, M3900), 0.2% v/v Biotin at 25 mg/ml, 0.05% v/v Folic Acid at 1 mg/ml, 0.045% v/v Nicotinic Acid at 10 mg/ml, 0.04% v/v Thiamine-HCl at 1 mg/ml, 8% w/v sucrose. pH was adjusted to 5.7 with 1M KOH. Charcoal (0.2% w/v) was added to medium and brought up to final volume with

ddH2O. Agar (0.6% w/v) was added to each magenta box (3x3x4; LxWxH inches) containing 100ml of medium, which were then capped and autoclaved for 35 min. Medium was left to cool in sterile bio-safety cabinet and gently swirled every 10 min, (to keep charcoal in suspension) until medium was solid.

Potato explants from the shoots prepared above were used to grow microtubers in this medium. Explants were obtained in the same fashion and planted the same way except 25 explants were planted in each magenta box in a 5 x 5 pattern. These explants were left in a growth chamber at 20°C under 24 h dark cycle for 9 weeks.

2.3 Microtuber Harvest

At the end of the 9 week growth period, microtubers were harvested from the explants under sterile conditions in a bio-safety cabinet. The microtubers were divided into 5 petri dishes corresponding to the 5 timepoints used in the study. Immediately after harvesting, all the petri dishes were put into the 20°C growth chamber in continuous darkness for 2 weeks. After the 2 week period, the micotubers were removed from the cabinet and the first meristems were ready to be harvested. The rest of the petri dishes, representing the other dormancy emergence timepoints, were stored at 4°C and meristems sampled periodically over 32 weeks. However, before harvesting the meristems, the tubers must sit at 20°C under 24h darkness for 1 week. After that 1 week, they were removed from the growth cabinet and meristems harvested. This process was repeated at 4 week intervals, placing the remaining microtubers into warm conditions for 1 week, before collection of meristems.

2.4 Meristem Harvest

Meristems of interest were those located in the equatorial region of the microtuber and apical meristems were to be excluded when harvesting as described in Figure 4. The dish of microtubers was placed under a dissecting microscope and equatorial meristems were located. They were excised from the tuber using a scalpel and forceps. It is important to collect the meristem and about a 1 mm cube of potato that the meristem is sprouting from. Once harvested, the meristems were stored in 800 µl of Trizol in a 2 ml snap top tube on ice. Once harvesting was completed, the Trizol containing the meristems of that timepoint was flash frozen in liquid N_2 and stored at -80°C until the RNA extraction.

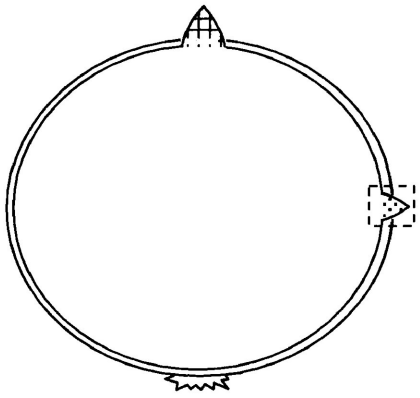

Figure 4: Cross section of potato microtuber including apical (checkered), and axial meristems (dotted). "Burst" at the base represents point of attachment to the stolon. Dashed line represents section of axial meristem harvested for further study.

2.5 RNA Extraction

The solution of Trizol containing the meristems was homogenized using a Power Gen 500 (Fisher Scientific) tissue homogenizer, and clarified by centrifugation at 4°C for 10 min at 12,000 g. The supernatant was transferred to a new 1.5 ml snap top centrifuge tube and care was taken not to disturb the pellet of tissue at the bottom of the tube. Chloroform (160 µl) was added to the clarified homogenate and then vigorously shaken for 15 s and left to incubate at room temperature for 3 min. Next, the samples were again centrifuged at 4°C for 15 min at 12,000 g. The tubes were removed from the centrifuge and the aqueous phase was transferred to a new 1.5 ml tube. To the aqueous phase, 200 µl of isopropanol and 200 µl of salt solution (1.2 M NaCl, 0.8 M sodium citrate) were added and mixed by inversion. The new solution was again centrifuged at 4°C for 10 min at 12,000 g. The supernatant was removed being careful not to loosen the RNA pellet. The pellet was then washed with 800 µl of cold (-20°C) 75% EtOH. The ethanol solution containing the RNA pellet was then lightly vortexed to dislodge the pellet from the tube. The solution was then left at -20°C for 1-24 h. After the time was up, the solution was centrifuged at 4°C for 5 min at 7,500 g. The ethanol was removed and the pellet was allowed to dry for up to 15 min. Finally, 20 µl of DEPC treated water was added to the pellet and allowed to solubilize the RNA, which was flash frozen and stored at -80°C.

2.6 Synthesis of cDNA

Before cDNA synthesis, the quality and the quantity of the RNA was tested using the BioRad Experion system. Samples with an RNA quality indicator number (RQI) above 7.5 were accepted as good quality and used in the study. The RQI is an estimate of eukaryotic total RNA degradation based on comparison of the electropherogram to a

series of standardized, degraded RNA samples. Samples failing the RQI were not used. A basic standard sensitivity RNA chip (accurate within 5-500ng/µl RNA) and protocol were used following the BioRad guidelines. Once samples passing the RQI threshold were identified, they were diluted to 100 ng/µl using DEPC H_20 for use in cDNA synthesis and further analysis.

In a 0.2 ml PCR tube 100 ng of RNA (1 µl) was mixed with 0.25 ug/µl of oligo DT primer and 0.01 ug/µl of random hexamer primer and brought up to a final volume of 10.5 µl using 0.1% v/v DEPC treated water. The solution was mixed gently by pipetting then centrifuged briefly to bring contents to the bottom. It was then incubated in a thermocycler at 65°C for 5 min and then cooled at 4°C for 2 min. Next a master mix was made containing: 42% v/v of Fermentas 5X reaction buffer, 2.1 U/µl of Fermentas Ribolock RNAse inhibitor, 2.63mM Fermentas dNTP mix, 21 U/µl of Maxima Reverse Transcriptase, and made up to a volume of 9.5 µl with 0.1% v/v DEPC treated water. This solution was mixed gently and centrifuged to bring contents to the bottom. Master mix (9.5 µl) must be added to each reaction previously prepared and incubated. This new RNA/Master mix solution was then incubated in a thermocycler at 50°C for 30 min and then at 85°C for 5 min to terminate the reaction. The resulting cDNA solution was stored at -80°C until use.

2.7 Protocol for qPCR

The same protocol for real time PCR was conducted in each of the gene expression analyses with exception of different primers specific for the different genes as well as different temperatures dependent on the most efficient binding temperature. A list of all the primers used and the corresponding genes can be found in Tables 1 and 2.

The qPCR reaction volume used was 10 µl. The master mix used was as follows: 5 µl of Fermentas Maxima SYBR Green/ROX qPCR Master Mix (2X) (K0223), 0.8 uM of the specific forward primer, 0.8 uM of the specific reverse primer, made up to 10 µl with 0.1% v/v DEPC H_20. This was enough for one reaction and the amounts were increased respective of the number of samples being analyzed. Bio-Rad 96 well un-skirted low profile plates (MLL9651) were loaded with 1 µl of 2X diluted cDNA sample described above. Once the cDNA was in the plate, 9 µl of the above master mix was aliquoted into each well containing cDNA to be analyzed. The plate was analyzed using the BioRad CFX96 Real Time System. The run protocol consisted of an initial enzyme activation step of 95°C for 10 min, next the amplification cycles were 95°C for 15 s to denature, then the specific primer annealing temperature (Table 1 and 2) for 60 s to allow extension, this cycle was repeated 40 times. After the 40 cycles a melt curve was constructed with the temperature starting from the specific annealing temperature rising to 95°C at 0.5°C intervals every 5 s.

2.7.1 Primers used in qPCR

The sequences of the primers used in this gene expression analysis can be seen in Table 1. This table also contains the annealing temperature used in qPCR and the expected amplicon size. The primers were designed based on specificity to the gene of interest using the NCBI Primer-BLAST tool (Ye, *et al.*, 2012). Restrictions when selecting primers included primer lengths 20-25bp, and GC content between 40-60%. The amplicon of interest was selected to be between 100-200bp, and primers were checked for any potential secondary structure that may interfere with DNA binding. There are two sets of primers for WUS and STM that were used for the nested PCR

performed to provide enough starting DNA for relative expression quantification by qPCR. For these genes, primers designated (O) are outer primers yielding a larger product, primers designated (I) are for the inner product amplified in the qPCR reaction.

Table 1: Primers and annealing temperatures used in qPCR.

Designation	Sequence	Specific Gene	T_A (°C)	Amplicon Size (bp)
L2 (F)	GGCGAAATGGGTCGTGTTAT	L2	82	121
L2 (R)	CATTTCTCTCGCCGAAATCG			
Efla (F)	ATTGGAAACGGATATGCTCCA	EF1a	79	101
Efla (R)	TCCTTACCTGAACGCCTGTCA			
ABI5-F	TGGAGAAGGTCGTTGAAAGG	ABI5	60	171
ABI5-R	GTTTCGCGACTTCTGCTTCT			
CUC1-F1	GTATCGCCTTGATGGCAAAT	CUC1	60	204
CUC1-R1	GAGTGCGGAAGGTGAGAGAC			
DHN-F1	GGAATCCAGTCCGCCAAAC	DHN	60	103
DHN-R1	GCCTGAGTTCCATAGCCGGT			
PKL-F2	TTCCAGCTTCCGGAGTTCC	PKL	60	150
PKL-R2	CATGCGAAAGTTCAGCTCCAC			
WUSP3-F	TTATTATCAGTGGCAGAGGCG	WUS (O)	50	238
WUSP9-R	GGCAAAGTACTAGCCGTTGG			
WUSP4-F	GCCAACGAGCGATCAGATA	WUS (I)	54	192
WUSP4-R	GCAGCAATGAGCCTCTTCTT			
STMNest-F1	TCATTGGAGAAGATCTGGCAC	STM (O)	50	621
STMNest-R1	TCAGAGTAGAGACGGTGTC			
STM-R1 (R)	AGGCTTCCCAAGTACCCACT	STM (I)	50	152
STM short (F)	CTTCTCATGAATCTGCTCTGG			

2.7.2 SSH Gene Primers

The primer sequences used in the quantification of the gene expression can be found in Table 2. The primers were selected based on an amplicon size of 80-150 bp. The primer length was selected to be between 20-25bp with a GC content ~50%. Also, the difference in T_m between the forward and reverse primer was selected to be within 3°C (Ye, *et al.*, 2012). The primer sequences were checked for any secondary structures that may interfere with DNA binding.

Table 2: Sequences and annealing temperatures of primers used in qPCR of genes identified from the subtractive libraries.

Designation	Sequence (5'-3')	Specific Gene	T_A(°C)	Amplicon Size (bp)
bypass-D12-FWD	AGCAAAGCATCGCGTCCAGT	BPS1	59	133
bypass-D12-REV	ACGCGGCACAGTTGGAACTTCT			
cyc07-B07-FWD	GGAACCTCGGCCTCTGCCTCA	cyc07	59	101
cyc07-B07-REV	GGCAAGCTGATGGAGGTTCATGGT			
cyc07-A12-FWD	TGGCAAGCTGATGGAGGTTCATGG	cyc07	61	102
cyc07-A12-REV	GGAACCTCGGCCTCTGCCTC			
DREB2a-E06-FWD	TGCTGCTGCTGCGGTTTTGGA	DREB2a	57	128
DREB2a-E06-REV	CGCCTTTACATTCCTCCGTTGATGC			
RUB1-C10-FWD	CGTTTGCCCGACATAGCCCCC	RCE1	56	81
RUB1-C10-REV	GCTGCTGTATTGAGAGACAACCCG			
RUB1-F09-FWD	TCGAGAAGACTGGAAGCCTGTGC	RCE1	59	108
RUB1-F09-REV	GCAGCTGCATCGTGATTGAGGGG			
WDrepeat-H11-FWD	AGGGTGACCATCCAAGCCGC	WDR	59	97
WDrepeat-H11-REV	GGCGAACAAGTGACTGGAGCCT			
MADS-E06-FWD	CCAAAGGGGGTCGAAGGCCA	MADS	56	133
MADS-E06-REV	AGTCATGCAAACCAAAGCGTCCC			

2.7.1 Additional qPCR methods

Specific annealing temperatures for each primer set was determined by running the primers with a cDNA solution consisting of cDNA from each of the sample types. Each primer set was run in triplicate at eight different temperatures ranging from 55°C to 65°C and the temperature which yielded the most efficient and specific binding was selected for use in the experiment.

Primer efficiencies were determined by analyzing the amount of fluorescence the primer set gave at varying concentrations. Concentrations from 100 ng/µl to 0.001 ng/µl

of cDNA were analyzed and a standard curve was constructed. The primer efficiency was obtained using the slope of this curve on a log scale where $E=(10^{-1/slope}-1) \times 100\%$ (Pfaffl, 2001). Calculations for normalized expression can be found in Appendix A 7.1.

Genes that are expressed at an equal level throughout dormancy emergence were selected and used to normalize the qPCR data. The genes selected include *EF1α* and *L2*. Their expression over the course of the study can be found in Figure 18 of Appendix A.

2.8 Growth Measurement By ^{3}H Labeling

Meristems were harvested as described in section 2.4 Meristem Harvesting. Upon harvest the meristems were put into 1 ml of uptake buffer in three groups of 10 in three different 50 ml polypropylene tubes. The uptake buffer consisted of 10 mM MES/KOH (pH 5.7) and 50 ug/ml chloramphenicol. Once the meristems were harvested and divided into the 10 meristem triplicates, 185 kBq or 5 µl of [methyl,1',2'-^{3}H] labeled thymidine (MP Biomedical 2403901) was added. The meristems were then incubated at room temperature for 4 hours under low light on an oscillating shaker. After the incubation, labelled tissue was rinsed 3 times with 1 ml dH_2O, then twice with 1 ml 5 mM unlabeled thymidine in uptake buffer, and finally any residual liquid was blotted dry. The meristems (labeled tissue) were then homogenized with a mortar and pestle in 2.5 ml 10% w/v trichloroacetic acid (TCA) in a fume hood and then the nucleic acids were left to precipitate on ice for 2 hours. The homogenized tissue was then measured with a scintillation counter. A 1 ml aliquot of the extract was transferred to a vial containing scintillation fluid and a (Whatman 1822-025) glass fiber disk. An additional 1 ml aliquot of the extract was filtered onto a glass fiber disk pre wetted with wash buffer (20 mM sodium pyrophosphate and 5% (w/v) TCA dissolved in distilled water), then washed

twice with the wash buffer and once with 70% EtOH (Sambrook and Russell, 2001). The glass disk containing the filtrate was also put in a vial with scintillation fluid and analyzed in the scintillation counter.

2.9 Meristem Measurements

Microtubers had the meristems dissected then immediately measured under the microscope. Meristem length was measured manually with a nm measuring tool at 40X magnification. Lengths were measured from the base of the meristem to the tip. Measurements were recorded and pooled. Measurements were taken in the Lakehead University Instrumentation lab using the LEICA MS5 stereo Microscope.

2.10 Suppressive Subtractive Hybridization

The suppressive subtractive hybridization (SSH) procedure was done by RxBiosciences Ltd. (1151 Taft St, Suite 2, Rockville, MD USA). Two subtractive libraries were constructed for this project. One used RNA from T-0 (2 weeks post-harvest) as the control and RNA from T-1 (7 weeks post-harvest) as the tester sample. The second used T-0 for a control again, but T-2 (11 weeks post-harvest) as the tester sample.

RNA samples were checked for quality and quantity using the BioRad Experion. Samples of sufficient RNA and high quality were selected for analysis. Three samples from each time treatment (6 for the control) were selected and pooled in equal amounts to get a good representation of RNA present at those timepoints.

The library to be constructed would give differentially expressed genes, present in T-1 (7 weeks post-harvest) but not T-0 (2 weeks post-harvest), and a second library of

genes present in T-2 (11 weeks post-harvest) but not in T-0 (2 weeks post-harvest). The sequences that were returned were analyzed using the NCBI BLAST tool. Sequences homologous to potential genes affecting dormancy were further analyzed using qPCR.

2.11 Statistics

All significance testing was performed using the statistical software R. For the significance in the measurements including the length and ^{3}H % incorporation, the samples were all compared to the control which is designated T-0 (2 weeks post-harvest). Therefore, upon testing of parametric assumptions, a Dunnett's test was preformed if parametric assumptions were met (Hothorn, *et al.*, 2008), and if parametric assumptions were not met, then a multiple Behrens-Fisher Test was performed (Munzel and Hothorn, 2001). The confidence interval for acceptance of significant difference was 95%.

The same statistical method was utilized when analyzing the qPCR data as well. All the comparisons were done relative to the control point T-0 (2 weeks post-harvest). If parametric assumptions were not met, then a multiple Behrens-Fisher Test was performed (Munzel and Hothorn, 2001), if they were met, then a Dunnett's test was done (Hothorn, *et al.*, 2008). Again, a 95% confidence interval was used in determining statistical significance.

The traditional way of calculating normalized expression condenses the sample values obtained into means and standard deviations,(Pfaffl, 2001) making it difficult to perform the significance testing. Therefore, pseudo normalized expression values were constructed for each individual sample replicate in order to generate a data set to analyze. This was done by using the mean of the control group T-0 and transforming each value as

one would normally do to find normalized expression. When plotted, the values obtained, as well as thus that simply use the means, showed similar results, with slightly higher standard deviations in the pseudo values.

3.0 RESULTS

3.1 Meristem Growth

In order to assess dormancy emergence, the growth of the microtuber meristem was determined by the level of [^{3}H]-thymidine incorporation into DNA, and change in meristem length over a 32 week period post harvest (Fig. 5). The data are presented as relative growth, (growth of the sample divided by the growth of the control -2 weeks post-harvest). With this representation, 1 represents the length of the T-0 control meristems and anything higher or lower than one is larger or shorter than the control respectively. The length of the meristem was measured in mm and shows significant differences in length of the control point compared to all other timepoints measured. The radiolabeling is measured by the amount of tritium in the sample due to uptake in DNA replication. There is a noticeable trend in the data with tritium incorporation, with incorporation increasing as the meristem emerges from dormancy. However, no significant differences were found due to variability in the data.

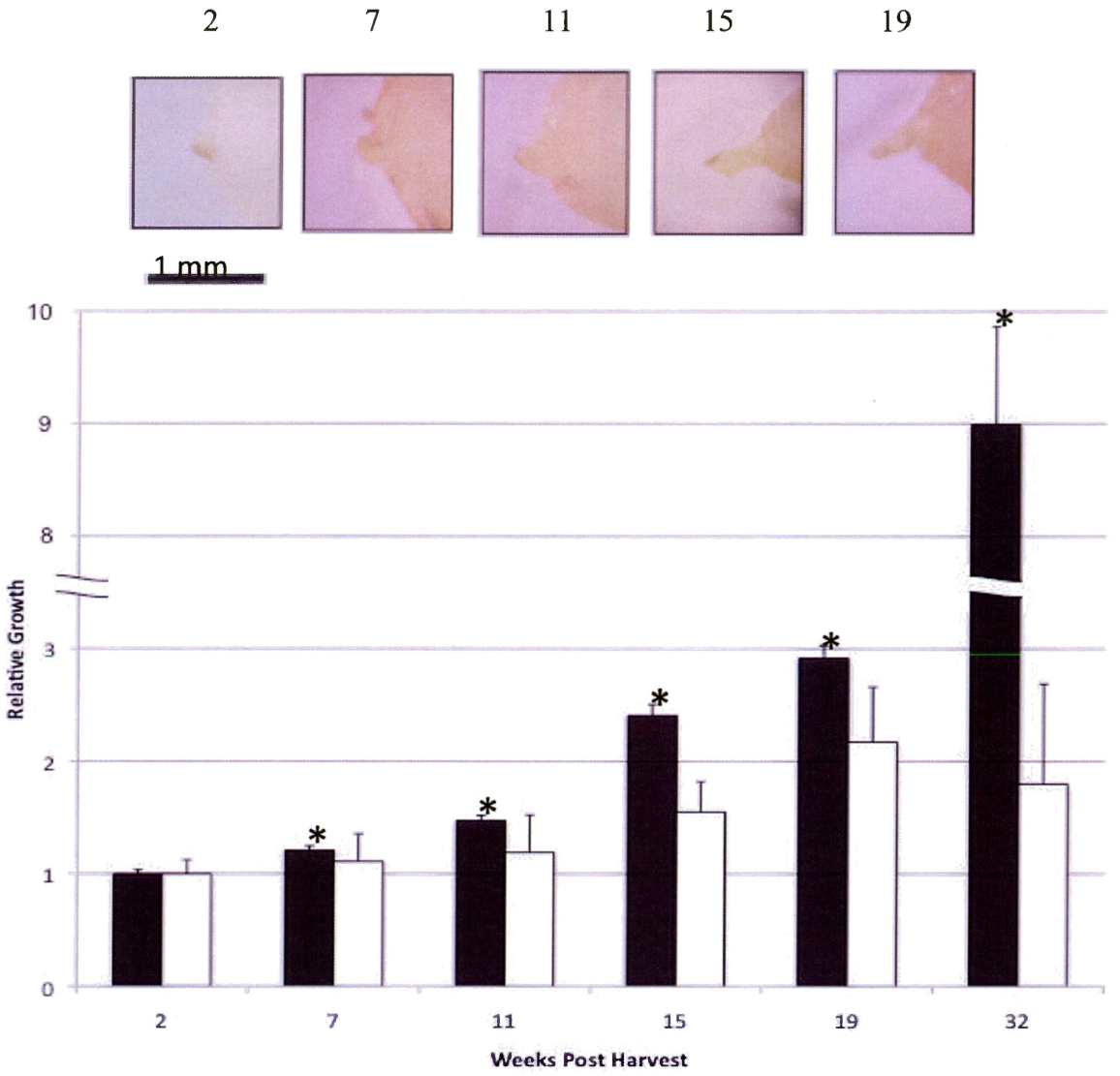

Figure 5: Meristem growth through dormancy emergence measured in growth relative to the control point (2 weeks post-harvest). The black bars represent the relative length of the meristems in mm. The white bars represent the relative % incorporation of tritium into the DNA of the growing meristems. Asterisks represent significant differences from the control sample ($p<0.05$) according to a Dunnett's test, and error bars represent standard error measure. Pictures of SAMs for each respective timepoint are present above their data series.

3.2 Expression Analysis for Previously Identified Genes

Various genes were studied through the progression of dormancy emergence in the potato meristem. They were selected based on previous studies done in potato as well

as other plants that implicated these genes in the process of dormancy and are described in detail in the introduction. They are thought to be involved in dormancy breakage through the action of meristem activation. They code for various transcription factors regulating meristem growth and maintenance.

Table 3: Genes related to SAM dormancy and their function as identified in past literature.

Gene Name	Function	Reference
WUSCHEL	Chromatin Remodelling protein	(Kieffer, *et al.*, 2006; Barton, 2010)
SHOOTMERISTEMLESS	Homeodomain transcription factor	(Schoof, *et al.*, 2000; Barton, 2010)
PICKLE	CHD3 chromatin remodelling protein	(Woodage, *et al.*, 1997; Tong, *et al.*, 1998)
CUP-SHAPED COTYLEDON 1	NAC domain transcription factor	(Takada, *et al.*, 2001)
ABSCISIC ACID INSENSITIVE 5	bZIP transcription factor	(Holdsworth, *et al.*, 2008; Liu and Stone, 2010)
DEHYDRIN	Protein stability	(Campbell and Close, 1997)

Expression of the various dormancy related genes was measured at 5 different timepoints over 19 weeks post tuber harvest, representing endodormancy emergence. The post dormant meristems were measured for expression values at 7, 11, 15, and 19 weeks post-harvest and compared to dormant meristems at T-0. These meristems represent tissue emerging from dormancy and resuming normal growth and function. The normalized expression that is presented in Figures 6-16 was calculated as described by Pfaffl (2001) (Pfaffl, 2001) and the methods are described in Appendix A 7.1.

3.2.2 WUSCHEL

The expression of the *WUS* gene increases early in dormancy emergence and its highest expression is seen at 11 weeks post-harvest with nearly a 4 fold increase (Figure

6). After the 11 week mark, the expression drops again to levels below that seen in the dormant meristem and then begins to increase again at 19 weeks post-harvest. The expression of *WUS* at 11 weeks post-harvest is significantly greater relative to the dormant control (2 weeks post-harvest) meristems.

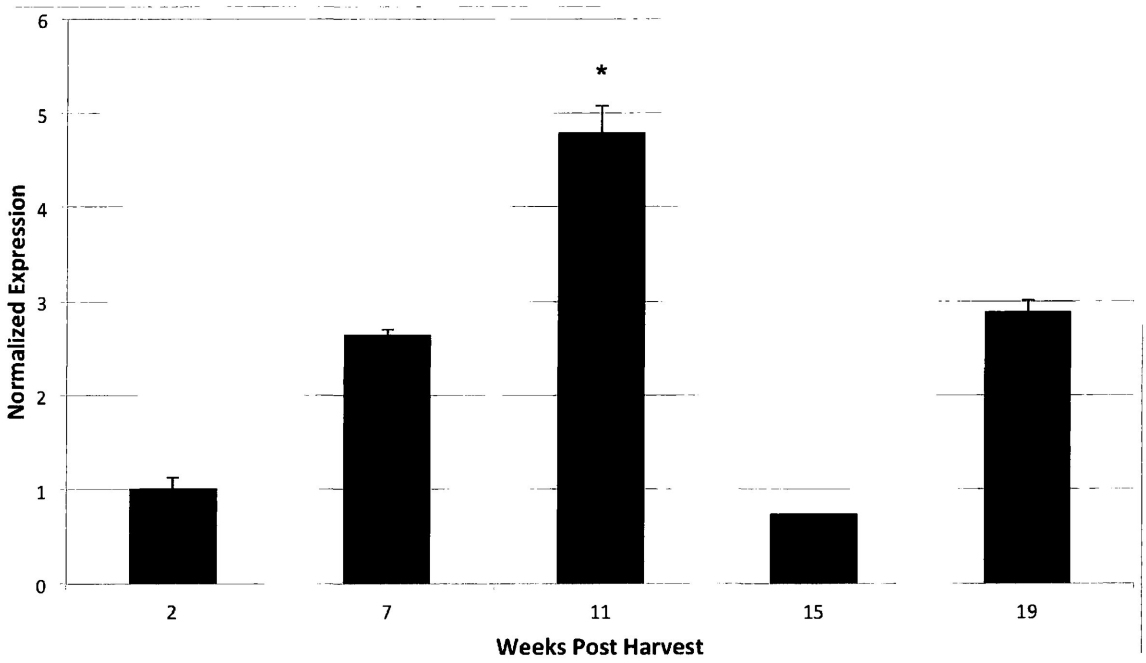

Figure 6: Normalized expression of *WUS* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM). Asterisks represent significant differences between the sample of interest and the control sample ($p<0.05$), n=7.

3.2.3 SHOOTMERISTEMLESS

Similar to the *WUS* expression pattern, *STM* increases post dormancy to a maximum expression at 11 weeks post-harvest (Figure 7). After this time, the expression of the gene drops slightly and seems to plateau at levels ~2.5x greater than that of the dormant sample. While there was an increase in *STM* expression through dormancy

emergence, no statistically significant differences were detected due to variability in the data.

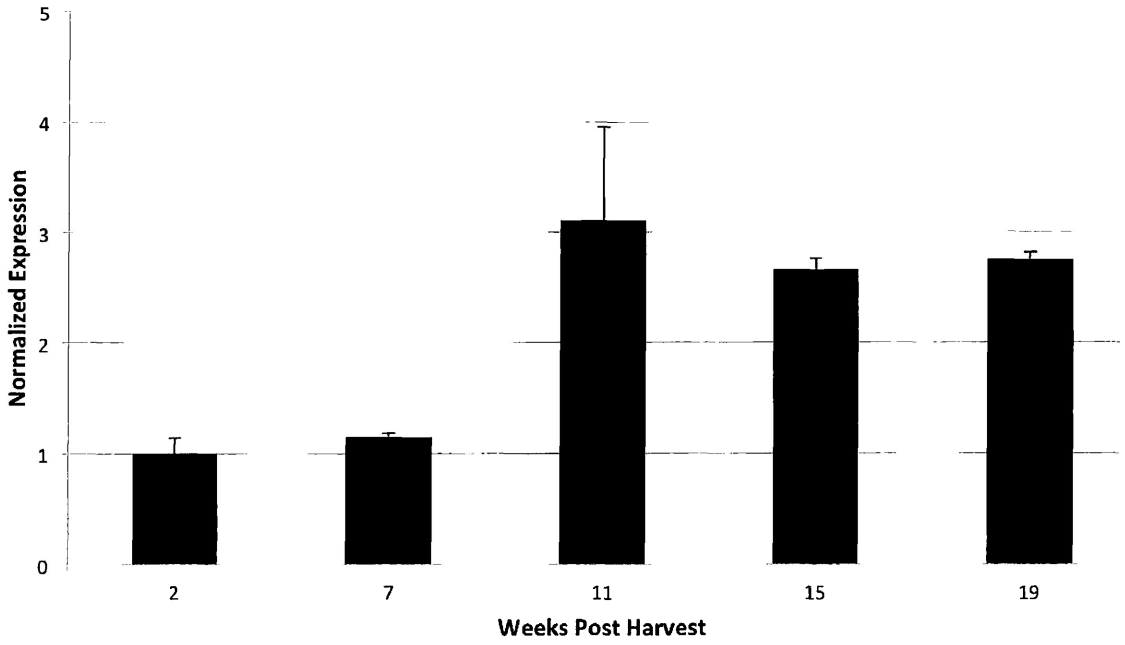

Figure 7: Normalized expression of *STM* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM), ($p<0.05$), n=7.

3.2.4 PICKLE

This expression data shows a slight increase in *PKL* expression at 11 weeks post-harvest compared to the dormant control meristems (Figure 8). After 11 weeks the expression of *PKL* drops to significantly lower levels relative to the dormant meristem. There is then a slight increase in *PKL* expression at the latest date tested, 19 weeks post-harvest, however the expression at this time is still lower then that seen in the dormant meristem. The overall general expression pattern of this gene is a decrease in expression as the meristem emerges from dormancy.

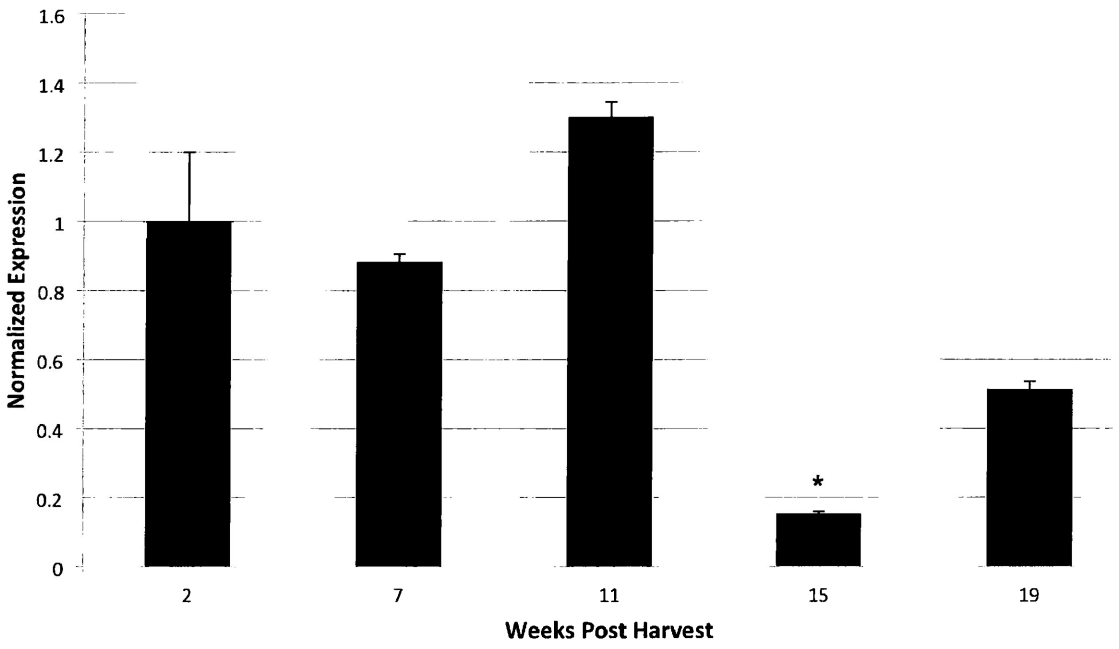

Figure 8: Normalized expression of *PKL* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM). Asterisks represent significant differences between the sample of interest and the control sample, ($p<0.05$), n=7.

3.2.5 ABSCISIC ACID INSENSITIVE 5

The highest expression of *ABI5* is in the dormant control meristems (Figure 9). There is a slight decrease in the expression of *ABI5* at 7 and 11 weeks post-harvest. At 15 and 19 weeks post-harvest, there is significant decline in *ABI5* expression with significantly lower expression then that seen in the control meristems. The pattern of *ABI5* expression shows a decrease in mRNA present in the meristems as they emerge from dormancy.

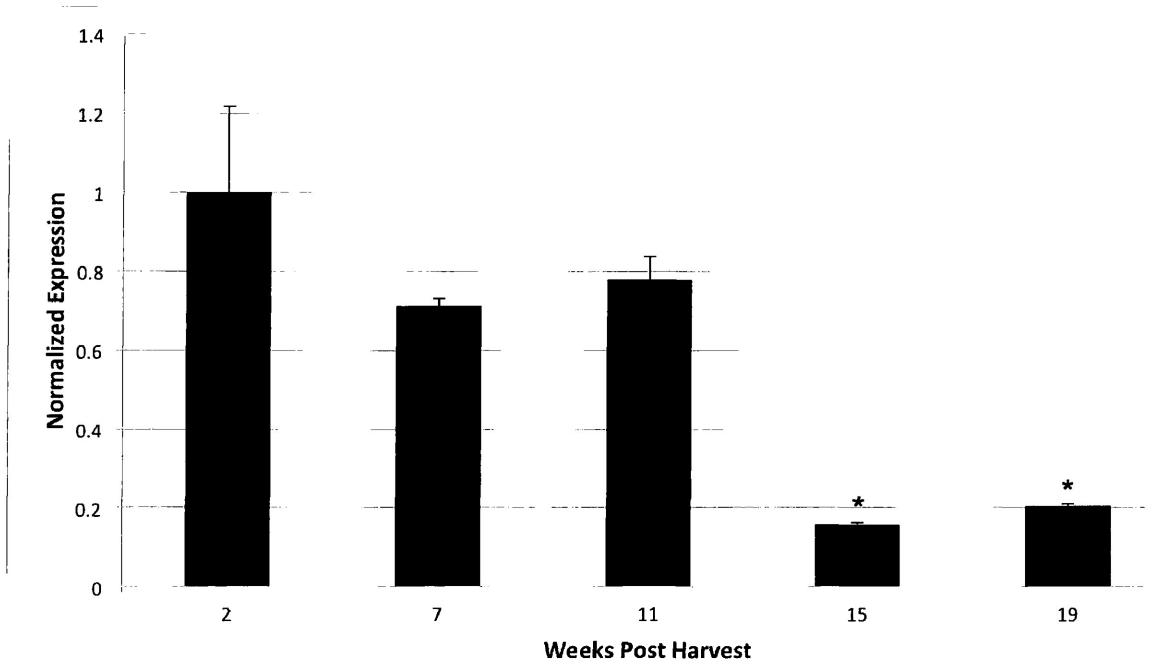

Figure 9: Normalized expression of *ABI5* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM). Asterisks represent significant differences between the sample of interest and the control sample ($p<0.05$), n=7.

3.2.6 DEHYDRIN

The highest expression of *DHN* is observed in the dormant control meristems (Figure 10). The meristems harvested after this show lower levels of *DHN* expression. At 7 and 11 weeks post-harvest there are slightly lower levels of *DHN* expression when compared to the control point. Later on, at 15 and 19 weeks post-harvest, the expression of *DHN* is significantly lower than its expression in the dormant meristems. The general trend observed in the expression of *DHN* is decreasing expression as dormancy emergence from dormancy progresses.

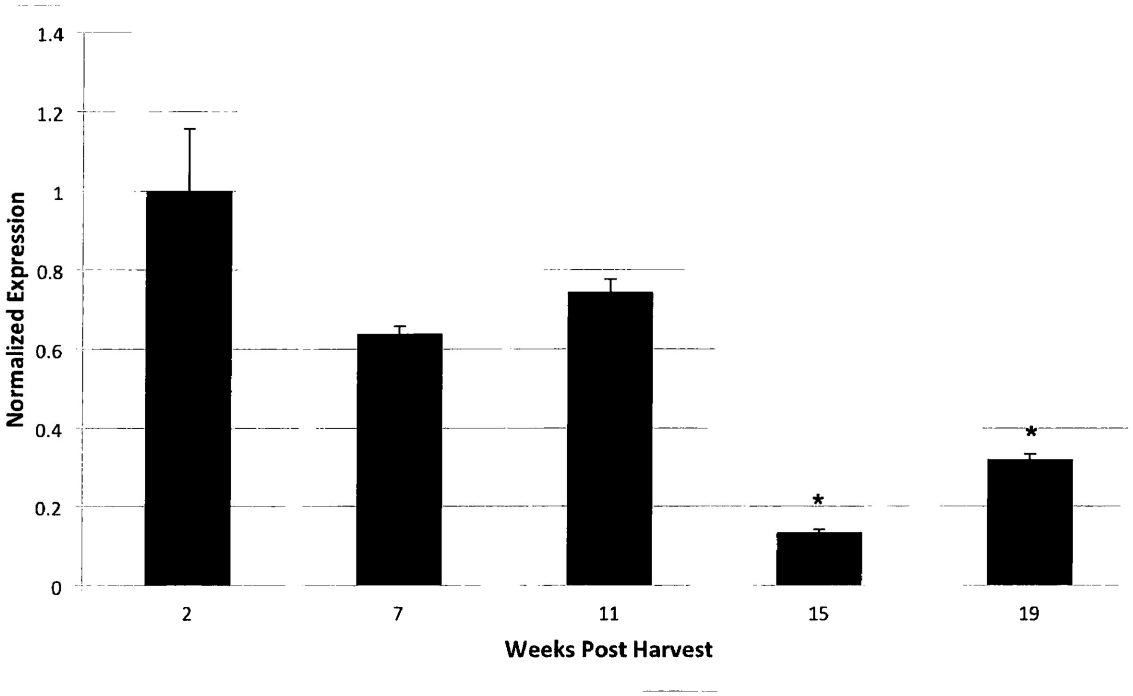

Figure 10: Normalized expression of *DHN* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM). Asterisks represent significant differences between the sample of interest and the control sample ($p<0.05$), n=7.

3.2.7 CUPSHAPED COTYLEDON1

The highest level of *CUC1* expression is seen at the latest timepoint analyzed, 19 weeks post-harvest (Figure 11). There is no discernible trend in the *CUC1* expression data, rather the expression of the gene seems to fluctuate during meristem dormancy emergence. During the progression from the dormant control to non-dormant meristems, expression drops at 7 weeks post-harvest then rises again at 11 weeks post-harvest almost to the levels present in the dormant meristems. At 15 weeks post-harvest, there is significantly lower expression of *CUC1* compared to the dormant control. The latest timepoint shows a spike in *CUC1* expression with nearly a 1.8 fold increase in expression compared to the control.

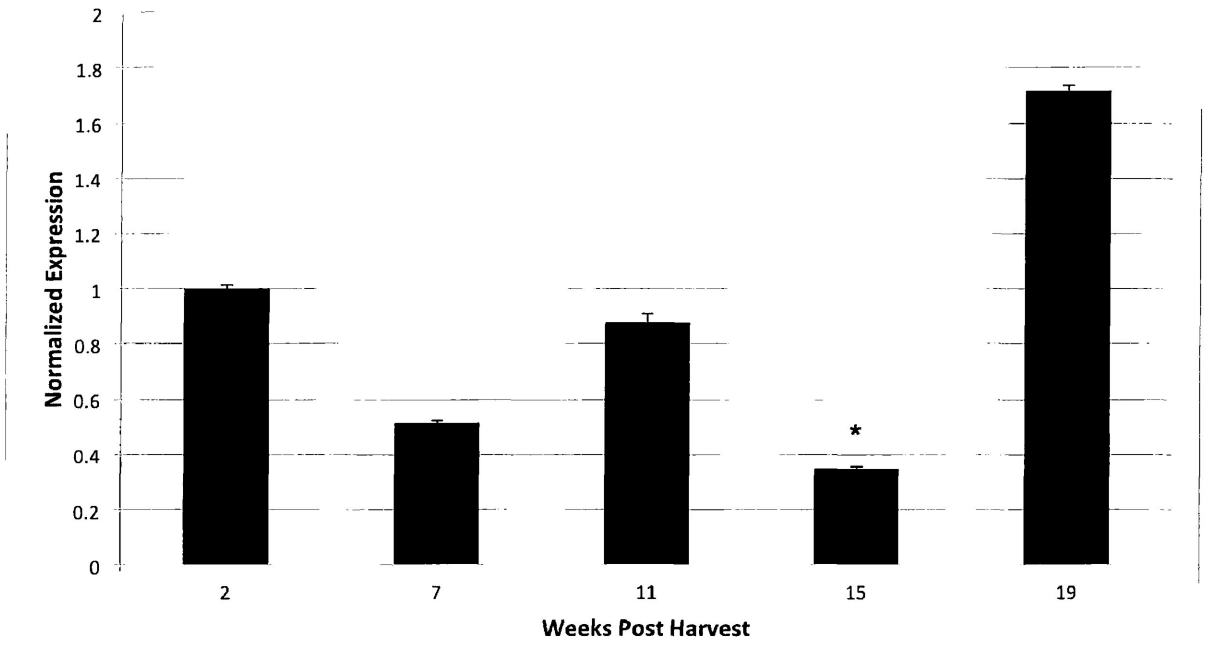

Figure 11: Normalized expression of *CUC1* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM). Asterisks represent significant differences between the sample of interest and the control sample ($p<0.05$), n=7.

3.3 Suppression Subtractive Hybridization

The qPCR gene expression results presented above show a large spike in gene expression of *WUS* and *STM* relatively early on during emergence from dormancy, specifically after around 7 to 11 weeks of microtuber storage. This suggests that the meristem has emerged from dormancy at this point initiated outgrowth of the meristem. A new question presented by this information is the identity of the genes involved in the activation of these transcription factors allowing for the growth of the meristem. This was investigated by construction of a subtractive cDNA library using suppression subtractive hybridization. Two libraries were constructed, one based on differentially expressed genes between the dormant 2 week post-harvest meristems and the 7 week post-harvest

meristem, and a second based on differences in the dormant 2 week post-harvest meristems and the 11 week post-harvest meristems. The results were compared and pooled to show some of the more interesting genes found from this analysis in Table 4.

Table 4: Gene identities of the sequences returned from the SSH. Sequence ID is the identification number of the clones form the SSH process. The E-value, Gene ID, and Gene Accession Number are the results of the homologous sequence search using the NCBI BLAST.

Sequence ID	Gene ID	Gene Description	E-Value	Gene Accession Number
2A-CAN-P-2-T7-B2-T7_B02	V-type proton ATPase proteolipid subunit c4 [*Arabidopsis thaliana*]	Role in plant development and involved with signaling networks controlling the plants life cycle. The plasma membrane proton pump ATPase (H+-ATPase) plays a major role in the activation of ion and nutrient transport and is suggested to be involved in several physiological processes, such as cell expansion and salt tolerance (Padmanaban, *et al.*, 2004).	1.806E-26	GI:9652288
2A-CAN-P-2-T7-B7-T7_B07	cyc07-like protein [*Solanum tuberosum*]	Cell cycle dependent gene, expressed specifically expressed in the S-phase of the cell cycle. Thought to play a role in cell proliferation in higher plants. Gene is conserved in plants and animals as well as yeast. The gene is dependent of DNA replication in the dividing cells, therefore not expressed when DNA replication is stopped. AA's and expression is similar to histone coding genes. It is a meristem specific gene (Ito, *et al.*, 1991; Lepetit, *et al.*, 1992).	1.299E-53	GI: 82400151
2A-CAN-P-2-T7-C10-T7_C10	Auxin-responsive protein IAA13, putative [*Ricinus communis*]	IAA proteins are short lived nuclear proteins that repress the expression of auxin responsive genes. Repression is thought to occur through the IAA proteins dimerizing with auxin response factor (ARF) transcriptional activators that reside on auxin-responsive promoter elements (Weijers, *et al.*, 2005).	5.949E-72	GI:223534102

2A-CAN-P-2-T7-A3-T7_A03	molecular chaperone Hsp90-2 [*Solanum lycopersicum*]	Heat shock protein 90 is molecular chaperone that is responsible for activating a variety of signalling proteins. It is able to under go conformational changes through ATPase activation, as well as interacting with other chaperones to give it a wide range of target proteins (Pearl and Prodromou, 2006).	2.278E-47	GI:170456
2A-CAN-P-2-T7-C3-T7_C03	bypass1 [*Nicotiana benthamiana*]	Involved in root to shoot signalling. Possibly involved in a signaling pathway for communicating between the root and the shoot. Bps1 mutant shoots induce growth arrest in shoots (Van Norman, *et al.*, 2011).	6.429E-85	GI: 160346972
2A-CAN-P-2-T7-D3-T7_D03	transcription factor DREB2a [*Nicotiana benthamiana*]	Involved in cold and drought responsive gene expression. It is a DRE binding protein and activates downstream genes driven by the DRE/CRT sequence. DREB2 is thought to be involved in drought response while DREB1 is involved with cold response. DREB2 has a conserved ERF/AP2 DNA binding domain. DREB2a found to have a C-terminal transcriptional regulating domain. DREB2a up regulation shows growth retardation in *Arabidopsis* (Yamaguchi-Shinozaki and Shinozaki, 1994; Maruyama, *et al.*, 2004).	2.242E-66	GI:317448462
2A-CAN-P-2-T7-F10-T7_F10	serine/threonine protein kinase-like [*Solanum tuberosum*]	SRK are a type of receptor protein that is thought to mediate many processes in the plant including cell growth and differentiation. It has been suggested that they function in the perception of self and nonself between pollen and stigma (Stein and Nasrallah, 1993).	4.795E-156	GI:81075764

2A-CAN-P-2-T7-E6-T7_E06	putative MADS-domain transcription factor MpMADS9 [*Magnolia praecocissima*]	MADS-box family of TF found in floral development. They assemble together to form multimeric organ specific proteins. Found to be crucial in the switch from vegetative growth to inflorescence and floral formation in the meristem. Function in meristem as an organ differentiation TF (Parenicova, *et al.*, 2003).	7.549E-38	GI:16549073
2A-CAN-P-2-T7-F7-T7_F07	Upstream activation factor subunit UAF30, putative [*Ricinus communis*]	Upstream activating factor (UAF) is a multisubunit complex that functions in the activation of ribosomal DNA (rDNA) transcription by RNA polymerase I (Pol I). It also silences the expression of polymerase II. Cells lacking the UAF30 subunit of UAF reduce the rRNA synthesis rate (Siddiqi, *et al.*, 2001).	9.8E-63	GI:223538407
2A-CAN-P-2-T7-G5-T7_G05	short-chain dehydrogenase/reductase [*Populus tremula*]	SDR enzymes have critical roles in lipid, amino acid, carbohydrate, cofactor, hormone and xenobiotic metabolism as well as in redox sensor mechanisms. The conserved fold and nucleotide binding emphasize the role of SDRs as scaffolds for an NAD(P)(H) redox sensor system, of importance to control metabolic routes, transcription and signalling (Kavanagh, *et al.*, 2008).	2.754E-126	GI:193795660
2A-CAN-P-2-T7-F9-T7_F09	RUB1-conjugating enzyme-like protein [*Solanum tuberosum*]	Protein is implicated in auxin response. Involved in the posttranslational modifications to other proteins in auxin response pathways. Act by conjugating the ubiquitin like protein RUB to other proteins (Wilkinson, 2000).	7.118E-121	GI:82623385
2A-CAN-P-2-T7-H11-T7_H11	WD-repeat protein, putative [*Ricinus communis*]	Protein utilized in protein-protein interactions, can act as a scaffolding unit for proteins to bind to. May also have an enzymatic domain (van Nocker and Ludwig, 2003).	2.135E-131	GI:223536868

2A-CAN-P-2-T7-G2-T7_G02	MAP kinase [*Arabidopsis thaliana*]	MAP kinases act in phosphorylating signal transduction cascades. They are a specific type of serine/threonine protein kinase. This type of signalling is used in cellular responses including cell division, differentiation, and stress response. It is suggested that the some methods of hormone signalling use a MAPK signal transduction pathway (Mishra, *et al.*, 2006)	1.571E-59	GI:2191126
1C-CAN-P-1T7-C4-T7_D10	CBL-interacting protein kinase [*Solanum chacoense*]	Calcineurin B-like proteins (CBL) and CBL-interacting protein kinases (CIPK) mediate plant responses to external stresses. They play a role in the calcium signalling pathway that responds to environmental stimuli (Kolukisaoglu, *et al.*, 2004).	1.48E-95	GI:329025151
1C-CAN-P-1T7-E5-T7_F11	receptor-like cytoplasmic protein kinase 1 [*Capsicum annuum*]	A class of transmembrane spanning receptor protein. They have been implemented in plant processes including meristem development controlled by CLV1 (Shiu and Bleecker, 2001).	2.49E-64	GI:304442675
2A-LIU-P-3-2T7-B9-T7_B09	WRKY transcription factor 1 [*Solanum lycopersicum*]	WRKY proteins are a type of zinc finger transcription factor. They are involved with many plant processes including defense against biotic and abiotic stressors, drought, heat and cold response, senescence, dormancy, plant growth, and metabolic pathways (Zhang and Wang, 2005).	1.681E-23	GI:224041531

2A-LIU-P-3-2T7-C7-T7_C07	notchless-like protein [*Solanum chacoense*]	Notchless is a WD-repeat protein involved in the notch signaling pathway. In plants, it plays a role in the biogenesis of the 60S ribosomal subunit, important in proper plant growth and development (Chantha and Matton, 2007).	1.178E-58	GI:40557600
2A-LIU-P-3-2T7-F7-T7_F07	ACRE 276-like protein [*Solanum tuberosum*]	Involved in disease resistance in tomato and tobacco. ACRE276 encode U-box proteins that function as ubiquitin E3 ligases targeting proteins for degradation by the 26S proteasome (Gonzalez-Lamothe, *et al.*, 2006).	2.818E-121	GI:118490014
3A-CAN-P-1T7-E10-T7_E04	NAC-like transcription factor [*Arachis hypogaea*]	Involved in xylem differentiation. A number of NAC domain transcription factors from several plant species play critical roles in diverse processes in addition to xylem cell specification, such as the establishment of the shoot apical meristem, lateral root formation, flowering time, senescence, abiotic stress responses, and defense responses (Olsen, *et al.*, 2005).	5.97E-96	GI:1944131
3A-CAN-P-1T7-A9-T7_A03	putative protein kinase ADK1 [*Oryza sativa* Japonica Group]	Acts upstream of serine/threonine kinases, phosphorylating them. Therefore, part of signalling involved in regulation of cellular growth and development (Ali, *et al.*, 1994).	7.07E-122	GI:50252390
2A-CAN-P-2-T7-C7-T7_C07	GATA transcription factor 15 [*Arabidopsis thaliana*]	GATA transcription factors such as HAN are involved in meristem development. It is a zinc-finger containing transcription factor. This GATA transcription factor is involved in cell proliferation and differentiation (Zhao, *et al.*, 2004).	4.337E-24	GI:71660789

From the genes described in Table 4, six were selected for further characterization using qPCR. These genes include the cyc07-like protein, bypass1, transcription factor DREB2a, RUB1-conjugating enzyme like protein, WD-repeat protein, and the MADS-domain transcription factor. These genes were selected based on the likelihood of their identification via BLAST characterized by the E-value, and their prevalence in the SSH results. The results from the qPCR are described in the following section. However, the MADS-domain transcription factor did not yield useable results due to the fluorescence levels falling outside the range of efficient quantification. The data from the MADS transcription factor can be found in Appendix A 7.2.

3.3.2 BYPASS1

The 2 week post-harvest timepoint represents the dormant control meristems and the other sample points are expression relative to this time. The expression of *BPS1* decreases as the meristem is emerging from dormancy (Figure 12). By weeks 15 and 19 there is a 50% decrease in the expression of *BPS1* and these points represent significantly lower expression compared to the control point.

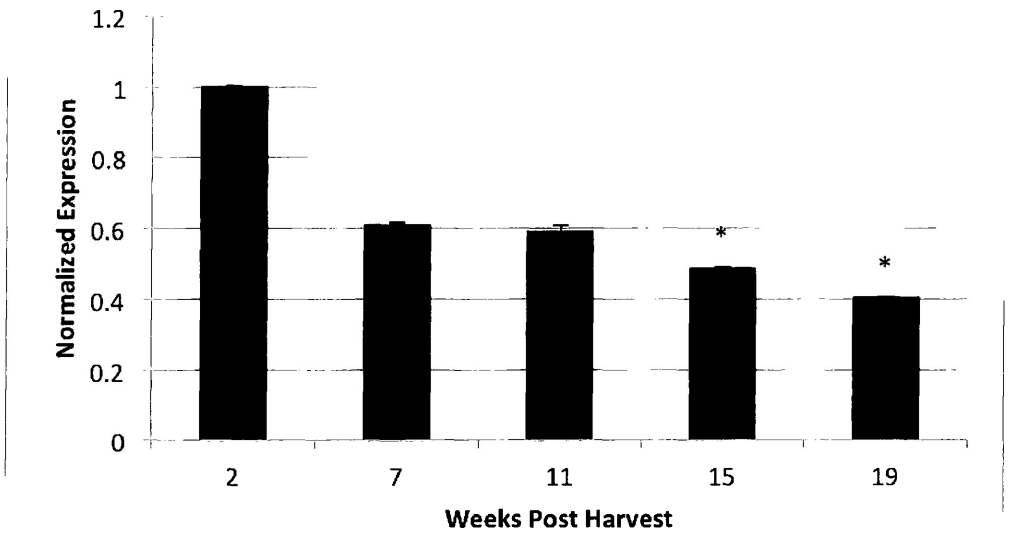

Figure 12: Normalized expression of *BPS1* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM). Asterisks represent significant differences between the sample of interest and the control sample ($p<0.05$), n=6.

3.3.3 Cell Cycle-Dependent Gene

There are two separate data series representing *cyc07*, they represent the expression of two different sequences (Figure 13). From the SSH there were two sequences that yielded the same BLAST result, since the two sequences follow the same expression pattern and there are no significant differences ($p<0.05$) between the two, it is likely that they are the same gene. Overall, the expression of *cyc07* decreases as the tuber meristems emerge from dormancy (Figure 13). There is an initial drop during the early stages of emergence from dormancy. The subsequent timepoints show a similar level of expression. The lowest expression of *cyc07* occurs at 15 weeks post-harvest, where the expression is significantly lower than that in the dormant control meristems.

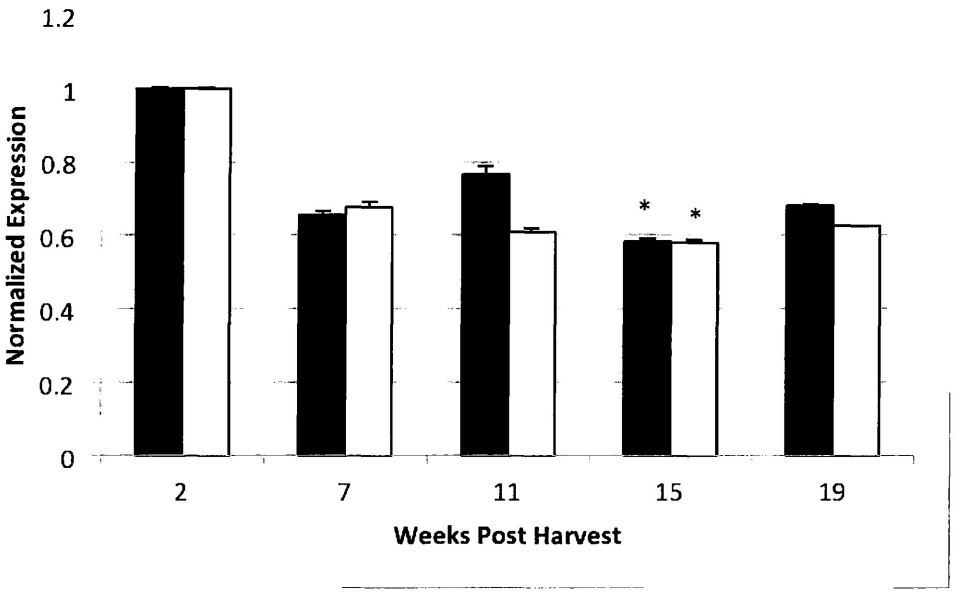

Figure 13: Normalized expression of *cyc07* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM). Asterisks represent significant differences between the sample of interest and the control sample ($p<0.05$), n=6. The black series represents a portion of the cyc07 gene sequence designated "A12" and the white series represents a portion of the cyc07 sequence designated "B07".

3.3.4 Related to Ubiquitin1-Conjugating Enzyme

There were two separate sequences identified by SSH as being part of *RCE1*, the expression of both of these sequences is represented (Figure 14). The similar expression patterns and lack of significant differences ($p<0.05$) between expression of the two sequences suggests that they are indeed expressed at the same time, and likely part of the same gene. The overall expression of *RCE1* decreases as emergence from dormancy progresses (Figure 14). There is a slight increase in the expression at 11 weeks post-harvest compared to the control, however not by much. The expression continues to decrease in the later weeks post-harvest and the ~50% reduction seen at 19 weeks post-

harvest is significantly different then the normalized expression of the dormant meristems.

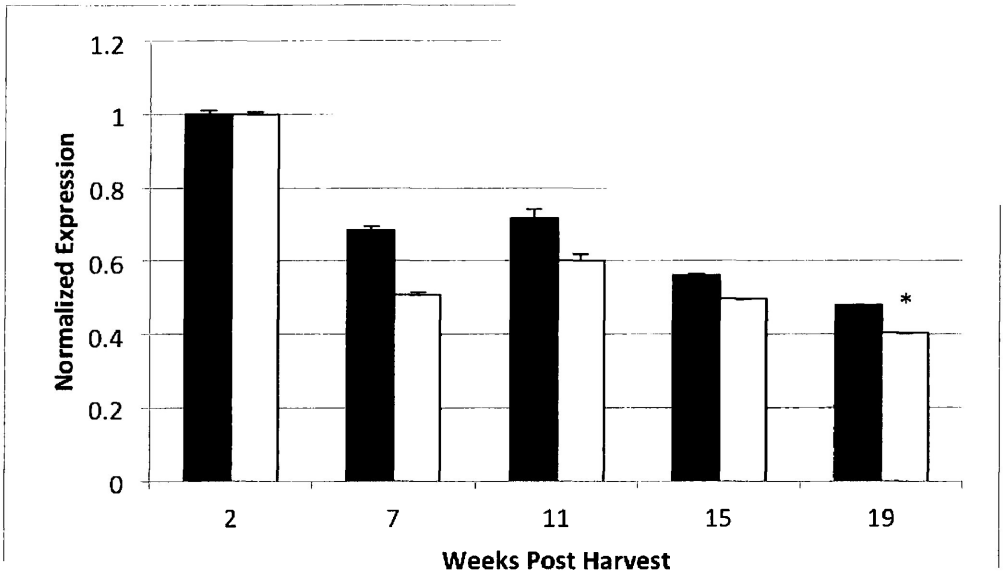

Figure 14: Normalized expression of *RCE1* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM). Asterisks represent significant differences between the sample of interest and the control sample ($p<0.05$), n=6. The black data series represents the RCE1 sequences designated "C10", the white data series represents the RCE1 sequences designated "F09".

3.3.5 Dehydration Responsive Element Binding 2

The expression of *DREB2a* fluctuates in the initial period of dormancy emergence (Figure 15). It drops slightly at 7 weeks post-harvest and then recovers to levels similar to that present in the dormant meristem again at 11 weeks. After the 11 week mark, the expression decreases steadily until the final 19 week timepoint, where the expression is significantly lower than that seen in the dormant control meristems (Figure 15).

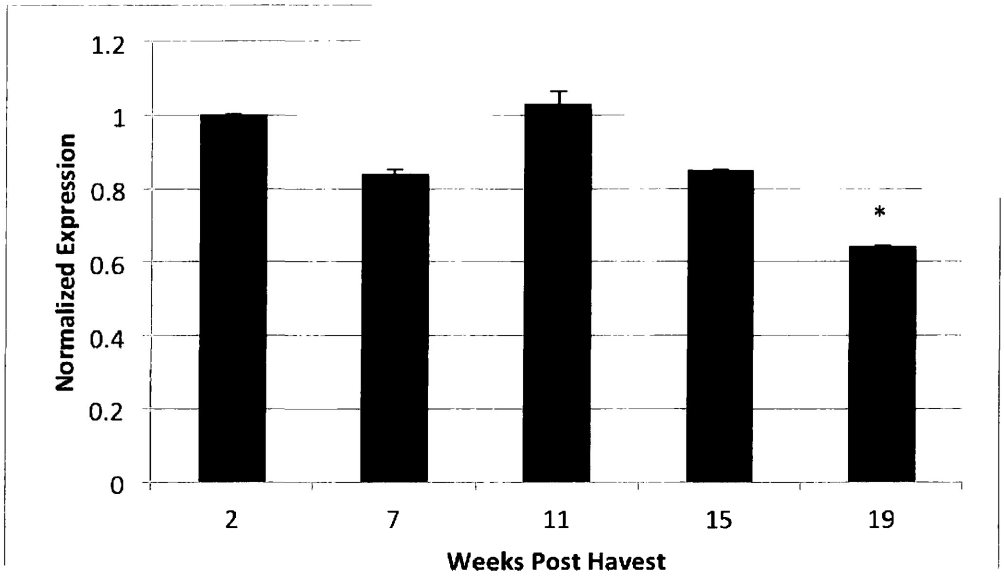

Figure 15: Normalized expression of *DREB2a* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM). Asterisks represent significant differences between the sample of interest and the control sample ($p<0.05$), n=6.

3.3.6 WD-Repeat

The expression of *WDR* is relatively constant until week 11 when there is a slight increase in expression (Figure 16). The expression drops slightly at 15 weeks post-harvest, then recovers to the slightly higher level at week 19 again. The similarities in the expression of *WDR* over the 19 week period showed no significant differences.

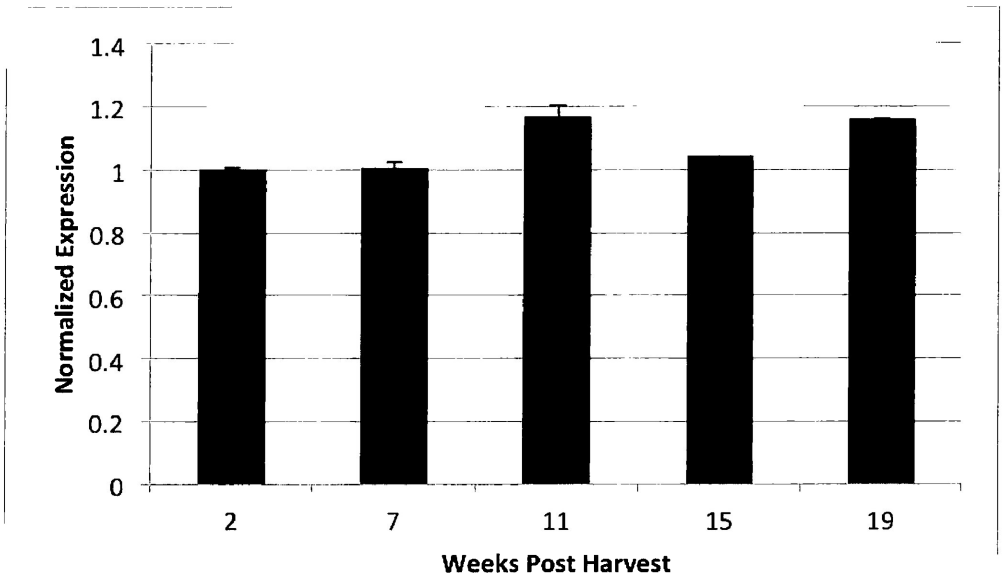

Figure 16: Normalized expression of *WDR* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM) ($p<0.05$), n=6.

4.0 DISCUSSION

4.1 Meristem Growth

Differences in meristem length, compared to the dormant control, are noticable as early as 7 weeks post-harvest (Figure 5), and growth continues thereafter. The early differences in meristem length indicate that the meristem has emerged from dormancy after 7 weeks and initiated the cell cycle. To further verify the activation of the cell cycle in the emerging meristems, ^{3}H thymidine was applied to the meristems and analyzed for uptake. If the cells are dividing, DNA replication is occurring, and therefore should utilize the applied ^{3}H thymidine in the newly formed DNA. An increase in the amount of ^{3}H thymidine is seen in the meristems as early as 7 weeks post-harvest (Figure 5). This indicates that the cells have started to grow and divide. The pattern seen in the ^{3}H % incorporation is less pronounced then the meristem length (Figure 5). This may be due to the fact that the ^{3}H incorporation only measures the cell replication in the meristem during the 4 hour incubation period. Since the actively growing and dividing cells are located in the meristem tip, it is likely that the ^{3}H incorporation would remain similar for all meristems post dormancy independent of the length. However, thymidine incorporation is greater with an increase in meristem length. This may be attributed to increasing growth rates in the meristem as time progresses. It is also possible that the amount of residual ^{3}H thymidine remaining after the wash process may be higher in the larger meristems due to their higher surface area.

4.2 Previously Identified Genes

4.2.1 WUS

The function of the WUS gene has been previously studied in *Arabidopsis* and is a known transcription factor that acts through chromatin remodelling in the meristem, to promote stem cell activity (Barton, 2010). The expression pattern of WUS in potato SAMs emerging from endodormancy (Figure 6) shows a large peak in expression at 11 weeks post-harvest. This suggests that the meristem is growing and also that the meristem has organized and new cells began propagating from the stem cell pool located at its tip. The down regulation of WUS after week 11 (Figure 6) may be due to the feedback loop existing with its antagonistic gene CLAVATA3 (Schoof, *et al.*, 2000; Muller, *et al.*, 2006; Fletcher, *et al.*, 1999; Brand, *et al.*, 2001). While quantification of the expression of CLV3 was attempted, the lack of sequence data available for this gene beyond that in *Arabidopsis* (Sharma, *et al.*, 2003) meant that qPCR was not successful (data not shown). The need for maintenance of the stem cell population also suggests that the cells in the meristem are metabolically active and growing once again after 7 weeks. This data correlates with meristem growth occurring as soon as 7 weeks post-harvest, as seen in the length and ^{3}H incorporation data.

4.2.2 STM

SHOOTMERISTEMLESS is a type of class 1 KNOX homeodomain containing transcription factor that is also involved in plant growth via cytokinin synthesis, and stemcell differentiation (Barton, 2010). The expression of STM over the 19 week period of study is similar to the expression of WUS in that they both have highest levels of expression at 11 weeks post-harvest (Figure 7). The up-regulation of STM at this time

may be due to the positive feedback loop existing between STM and cytokinin (Rupp, *et al.*, 1999). It is also possible that its expression is influenced by the growth of the stem cell pool, where it is needed to aid in proper development of these cells. It may be that the positive feedback loop existing between STM and cytokinin is in place for it to perceive the expression of WUS (which is involved in cytokinin synthesis) and, itself be expressed accordingly. Previous studies by Hartmann *et al.* (2011) showed an increase in endogenous cytokinin levels is associated with dormancy release in potato tubers (Hartmann, *et al.*, 2011), further enforcing this possibility. The expression of STM at these early timepoints post dormancy suggests that the meristem has begun to organize itself and initiate outgrowth.

4.2.3 PKL

PKL encodes a transcription factor that is involved in chromatin remodelling and is thought to negatively regulate transcription (Woodage, *et al.*, 1997). It may be involved in the gibberellic acid signalling pathway to suppress embryonic traits during germination (Ogas, *et al.*, 1999). Additionally, a *PKL* gene was identified that is thought to be involved in the repression of genes that promote meristematic activity (Sang, *et al.*, 2009). Therefore, in seeds it may play a role in germination, and in meristems it may play a role in development or function. From the gene expression data obtained in the study, the expression of the PKL gene is higher in the early weeks post dormancy and then decreases thereafter (Figure 8). It may be that the PKL transcription factor is highly expressed in the early stages to help with organizing the switch from cell arrest to active growth by suppressing certain genes until they are needed. The lower expression of PKL

later on may allow the expression of genes needed for growth that it was once repressing during dormancy, such as the KNOX genes (Sang, *et al.*, 2009).

4.2.4 ABI5

ABI5 is essential to ABA-dependent post germination growth arrest (Finkelstein, 1994). The purpose of this in the plant is to survive stress conditions that when present will be perceived via the ABA responsive pathway leading to ABI5 activation to prevent cell growth (Lopez-Molina, *et al.*, 2002). The expression pattern of ABI5 over the 19 week period of study (Figure 9) may be explained by considering that stress response and dormancy are both characterized by cell growth arrest. Dormancy itself may be considered a stress response mechanism, so it is possible that the same genes are utilized in both processes. ABI5 is expressed at the highest level in the dormant meristem tissue and its expression decreases as time progresses and dormancy emergence occurs (Figure 9). The down regulation of ABI5 is predicted to allow continued growth of the meristem, since the gene is known to function in dormancy and growth suppression in seeds (Lopez-Molina, *et al.*, 2002; Bensmihen, *et al.*, 2002).

4.2.5 DHN

DHN genes encode proteins with a conserved amino acid sequence that are thought to be support proteins for protection from environmental stressors. They are not thought to have any enzymatic function and may act to stabilize other proteins by interacting with hydrophobic surfaces (Campbell and Close, 1997). The results from the expression analysis (Figure 10) are resonable since DHN is expressed under stress conditions (Borovskii, *et al.*, 2002). Plant response to stress often involves cessation of cell growth and waiting for improved growth conditions. Dormancy is a period of growth

arrest so, it would be expected that stress response genes, enforcing growth arrest, would be expressed during this time. Over the 19 week period of study, the highest expression of DHN was seen in the dormant meristems (Figure 10). As time progressed and tubers emerged from dormancy, the expression of DHN decreased along with other stress response and dormancy enforcing genes including ABI5, and DREB2a.

4.2.6 CUC1

CUC1 is a DNA binding transcription factor that is thought to be involved in SAM formation and development (Aida, *et al.*, 1997). It is also implicated to act upstream of STM and be required for its expression (Takada, *et al.*, 2001). From the expression analysis (Figure 11), there is no apparent pattern in CUC1 expression during dormancy emergence. Furthermore, the expression of CUC1 is not correlated with STM expression. While CUC1 is able to promote STM expression, it may not be required. It is known that CUC1 is redundant with CUC2 (Aida, *et al.*, 1997) so it is possible that CUC2 may be acting to up-regulate STM in some manner. The only timepoint where CUC1 expression is higher then that of the dormant tissue is at 19 weeks post-harvest (Figure 11). Unfortunately, this was the last point in this study so expression patterns past this point are unknown. While CUC1 is predicted to be expressed highly after endodormancy break (Takada, *et al.*, 2001) this was not the case, unlike the expression patterns of STM (Figure 7) and WUS (Figure 6).

4.3 SSH Identified Genes

The subtractive cDNA library constructed in this study was meant to identify genes upregulated in tissues emerging from dormancy, 7- and 11-weeks post harvest. Therefore the gene expression data from the qPCR targeting these genes would be

expected to show higher expression at later points in time. Unfortunately, this was not the case. The genes from the SSH that were further characterized showed decreased gene expression as time progressed post dormancy and nearly all of them showed highest expression in the dormant tissue. The genes that were identified are likely dormancy enforcing genes rather than dormancy breaking genes. Characterization of these gene expression patterns as well as information from previous research, help give these genes a possible role in dormancy maintenance.

4.3.1 BPS1

The BPS1 gene has been found to negatively regulate the production of a mobile signalling molecule known as the BPS1 signal which is able to affect normal shoot and root development (Van Norman, *et al.*, 2011). In this study, it was found that the expression of the BPS1 gene is higher in dormant meristem tissue then that of the actively growing meristem (Figure 12). This is opposite to what has been previously described, where lower levels of BPS1 expression were associated with absence of meristem growth (Van Norman, *et al.*, 2004). It may be that the BPS1 gene is involved in much more then simply the production of the BPS1 signal. It has been shown that low BPS1 levels limit development presumably through the over production of the BPS1 signal (Van Norman, *et al.* 2011). However, in this study, the opposite was observed.

While *bps1* mutants produce shoots with stunted growth, a normal cell division pattern is retained (Van Norman, *et al.*, 2011), suggesting that it is the cell cycle that is being prevented rather then functional development. It is possible that this signalling system is in place to control growth based on available nutrients. The growth of the meristem from the tuber is driven by energy from the starch reserves in the potato. As it

continues to grow, it may be exhausting nutrients in its immediate vicinity, requiring it to slow its growth in order to maintain enough energy to survive until it is able to undergo autotrophic growth. The BPS1 gene has only been recently described (Van Norman, *et al.*, 2004) and its full function is still not completely understood. In the context of this study however, the expression of BPS1 seems to be downregulated when the tuber meristems emerge from dormancy (Figure 12). This suggests that the gene may be involved in regulating or maintaining the dormant state of the tuber.

4.3.2 Cyc07

The expression of the cyc07 like gene identified in the SSH also decreases with time of post harvest storage. The cyc07 gene has been described previously as a cell cycle dependent gene that is expressed only during the cell cycle (McKinney and Heintz, 1991). However, in stored potato microtubers, the expression of the identified cyc07 like gene was downregulated in growing meristems and was most highly expressed in the dormant tissue (Figure 13). It may be that the gene amplified has similar sequence to the cyc07 gene but has different regulatory properties. There are other examples of this in plant growth and development studies. For example, ABI5 that has a structural homolog called EEL that acts antagonistically to ABI5 depending on the plant developmental stage (Bensmihen, *et al.*, 2002). The cyc07-"like" gene we examined may be expressed in the dormant meristem to prevent the normal function of a cyc07 or any cell cycle dependent gene in order to maintain the dormant state.

4.3.3 RCE1

The RUB1 conjugating enzyme attaches RUB to an E3 complex for binding of ubiquitin to a target protein (Wilkinson, 2000). Previous studies have found a link

between this complex and auxin signalling that affected vegetative growth (Bostick, *et al.*, 2004). The expression of the RCE1 gene in this study decreased as the meristem emerged from dormancy (Figure 14). This suggests that RCE1 may be are required for maintaining the dormant state of the meristem. RCE1 is part of SCF^{TIR1} complex which may be involved in degradation of auxin response proteins that regulate cell growth (Gray and Estelle, 2000). It is possible that RCE1 is upregulated in the dormant tissue as part of this complex to help suppress cell growth. The decrease in expression (Figure 14) may be due to the down regulation of this pathway in actively growing SAMs. There are other indications that the expression pattern of the RCE1 gene is suggestive of activation of the 26S proteasome in the dormant meristem. Some of the genes found in the SSH (Table 4) are involved in this pathway such as the ACRE 276-like protein (Gonzalez-Lamothe, *et al.*, 2006). It has also been found that ubiquitination can lead to conformational changes in the chromosome and ultimately to gene silencing(Liu, *et al.*, 2007). However, since the expression of RCE1 is highest in the dormant tissue (Figure 14), it is likely that it is involved in protein degradation rather than gene expression. If it were involved in gene expression, it would likely be upregulated in the post dormant phase, relative to dormant tuber SAMs.

4.3.4 DREB2a

The DRE binding transcription factors are known to be ABA independent and important in abiotic stress response (Yamaguchi-Shinozaki and Shinozaki, 1994). DREB2a is a TF that is involved with drought response in plants (Liu, *et al.*, 1998). In the early stages of post dormancy, there is a fluctuation in the expression of DREB2a, and later at 19 weeks post-harvest, there are significantly lower amounts of DREB2a present

(Figure 15). DREB2a undergoes post-translational modification, via phosphorylation, in order to function in drought response (Agarwal, *et al.*, 2007). In potato SAMs, when it is expressed after dormancy emergence, it may thus be in its inactive state. Considering what is known about DREB2a, it should be expressed in higher amounts in the dormant meristem since it is a stress responsive gene. As the meristem begins to grow, the environmental change would lead to downregulation of DREB2a expression. Previous studies have looked at several genes that are upregulated in response to DREB2a, which included the expected stress response genes but also molecular chaperones and other enzymes(Sakuma, *et al.*, 2006). The higher expression of DREB2a in the early post dormant tissue (11 weeks post-harvest) (Figure 15) may be to activate genes of this nature to aid in growth resumption.

4.3.5 WDR

WD repeat type proteins are involved in a number of cellular processes including signal transduction, chromatin modification, and transcriptional regulation to name a few (van Nocker and Ludwig, 2003). Since there is such a wide variety of these proteins, the gene expression pattern could increase or decrease depending on which WDR gene is being examined. While the expression of this WDR gene increased as dormancy emergence occurred (Figure 16), no significant differences were seen. It is difficult to link this gene to a certain process, because there is a large amount of metabolic change during dormancy emergence. However, it is possible that it is part of a complex involved in growth of the meristem. Since it is evenly expressed during the period of meristem growth post dormancy (Figure 16), it is suggested that it is likely involved with growth promotion rather than suppression. WDRs are known to be parts of chromatin

remodelling complexes and there are other chromatin remodelling genes involved with dormancy break such as WUS (Figure 6) that is described above (Barton, 2010). The highest expression of WUS was seen at 11 weeks post dormancy which is also when the highest expression of WDR occurs. It is possible that the WDR protein examined here is acting in chromatin remodelling and transcriptional regulation, potentially in the same pathway as WUS.

5.0 CONCLUSION

Many of the genes whose expression was examined here were identified from previous studies implicating these genes in other plant dormancy systems (e.g., *WUS*, *STM*, *ABI5*, *CUC1*, *PKL*, and *DHN*). The observed gene expression profiles are expected if considering their known roles in plant dormancy. Interestingly, the expression of *WUS* and *STM* (Figure 6 and 7) is greatly up regulated in the early stages of meristem growth. This suggests that the meristem has emerged from dormancy and is beginning to organize itself to grow and is confirmed by meristem growth (Figure 5) occurring as early as 7 weeks post-harvest. The next stage of the study was to construct a subtractive library between the control (dormant) meristems and the early emerging meristems (7 weeks post-harvest) to look for differences in novel gene expression at this early stage of growth (Table 4). However, there was an issue with the library construction where the genes sequenced were down regulated upon dormancy emergence rather then up regulated. This is seen in the qPCR expression analysis where the expression of the SSH identified genes are down regulated as growth resumption occurs (Figures 12-15). While this is unfortunate, it did provide further insight into the mechanisms at work in the dormant meristem. Identification of the *RCE1* gene suggests a possible role for protein degradation in dormancy maintenance. It may be targeting auxin response proteins for degradation to promote growth suppression (Bostick, *et al.*, 2004). DRE binding elements were also discovered to contribute to the dormant state of the meristem. Another interesting finding was the expression pattern of *BPS1*. This newly discovered gene is thought to be involved in root to shoot signalling through negatively regulating the production of the BPS1 signal, which in turn can negatively influence growth and development (Van Norman, *et al.*, 2011). The findings in this study are opposite to the

previous findings indicating that BPS1 may be involved with other plant developmental mechanisms. The information obtained through this study helps provide a better understanding of the molecular mechanisms at work during endodormancy maintenance and release. Expanding knowledge in this field may lead to better tuber growing and storage practices.

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7.0 APPENDIX A

7.1 qPCR Calculations

1. "**Relative Quantity**" is the expression value of a treatment in relation to a control sample.

$$\text{Relative Quantity} = \mathbf{E}^{(\text{"Ct control"} - \text{"Ct treatment"})}$$

Where: **E** is the primer efficiency and is calculated by → (% Efficiency x 0.01 + 1)

"Ct Control" is the average Ct value of the control sample

"Ct Treatment" is the average Ct value for the treatment

The % efficiency is calculated separately by creating a dilution series of template RNA and running the qPCR with the primers for the gene of interest. The concentrations of RNA used should encompass all possible Ct values that may occur in the experiment. Therefore, relative quantity is based on the primer efficiency to the power of the difference between the control and the treatment. The Relative Quantity needs to be determined for the gene of interest, and all the housekeeping genes used to normalize the data.

2. The Relative Quantity of the housekeeping genes can then be used to determine the "**Normalization Factor**" which is used to normalize the relative quantity of the gene of interest.

$$\text{Normalization Factor} = (\text{Relative Quantity}_{(HKG1)} \times \text{Relative Quantity}_{(HKG2)} \times \ldots \times \text{Relative Quantity}_{(HKGn)})^{1/n}$$

The normalization factor is the product of the housekeeping gene Relative Quantities determined in the first step to the power of 1/n (n= number of housekeeping genes being used).

3. Next the "**Normalized Expression**" can be calculated using the Relative Quantity of your gene of interest and the Normalization factor.

$$\text{Normalized Expression} = \frac{\text{Relative Quantity}_{(gene\ of\ interest)}}{\text{Normalization Factor}}$$

The normalized expression is the value that will be used to describe the gene expression.

4. Now that the gene expression has been quantified, the standard deviation needs to be determined. The "**SD of Relative Quantity**" needs to be determined first.

SD of Relative Quantity = (SD of Ct Values) × (Relative Quantity) × (Ln(**E**))

The SD of the Relative quantity is determined using the standard deviation of the original Ct values obtained (the ones that make up the average Ct in the relative quantity). It is multiplied by the Relative quantity determined in equation 1, and by the Ln of the **E** value determined. This is done for each individual gene of interest and for the housekeeping genes used to normalize the data.

5. Next the standard deviation of the normalization factor is determined, the "**SD Normalization Factor**". This value represents the standard deviation observed in the normalization factor that was calculated earlier.

$$\text{SD Normalization Factor} = \sqrt{\left(\frac{\text{SD Rel Quant}_{(ref\ gene\ 1)}}{\text{n} \times \text{Rel Quant}_{(ref\ gene\ 1)}}\right)^2 \times \left(\frac{\text{SD Rel Quant}_{(ref\ gene\ 2)}}{\text{n} \times \text{Rel Quant}_{(ref\ gene\ 2)}}\right)^2 \times \ldots \times \left(\frac{\text{SD Rel Quant}_{(ref\ gene\ n)}}{\text{n} \times \text{Rel Quant}_{(ref\ gene\ n)}}\right)^2}$$

The values needed to calculate the **SD Normalization Factor** are the SD Relative Quantities of the reference genes that were calculated earlier, and the Relative Quantity of the reference genes that was initially calculated. "n" in this equation represents the

number of reference (or housekeeping) genes used to normalize the gene expression. The **SD Normalization Factor** is needed to calculate the final standard deviation of the normalized gene expression.

6. Finally the standard deviation of the normalized expression is calculated and is labelled the "**SD Normalized Expression**". This value represents the standard deviation of the values used to calculate the mean normalized gene expression.

$$\text{SD Normalized Expression} = \textbf{Normalized Expression} \times \sqrt{\left(\frac{\text{SD Normalization Factor}}{\text{Normalization Factor}}\right)^2 \times \left(\frac{\text{SD Rel Quant}_{(gene\ of\ interest}}{\text{Rel Quant}_{(gene\ of\ interest}}\right)^2}$$

To calculate the **SD Normalized Expression** you need the SD Normalization factor that was previously calculated as well as the Normalization factor. This equation also incorporates the SD Relative Quantity for the gene of interest and the Relative Quantity of the gene of interest that was calculated earlier.

The important values to come out of this procedure are the **Normalized Expression** and the **SD Normalized Expression**. The normalized expression is the value that will be used to quantify and describe the gene expression data. This value has been normalized by both the primer efficiencies and also the quantity of housekeeping or reference genes present in each trial. Since the initial relative quantity calculation uses the difference between the control and the treatment Ct values, the normalized expression will be relative to control sample. If desired, the normalized expression of the control sample can be calculated as well. To do this, the initial relative quantity calculation for the control sample will be:

$$\text{Relative Quantity} = \mathbf{E}^{(\text{"Ct control"}-\text{"Ct control"})}$$

Therefore, the relative quantity will be **1**. A similar result will be seen when the relative quantity of the reference genes is calculated, therefore the normalized expression for the control will be **1**. However since there is variation among the values used to calculate these means, there will be variation in the standard deviation of the average control sample.

7.2 *MADS* Gene Expression Data

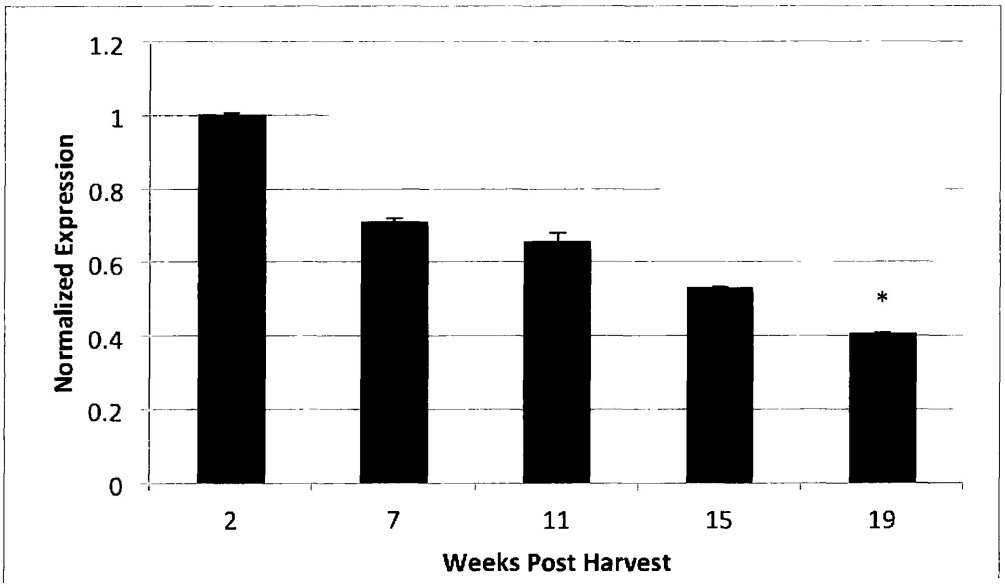

Figure 17: Normalized expression of *MADS* in microtuber meristems through dormancy emergence. Normalized expression is relative to the dormant control sample (2 weeks post-harvest). Error bars represent the standard error of the mean (SEM) ($p<0.05$), n=6.

7.3 Housekeeping Gene Expression used to Normalize qPCR

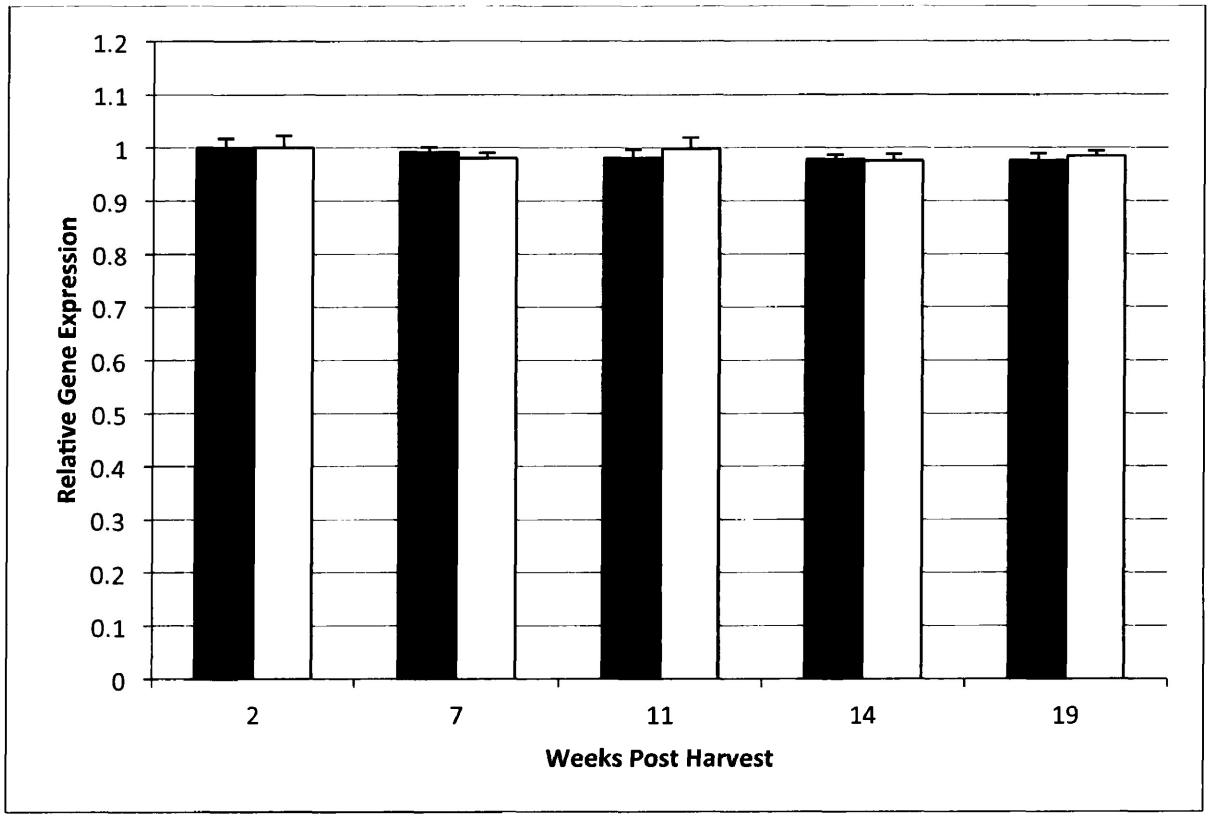

Figure 18: Normalized expression of the housekeeping genes used in the qPCR to normalize the results. The black data series represents the gene *EF1α,* the white series represents the gene *L2.*