Key players associated with tuberization in potato: potential candidates for genetic engineering

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Key players associated with tuberization in potato: potential candidates for genetic engineering

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ABSTRACT
Tuberization in potato (Solanum tuberosum L.) is a complex biological phenomenon which is affected by several environmental cues, genetic factors and plant nutrition. Understanding the regulation of tuber induction is essential to devise strategies to improve tuber yield and quality. It is well established that short-day photoperiods promote tuberization, whereas long days and high-temperatures inhibit or delay tuberization. Worldwide research on this complex biological process has yielded information on the important bio-molecules (proteins, RNAs, plant growth regulators) associated with the tuberization process in potato. Key proteins involved in the regulation of tuberization include StSP6A, POTH1, StBEL5, StPHYB, StCONSTANS, Sucrose transporter StSUT4, StSP5G, etc. Biomolecules that become transported from “source to sink” have also been suggested to be important signaling candidates regulating the tuberization process in potatoes. Four molecules, namely StSP6A protein, StBEL5 RNA, miR172 and GAs, have been found to be the main candidates acting as mobile signals for tuberization. These biomolecules can be manipulated (overexpressed/inhibited) for improving the tuberization in commercial varieties/cultivars of potato. In this review, information about the genes/proteins and their mechanism of action associated with the tuberization process is discussed.

INTRODUCTION
Presently, potato (Solanum tuberosum L.) is the world’s third most important crop (after wheat and rice) in terms of human consumption [1–3]. Tubers of the potato plant have been used as a primary nutrient and a carbohydrate source in many diets and is the basis for a variety of processed products throughout the world. Owing to climate change, potato production is expected to fall in the coming decades, predictions assert that global potato yields will decrease by 9–18% in most parts of the world [4]. The availability of new varieties less sensitive to these environmental cues is thus crucial in order to overcome the negative effects of global warming. Of various developmental phases, tuberization is the most sensitive stage in potato growth that limits its climate-associated geographical distribution and actual yield. Hence, an understanding of the tuberization process becomes even more important in the face of the changing global climate.

Potato is formed from an underground stem called a stolon through a process known as tuberization. The initiation of tubers involves a shift in growth of the stolon from extension growth to radial growth. The mechanism of tuberization has been the subject of considerable investigation by plant scientists in recent decades. However, the controlling factors involved in the tuberization process are not precisely clear. Several environmental factors, the most important of which are day length and temperature, are known to regulate the tuberization process in potato [5,6]. Besides environmental factors, phytohormones have been found to influence the tuberization process in potato [7,8]. The action of gibberellins (GAs) has been implicated in different aspects of potato tuber formation. Several studies have shown that the noninduced state in potato plants is correlated with high endogenous GA levels [9,10]. High levels of GA in the stolon tip has been found to favor elongation of stolon meristems, whereas decreasing levels of GA are required for the initiation of tuberization [11]. Implications of cytokinin in the creation of metabolic sinks have raised curiosity to study their role in tuberization. In general, cytokinin favors tuberization under tuber-inducing conditions in vitro, especially increasing the number of tubers produced either when...
exogenously added or their biosynthesis has been increased by transgenic expression of ipt gene, encoding a main regulatory enzyme of the cytokinin biosynthesis pathway [12–14]. The effects of these factors on tuberization and the biomolecules including proteins, genes and RNAs regulating or associated with tuberization process are reviewed and described in this document.

The photoreceptor phytochrome B plays an important role in photoperiod mediated tuberization in potato and is required to inhibit tuberization during the long days. Like in many other complex biological processes, transcription factors (TFs) have been found to play roles in the tuberization process in potato. These TFs regulate the expression at transcriptional levels of certain genes that are associated with processes influencing the tuberization. CONSTANS protein that is an important regulator of photoperiodic-mediated plant developmental processes acts as a negative regulator of tuberization in the potato. On the other hand, Flowering locus T (FT), heterodimer of BEL1 and KNOX transcription factors have been found to positively regulate tuberization. Deciphering the key molecules and the associated mechanisms involved in transducing tuberization signals from site of perception (i.e. leaves) of the external cues such as photoperiod and temperature to the stolon tips to regulate tuberization has also been an important area of research. Besides proteins, RNA molecules, which are transported from source to sink, have also been suggested to be important signaling candidates regulating the tuberization process in potato. The RNA levels of the sucrose transporter 4 (SUT4) has been shown to follow a diurnal rhythm and has been suggested to be involved in regulating tuberization in potato. Recently, mRNA of BEL5 transcription factor has been found to move through the phloem to the stolon tip to induce tuberization. Micro-RNA miR172 has also been associated with tuberization in potato and found to induce tuberization. Various biotechnological tools such as gene silencing, RNAi, genome editing, etc. may be exploited for over-expression or inhibition of the identified key regulatory genes/proteins for increasing the yield of potato by improving tuberization behavior of commercial potato varieties under prevalent/changing environmental conditions. In the paper, the literature available has been reviewed and compiled.

### Environmental factors affecting tuberization

Among various environmental factors, temperature and photoperiod are most important cues affecting tuberization in potato. High temperatures are inhibitory for tuberization and affect partitioning of assimilates by decreasing the amount going to the tubers and increasing the amounts to other parts of the plant. Extensive studies have been carried out in order to understand the effects of temperature on tuberization [6,15–18]. All the stages and phases of tuberization, that is, tuber induction, tuber set, tuber bulking, tuber number, size and yield have been found to be affected by temperature. Optimal temperatures for various processes of potato plant development have been defined (Table 1). Higher temperatures delay or even inhibit tuberization in comparison with lower temperatures [17,19]. In particular, night temperature has a strong influence on tuberization. Similarly, photoperiod plays an important role in the induction and initiation of tuber formation in the potato and has been the most intensively investigated environmental cue. The potato is a short-day plant, although the critical night length for tuberization and the strength of the photoperiodic response varies with different genotypes [20]. Detailed studies have been carried out on the effects of photoperiod on potato tuberization [17,21–24]. It has been suggested that both temperature and photoperiod cues converge at some point, probably by controlling common component(s) of the day length pathway [25].

### Growth regulators controlling tuberization

The process of tuber initiation has been extensively studied in relation to endogenous levels of plant

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**Table 1.** Optimal temperatures (in °C) for maximum rates of different processes in the potato plant (adapted from Timlin et al. 2006 [18].

<table>
<thead>
<tr>
<th>Process</th>
<th>Optimal temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprouting</td>
<td>16–20</td>
</tr>
<tr>
<td>Sprout growth</td>
<td>20–25</td>
</tr>
<tr>
<td>Emergence</td>
<td>20–25</td>
</tr>
<tr>
<td>Early shoot growth</td>
<td>24</td>
</tr>
<tr>
<td>Leaf primordial development</td>
<td>35</td>
</tr>
<tr>
<td>Leaf appearance</td>
<td>28</td>
</tr>
<tr>
<td>Individual leaf growth</td>
<td>25</td>
</tr>
<tr>
<td>Leaf area development</td>
<td>20–25</td>
</tr>
<tr>
<td>Stem elongation</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Shoot growth</td>
<td>32</td>
</tr>
<tr>
<td>Progress to flowering</td>
<td>30</td>
</tr>
<tr>
<td>Photosynthesis leaf</td>
<td>24</td>
</tr>
<tr>
<td>Whole plant photosynthesis</td>
<td>20–24</td>
</tr>
<tr>
<td>Dry matter production</td>
<td>20</td>
</tr>
<tr>
<td>Stolon initiation</td>
<td>25</td>
</tr>
<tr>
<td>Stolon growth</td>
<td>25</td>
</tr>
<tr>
<td>Stolon branching</td>
<td>25</td>
</tr>
<tr>
<td>Tuber induction</td>
<td>15</td>
</tr>
<tr>
<td>Tuber initiation</td>
<td>22</td>
</tr>
<tr>
<td>Tuber set/onset of tuber growth</td>
<td>15</td>
</tr>
<tr>
<td>Dry matter partitioning to tubers</td>
<td>20</td>
</tr>
<tr>
<td>Tuber bulking</td>
<td>14–22</td>
</tr>
<tr>
<td>Tuber yield</td>
<td>20–24</td>
</tr>
<tr>
<td>Starch synthesis</td>
<td>21.5, 25 or &gt;35</td>
</tr>
<tr>
<td>Breaking of dormancy</td>
<td>28</td>
</tr>
</tbody>
</table>
growth regulators (cytokinins, gibberellins, auxins, abscisic acid). Numerous studies have implicated the growth regulators as both inhibitor and promoter working coordinately to control tuber induction. The relevant literature has been reviewed from time to time [7,8]. The action of gibberellins (GAs) has been implicated in different aspects of potato tuber formation. Several workers have shown that the noninduced state in potato plants is correlated with high endogenous GA levels [9–11,26]. GA levels in the leaf decrease under short-day photoperiods and increase under long-day conditions [26–28]. Initial evidence at molecular levels about the role of GA in tuberization emerged from the studies by Carrera et al [29]. They isolated three potato cDNA clones encoding GA 20-oxidase (StGA20ox1–3), a key regulatory enzyme in the GA-biosynthetic pathway and observed that StGA20ox1 mRNA was expressed at higher levels in leaves. They suggested that night-break induction of this gene might play a role in the control of tuberization by regulating endogenous levels of GAs in response to day length conditions. Similarly, Kloosterman and his group studied the role of a potato GA 2-oxidase gene (StGA2ox1) in tuber formation [30]. StGA2ox1 was found to be upregulated during the early stages of potato tuber development prior to visible swelling and was predominantly expressed in the subapical region of the stolon and growing tuber. They proposed a role for StGA2ox1 in early tuber initiation by modifying GA levels in the subapical stolon region at the onset of tuberization, thereby facilitating normal tuber development and growth.

Implications of cytokinin in the creation of metabolic sinks have raised curiosity to study their role in tuberization [31–33]. A number of studies have been carried out to analyze the role of cytokinin in tuberization [13,14,34–37]. In general, cytokinins have been considered to favor tuberization under tuber inducing conditions.

Since auxins were long known to have pronounced effects on many plant developmental processes, a role for auxin in tuber initiation has been suggested. Increases in auxin levels in the stolon, prior to tuberization, remain relatively high during subsequent tuber growth. This suggests a promoting role for auxins in tuber formation [38]. Furthermore, in vitro tuberization experiments have shown higher levels of auxin synthesis in B33:StPIN1 expressing transformants. The organ-specific increase in auxin synthesis in B33:StPIN1-transformants accelerated and intensified the process of tuber formation, reduced the dose of carbohydrate supply required for in vitro tuberization and decreased the photoperiodic dependence of tuber initiation. Overall, a positive correlation was observed between tms1 expression, the IAA content in tubers and stimulation of tuber formation.

Abscisic acid (ABA) is normally regarded as a regulator that reduces GA-promoted processes in plant development. It was speculated that ABA is a promoting hormone in potato tuberization [42–44]. However, the functions of ABA with respect to stolon elongation, tuber initiation and tuber growth are not clear. Initial observations [(a) higher endogenous ABA content under short-day conditions; (b) under long-day conditions tuberization may be induced by ABA application; (c) ABA application following GA application induces tuberization and (d) ABA application to the stolons of plants growing under noninductive conditions induces tuberization] indicated that ABA is a factor counteracting the GA effect on tuberization [34,45]. Results of the studies on the effects of exogenous applications of ABA to potato plant foliage and to cultured plant sections have been inconsistent. Foliar sprays and the incorporation of ABA into aqueous nutrient media has been reported to stimulate tuberization by several researchers. However, in contrast, no effect of ABA was observed on sprout or stolon sections cultured in vitro [46–48]. Although jasmonic acid plays important role in plant development and defence, its involvement in the tuberization process has not been reported. Currently, there are only two reports suggesting promotion of tuberization by jasmonic acid [49,50].
Proteins associated with tuberization

Most of the biological processes are catalyzed and hence regulated by proteins, and tuberization is no exception. Various proteins have been identified as key components for the regulation of tuberization. The literature reporting the proteins and their role in tuberization in potato is briefly described below.

Phytochrome B

In 1982, Batutis and Ewing tested the hypothesis that phytochrome is involved in the regulation of potato tuberization [51]. They observed that when red light was given in the middle of the dark period to which whole plants were exposed daily, the percentage of tuberization decreased. Subsequently, the photoreceptor phytochrome B (PHYB) was shown to be involved in the response of potato tuber induction to the photoperiod. In potato, PHYB (StPHYB) has been found to stably accumulate in the green leaves and suggested to be involved in sensing day-length duration. PHYB inhibits tuberization under long-day conditions. Using an antisense approach, Jackson et al. [52,53] showed that antisense StPHYB plants tuberize during long days and the effect was found to be graft transmissible (Figure 1). Reduced levels of StPHYB in transgenic antisense S. tuberosum ssp. andigena plants enabled them to tuberize in both short-day and long-day conditions, whereas wild-type plants formed only stolons and did not tuberize in long days. Tubers formed on the antisense plants with little or no stolon formation, even in continuous light, reflecting a strongly induced state of these plants to tuberize. Thus, the antisense plants lost the inhibitory effect on tuberization caused by long day and hence, StPHYB appeared to play a role in inhibiting tuberization in long day.

Three amino acid loop extension (TALE) superclass transcription factors

Three amino acid loop extension (TALE) superclass TFs are named so because of the presence of three amino acids: the proline–tyrosine–proline loop. These TFs are distinguished by very high levels of sequence conservation in the DNA-binding region, designated as homeodomain and consisting of three α-helices [54]. The proline–tyrosine–proline loop is present between helices I and II. The third helix, the recognition helix, is involved in DNA binding [55]. Of the several TFs in the TALE superclass, the two main groups in plants are the KNOX (knotted-like homeobox) and BEL types. KNOX gene family encodes TFs that are involved in regulating the developmental events in apical meristem [56,57]. Results from expression patterns and functional analysis of mutations have supported the involvement of knox genes in specific developmental processes in the shoot apical meristem (SAM). Kn1 (the KNOX gene family member) from maize has been implicated in the switch from indeterminate to determinate cell fates [58–60]. Also, overexpression of kn1 in Arabidopsis [61] and tobacco [62] has resulted in plants with altered leaf morphologies including lobed, wrinkled or curved leaves with shortened petioles and decreased the elongation of veins. The BEL1-like homeodomain (BLH) proteins TFs are ubiquitous among plant species and regulates a range of developmental processes including meristem and floral development [63–69].

BEL1 and KNOX TFs have been shown to interact in a tandem complex to regulate the expression of target genes. In potato, StBEL5 and its KNOX protein partner designated as POTH1 (potato homeodomain 1) regulates tuberization by targeting genes that control growth. In order to decipher the mechanism of action of these two TFs, Chen et al. (2003) analyzed and verified the interaction of POTH1 protein with all the seven members of the BEL1 family of TFs in potato [57]. Using deletion mutant analysis, 80 amino acids of the BELL domain were identified as the region involved in protein interaction with POTH1. The existence of so many unique BEL partners that bind to POTH1 implies that they are involved in a complex system of developmental control in potato. One of the BEL1 partners, StBEL5, consistently exhibited enhanced RNA levels in stems, leaves and stolons (but not roots) in response to a SD photoperiod. In situ hybridization results placed the RNA of both POTH1 and StBEL5 in the vascular tissue of...
tuberizing meristems. Further, the overexpression of StBEL5 produced transgenic plants with an enhanced capacity to form tubers. The heterodimer of StBEL5 and POTH1 binds to a tandem TTGAC-TTGAC motif that is essential for regulating transcription [70].

POTH1 and StBEL5 have been shown to regulate plant growth by controlling GA synthesis. Rosin et al. (2003) observed that overexpression of a POTH1 gene of potato altered vegetative development by decreasing GA accumulation [71]. Overexpression of POTH1 produced dwarf plants with altered leaf morphology. Levels of intermediates in the GA biosynthetic pathway were altered, and the bioactive GA, GA(1), was reduced by one-half in sense mutants. Accumulation of mRNA for GA 20-oxidase1, a key biosynthetic enzyme, decreased in overexpression lines. In vitro tuberization was enhanced under both short- and long-day photo-periods in several POTH1 overexpression lines. Sense lines produced more tubers at a faster rate than controls. These results implied that POTH1 mediates the development of potato by acting as a negative regulator of GA biosynthesis. Further, application of GA resulted in partial reversal of the leaf phenotype and completely rescued the dwarf phenotype. Also, DNA-binding assays have demonstrated that StBEL5 and POTH1 bind to the regulatory region of GA 20 oxidase1 from potato, a gene encoding a key enzyme in the GA biosynthetic pathway. In tandem, StBEL5 and POTH1 had a greater binding affinity for the GA20 oxidase 1 promoter than either protein alone [70]. Transcription assays with the BEL and KNOX proteins has indicated that, in tandem, they bind-specific DNA sequences of GA20 oxidase1 to repress its activity by more than 50%. These results indicated that the tandem interaction of StBEL5 and POTH1 is essential for regulation of the expression of their target gene, GA oxidase1.

Sharma et al. (2014) identified seven StBEL1-type genes in potato [72]. One of these genes, designated StBEL5, has transcripts that move long distances in the plant and enhance tuberization and root growth. Phylogenetic analysis of the StBEL family demonstrated a degree of orthology with the 13 BEL1-like genes of Arabidopsis. Yeast two-hybrid experiments with KNOTTED1-like proteins and the new StBEL5s confirmed the interactive network between these two families.

**Flowering locus T and CONSTANS**

Photoperiodic control of tuberization shares a number of common elements with that of flowering regulation [19,25]. Inductive day lengths to these two responses are perceived by the leaves. Under favorable conditions, a systemic signal (initially named as florigen or tuberigen) is produced in the leaf vascular bundles and transported via the phloem to the vegetative shoot apex or the underground stolon tips, to respectively induce floral or tuberization transition. This signal was confirmed to be the FLOWERING LOCUS T (FT) protein [19,73,74]. Recently, it was shown that expression of the Heading date 3a (Hd3a), the FT ortholog in rice, induces strict short-day potato type to tuberize in log days [75]. They transformed Andigena plants with the rolC::Hd3a-GFP construct, which in rice promotes floral transition in long days. Lines expressing this construct were induced to flower and were able to tuberize in noninductive long days. When grafted to wild-type plants, these lines induced the wild-type controls to tuberize in long days. They detected the Hd3a-GFP protein, but not its transcript in the stolon of grafted wild-type stocks, demonstrating that the protein but not the RNA can move across the graft junction as a powerful tuberization inducer. They further provided evidence that the potato floral and tuberization transitions are controlled by two different FT-like paralogs (StSP3D and StSP6A) that respond to independent environmental cues and showed that an autorelay mechanism involving CONSTANS modulates expression of the tuberization-control StSP6A gene. Two additional FT family members from potato, StTFL1 and StSP5G, have also been related to the tuberization process. StTFL1 mRNA levels were found to be high in stolons before induction and decrease at early stages of tuber development. Overexpression of StTFL1 causes an increase in the number of tubers produced [76], suggesting a role in tuber induction or development. The expression pattern of StSP5G suggests that this protein might play an opposite role to that of StSP6A in tuberization control [75,77].

The other important proteins reported to be playing roles the in regulation of flower and tuberization is CONTANS. It belongs to a protein family comprising transcriptional regulators containing a B-box domain at their N terminus and a CONSTANS, CONSTANS-like, TOC 1 (CCT) region at their C terminus [78,79]. In Arabidopsis, CONSTANS (AtCO) is known to accelerate flowering in long-day conditions. Several genes belonging to the CO family play an important role in the photoperiodic responses. Within the CO family, the group containing CO also includes Heading date 1 (Hd1), which accelerates flowering under short day and delays it under long-day conditions in rice [79–81].

In potato, CONSTANS TF has been found to inhibit tuberization [82,23]. Martinez-Garcia et al. (2002) have shown that constitutive overexpression of that gene in potato impairs tuberization under short-day conditions.
inductive conditions [23]. AtCO overexpressing lines of *S. tuberosum* subsp. *andigena* required longer exposure to short days before they could tuberize. Gonzalez-Schain et al. (2012) identified a potato CO-like gene (*StCO*) and overexpressed/silenced in potato [83]. The plants overexpressing *StCO* tuberized later than wild-type plants under a weakly inductive photoperiod. *StCO*-silencing promoted tuberization under both repressive and weakly inductive photoperiods but did not have any effect under strongly inductive short days, demonstrating that *StCO* represses tuberization in a photoperiod-dependent manner. The effect of *StCO* on tuber induction was transmitted through grafts. In addition, *StCO* affected the mRNA levels of *StBEL5*, a tuberization promoter and *StFT/StSP6A*, a protein highly similar to the FLOWERING LOCUS T (FT), which is a key component of systemic flowering signals in other species. Further, it was observed that *StFT/StSP6A* transcript levels correlate with the induction of tuber formation in wild-type plants. For these observations, it was concluded that *StCO* plays an important role in photoperiodic tuberization and, together with *StFT/StSP6A*, it promotes tuberization and indicates that the CO/FT module participates in controlling this process. Moreover, it supported the notion that *StCO* is involved in the expression of long-distance regulatory signals in potato. Based on the above described studies, Navarro et al. (2011) [75] have proposed a model for *StCO* and *StSP6A*-mediated tuber induction (Figure 2).

![Figure 2](image-url)

**Figure 2.** *StSP6A*-mediated regulation of tuberization in potato. Under long-day conditions *StSP6A* is repressed by *StCO*. A role of PHYB is being suggested in the modulation of this repressor activity. Transfer to short days induces a switch in *StCO* repressor function and activates *StSP6A* gene expression in the leaves. During transport this signal is amplified by an autorelay mechanism partially mediated by *StCO*. *StSP6A* activation in leaves and stolons promotes tuber formation (adapted from Navarro et al. 2011 [75]).

**DOF, MADS box and ABF transcription factors**

Recently, cycling DOF (DNA-binding with one finger) factor (CDF), MADS box and ABFs (ABA responsive element-binding factors) transcription factors have been shown to be involved in the regulation of tuberization in potato. Kloosterman et al. (2013) identified a central regulator underlying a major-effect quantitative trait locus for plant maturity and the initiation of tuber development [77]. They showed that this gene belongs to the family of DOF transcription factors and regulates tuberization and plant life-cycle length, by acting as a mediator between the circadian clock and the *StSP6A* (FT protein) mobile tuberization signal. CDF has been suggested to be post-transcriptionally regulated by the circadian clock gene *GI*GANTEA (GI) and blue light receptors. Further, it was observed that natural allelic variants of GI evade post-translational light regulation, allowing cultivation outside the geographical center of origin of potato.

**MADS box genes** are an example of a family of highly conserved TFs that have diverse roles during plant development. Kang and Hannapel (1996) isolated a novel MADS-box gene cDNA (*POTM1–1*) of potato expressed during the early stages of tuberization [84]. The deduced amino acid sequence of *POTM1–1* cDNA showed a putative transcription factor containing a MADS-box domain and a K-box domain and shared high homologies to those of flower-specific homeotic proteins, TM4 of tomato and AP1 of Arabidopsis, indicating that the *POTM1–1* gene is a homolog of the AP-1 (activator protein 1) gene family. The levels of *POTM1–1* transcripts were high in axillary buds, underground stolons but not in mature tubers. Kang et al. (2003) performed in situ hybridization and RNA-blotting analysis to investigate the patterns of *POTM1–1* gene expression in the flower development and early tuber development [85]. In the early flowers, *POTM1–1* transcripts were abundant in the developing reproductive organs including the placenta of carpels and the pollen sacs of stamens. In contrast, the pattern of *POTM1–1* distribution during late flower development was different from that of early flower development. The *POTM1–1* transcripts were abundant in the sepal and petals of late flowers but were minimally expressed in the stamens and carpel. In the shoot apical meristem of the vegetative organs, transcripts were distributed throughout meristem domes, young leaves and developing vascular cambium. In the early tuberization, the transcripts were widely distributed in the swollen tips of the stolons. Taken together, the results suggested that *POTM1–1* gene expression was temporally and spatially
regulated in active growing tissues of both vegetative and floral organs with specific distribution patterns dependent upon the developmental stages of the tissue.

ABA responsive element-binding factors (ABFs) are a group of bZIP transcription factors that are involved in ABA mediated plant responses to various abiotic stresses. Muniz Garcia et al. (2014) evaluated the potential use of ABF genes to enhance tuberization and to determine the molecular mechanism involved [86]. For this purpose, transgenic potato plants expressing the Arabidopsis ABF4 or ABF2 genes were generated, and their tuberization capacity and response to tuberization-related signals were analyzed in vitro. The results indicated that both ABF4 and ABF2 proteins positively regulate potato tuber induction. However, only ABF4 expression significantly increases the number and weight of the tubers obtained, without stunting growth. ABF4 and ABF2 transgenic plants exhibit ABA hypersensitivity during tuberization, accompanied by a GA-deficient phenotype. ABF4 expression triggered a significant rise in ABA levels in stolons under tuber-inducing conditions as compared with wild-type plants and a transcriptional deregulation of GA metabolism genes. These results demonstrated that Arabidopsis ABF4 functions in potato ABA-GA signaling crosstalk during tuberization by regulating the expression of ABA- and GA-metabolism genes.

**Sucrose transporter SUT4**

Sucrose transporters belong to a large gene family. In potato, there are three known sucrose transporters viz SUT1, SUT2 and SUT4 which are colocalized and their RNA levels follow a diurnal rhythm and oscillate in constant light. In 2008, Chincinska et al. studied the role of SUT4 in potato [87]. They examined the physiological effects of RNA interference (RNAi)-inactivated StSUT4 expression on transgenic potato plants. The StSUT4-RNAi plants exhibited early flowering, higher tuber production and reduced sensitivity toward light enriched in far-red wavelength. Inhibition of StSUT4 led to tuber production of the strict photoperiodic potato *Solanum tuberosum* subsp. *andigena* even under noninductive long-day conditions. Accumulation of soluble sugars and sucrose efflux from leaves of transgenic plants were modified in StSUT4-RNAi plants, leading to modified sucrose levels in sink organs. It was further observed that StSUT4 expression of wild-type plants was induced by gibberellins and ethephon, and external supply of GA leads to even more pronounced differences between wild-type and StSUT4-RNAi plants regarding tuber yield and internode elongation, indicating a reciprocal regulation of StSUT4 and gibberellins. Also, it is well established that PHY B and light also regulate GA3 biosynthesis [88]. The phenotype of StSUT4-RNAi plants including decreased length of internodes and early tuberization leading to higher tuber yields was exactly described for plants with a reduced expression of StGA20ox1 [89]. In addition, StSUT4-RNAi plants showed early flowering. The overall phenotype of StSUT4-RNAi plants also included a reduced level of StGA20ox1 at the end of the day and in accordance with reduced biosynthesis of GAs. Based on these observations, a model was proposed showing StSUT4-mediated interconnection of the photoreceptor and the GA3-signaling pathway triggering tuberization and potato flowering. Further work showed that the StSUT4 gene affects the expression of circadian-regulated genes and ethylene production [90]. They observed that induction of early flowering and tuberization in the SUT4-inhibited potato plants correlated with increased sucrose export from leaves and increased sucrose and starch accumulation in terminal sink organs, such as developing tubers. SUT4 was also found to affect the expression of the enzymes involved in gibberellin and ethylene biosynthesis, as well as the rate of ethylene biosynthesis. Thus, it was concluded that StSUT4 controls circadian gene expression, potentially by regulating sucrose export from leaves. Furthermore, SUT4 expression affects clock-regulated genes such as StFT, StSOC1 (Suppressor of Overexpression of CONSTANS), and StCO, which might be also involved in a photoperiod-dependent tuberization. Based on these observations, a model has been proposed on the mechanism of action of StSUT4 in regulation of tuberization (Figure 3). The proposed model suggests the impact of the circadian-regulated StSUT4 on the photoperiod-dependent accumulation of StCO and StSP6A transcript levels.

**Other proteins associated with tuberization in potato**

In addition to above-described transcription factors and proteins, various other proteins have been reported to be associated with tuberization in potato. These include acid phosphatase (APase), protein phosphatase type 2A, reactive oxygen species catabolizing enzymes (superoxide dismutase, ascorbate peroxidase and catalase), patatin, proteinase inhibitors, calcium-dependent protein kinase, enzymes of sucrose metabolism (sucrose synthase, fructokinase, invertase). In this review, these are described briefly.

Higher expression of acid phosphatase (APase) have been found in swelling tubers as compared to that in elongated stolons and matured tubers [91]. Inhibition of
Figure 3. Regulation of tuberization by sucrose transporter StSUT4. StSUT4 affects the accumulation of StCO and StSP6A mRNA in a photoperiod-dependent manner. Under long-day condition StSUT4 induces StCO accumulation which in turn inhibits StSP6A accumulation and hence results in no tuberization. On the other hand, under short day conditions StSUT4 inhibits StCO accumulation, and hence, StCO-mediated inhibition of StSP6A is prevented and hence tuberization takes place (partially adapted from Chincinska et al. 2008 and Chincinska et al. 2013 [87,90]).

APase activity has been found to result in suppression of tuber swelling and moderately affected the stolon elongation and the tuberization frequency. Moreover, inhibition of APase activity has led to marked reduction in the sucrose content in tubers and further decreased starch accumulation, suggesting that the function of APase in regulating tuber swelling might be at least partially mediated by the sugar resorption [91]. Pais et al. (2010) analyzed the roles of protein phosphatase type-2A catalytic subunits (PP2Ac) in the leaf responses to conditions that affect tuberization [92]. Experiments using PP2A inhibitors, together with PP2Ac expression profiles under conditions that affect tuberization indicated that a high sucrose/nitrogen ratio, which promotes tuber formation, increases the transcript levels of Patabin and Pin2, by increasing the activity of PP2As without affecting PP2Ac mRNA or protein levels. GA, a negative regulator of tuberization, downregulates the transcription of catalytic subunits of PP2As from the subfamily I and decreases their enzyme levels. These results suggested that PP2As may positively modulate the signaling pathways that lead to the transcriptional activation of tuber-specific genes in leaves and act as molecular switches regulated by both positive and negative modulators of tuberization. Comparative proteome analysis of protein expressed at different development stages of potato has revealed that the expression of reactive oxygen species catabolizing enzymes, viz superoxide dismutase, ascorbate peroxidise and catalase, were induced during tuber initiation indicating their possible role during the developmental transition from stolons into tubers [93]. Kim et al. (2007) observed a higher concentration of H2O2 in the sense transgenic potato plant with the lily chCu, ZnSOD, whereas higher levels of O2(-) was detected in the antisense transgenic plant than the wild-type plant [94]. They hypothesized that a specific ROS acts as a signal transducer via GA biosynthetic pathways for the regulation of plant growth and tuber development of potato.

During the transition from stolons to tubers, a dramatic increase of patatin gene expression has been reported to be coincided with an increase in histone lysine acetylation suggesting that the patatin genes exhibit alterations in the chromatin state and differential transcriptional regulation during the developmental transition from stolons into tubers, in which there is an increased demand for protein storage [95]. Hendriks et al. (1991) have reported that patatin and four serine proteinase inhibitor genes are differentially expressed during potato tuber development [96]. The studies showed that the length of the day/light conditions differently influenced the expression level of the individual genes. In addition, the expression of each of these genes changed specifically during the development of the axillary bud to a tuber. In contrast to the expression of these proteinase inhibitor genes, patatin gene expression was only detectable from the day tuberization was manifested as a radial expansion of the axillary bud. Ribosomal protein genes TUBS19 and TUBL7 have also been shown to be differentially expressed during tuberization in potato. Taylor et al. (1992) studied the expression and sequence analysis of the ribosomal protein genes TUBS19 and TUBL7, which showed a 15- to 20-fold increase in transcript level in the stolon tip during the early stages of tuberization [97].

Appeldoorn et al. (2002) performed in situ analysis of enzymes involved in sucrose to hexose-phosphate conversion during stolon-to-tuber transition of potato to follow developmental changes in spatial patterns [98]. During the stages of stolon formation, high hexokinase and acid (cell wall-bound) invertase activities were found to be restricted to the mitotically active subapical region, suggesting a possible importance of these enzymes for cell division. At the onset of tuberization, sucrose synthase and fructokinase were shown to be strongly induced (visualized at transcriptional and translational level) and the acid invertase activities disappeared from the swelling subapical region as expected. The high degree of similarity in the spatial pattern and the temporal induction of sucrose synthase and fructokinase suggested a tightly coordinated coarse
upregulation, which may be subjected to a sugar-modulated mechanism(s) by which genes involved in the metabolic sucrose-starch converting potential are coordinately regulated during tuber growth. Viola et al. (2001) reported that tuberization in potato involves a switch from apoplastic to symplastic phloem unloading [99]. They studied phloem unloading in potato plants in real time during the early stages of tuberization. Analysis of invertase activity in non-tuberizing and tuberizing stolons revealed a marked decline in soluble invertase in the subapical region of swelling stolons, consistent with the switch from apoplastic to symplastic unloading.

**Long distance signaling mechanisms regulating tuberization**

Movement and accumulation of StBEL5 RNA have been consistently associated with enhanced tuberization even under long day conditions [57]. Of the 13 BEL genes identified in potato [72], StBEL5, functions as a signal mRNA in potato, is trafficked long distance through the sieve element system and is involved in the activation of tuberization [100]. RNA movement assays have demonstrated that StBEL5 transcripts move through the phloem to stolon tips, the site of tuber induction. StBEL5 mRNA originates in the leaf, and its movement to stolons is induced by a short-day photoperiod. Transcription of StBEL5 in leaves is induced by light but is insensitive to photoperiod, whereas in stolon tips growing in the dark, promoter activity is enhanced by short days. RNA-binding proteins, StPTB1 and StPTB6 have been suggested to mediate this movement [101]. Recently, POHT1 has also been suggested to be the potential candidate for acting as a long distance mobile signal and is transported from the leaves to the stolon leading to induction of tubers in association with StBEL5 [102].

The roles of micro RNA172 (miR172) in photoperiodic regulation of flowering, flower development, sex determination, meristem cell fate and vegetative phase change have been demonstrated by various studies [103,104]. All the genes targeted by miR172 encode members of a subset of the APETALA2 (AP2)-like transcription factor family that can both repress translation and induce degradation of its target mRNAs [105]. miR172 promotes flowering in Arabidopsis by negatively regulating AP2-like flowering repressors, such as TOE1, TOE2, SMZ and SNZ.

Martin et al. (2009) showed the effect of StmiR172 on potato developmental events [106]. They observed that StmiR172 levels were higher under tuber-inducing short days than under noninductive long days and were upregulated in stolons at the onset of tuberization. Overexpression of StmiR172 in potato-promoted flowering, accelerated tuberization under moderately inductive photoperiods and triggered tuber formation under long days. In plants with a reduced abundance of phytochrome B (PHYB), which tuberize under long days, both StBEL5 mRNA and StmiR172 levels were reduced in leaves and increased in stolons. This, together with the presence of StmiR172 in vascular bundles and the graft transmissibility of its effect on tuberization, indicated that either StmiR172 might be mobile or it regulates long-distance signals to induce tuberization. Consistent with this, plants overexpressing StmiR172 showed increased levels of StBEL5 mRNA, which has been identified and cloned a potato AP2-like gene, StRAP1 that contains a StmiR172 target site. An inverse correlation between the abundance of StRAP1 transcript and StmiR172 in several organs of wild-type plants and downregulation of RAP1 in miR172-overexpressing leaves and plants in which StPHYB was silenced was observed. These observations suggest that StRAP1 is a target of StmiR172 and that this miRNA induces the degradation of StRAP1 mRNA. From this study, it was concluded that StmiR172 acts downstream of PHYB (a tuberization repressors) and upstream of the BEL5 (tuberization promoter) to regulate tuber induction and that it acts as a long distance regulator of tuberization. Based on these observations, Martin et al. (2009) [106] proposed a model for the regulation of tuberization (Figure 4; for detailed description see [107]).

**Molecular network model for tuberization in potato**

Based on the above discussions, we propose a model for overall molecular regulation of tuberization in potato (Figure 5). The model is briefly described as follows. Under long-day conditions StPHYB induces StCO and suppresses StmiR172 (Figure 5(a)). As mentioned earlier, StCo has been suggested to be a specific inducer of StSP5G. Inturn StSP5G inhibits expression of StSP6A which is an important mobile signal transported from the leaves to stolon tips. StBEL5 and POTH1 have also been shown to be mobile (transported in the form of mRNA) and associated with transport to the stolon tip promoting tuber formation. StPHYB might repress the movement of StBEL5 mRNA from the leaves to stolons under long-day conditions resulting in the repression of tuberization. Suppression of StmiR172 (a mobile signal that transports from leaves to stolons and induces tuberization) by StPHYB results in nontuberization. Similarly, under long-day...
conditions, the sucrose transporter (StSUT4) has been suggested to induce StCO and thus inhibits tuberization. Also, under long-day conditions, certain blue light receptors and a clock gene protein GI (GIGENTEA) (GI) interacts and targets the StCDF protein for degradation and thus escapes the inhibition of StCO expression by StCDF (Figure 5(a)).

Under short-day conditions, StPHYB and StSUT4 expression is inhibited (Figure 5(b)). This further leads to the inhibition of StCO expression, and thus, StSP6A-a mobile tuber-inducing long distance signal expression takes place, which ultimately leads to the induction of tuberization. Also, inhibition of StPHYB, blue light receptors and the clock gene protein GI results in the removal of barriers to the expression of StmiR172, thus leading to the expression of StmiR172 and induction of tuberization. Further, StmiR172 induces the degradation of StRAP1 protein (which is a suppressor of StBEL5) and thereby results in the expression of StBEL5. In the absence of StPHYB, StBEL5 moves from the leaves to stolons and there leading (in association with POTH1) to reduction in GA biosynthesis and thereby promoting tuberization (Figure 5(b)). Plant growth regulators ABA, cytokinin and jasmonic acid have also been suggested to be promoters of tuberization; however, the exact mechanism of their action and the degree of the impact on the tuberization process still remains to be determined.

Figure 4. The regulation of tuber induction by phloem-mobile signals. The main candidates for mobile signals are the StSP6A protein, two RNAs (StBEL5 and StmiR172) and GAs. The production, and possibly the movement, of these four factors are regulated by a complex genetic network. StPHYB, StSUT4 and StCO repress tuberization in response to long days. GAs also seem to act as repressors, whereas StSP6A, StmiR172 and StBEL5 act as tuberization promoters under inductive short days conditions. Under long days, StPHYB represses the expression of StSP6A and StGA20ox1, which encodes an enzyme that catalyzes the synthesis of GA20 (partially adapted from Suarez-Lopez 2013 [107]).
Figure 5. Molecular regulatory networks of potato tuberization. (a) Gene/protein interactions and actions under long-day condition resulting in nontuberization. (b) gene/protein interactions/actions under short-day conditions leading to tuberization.
Molecular targets for genetic engineering of tuberization

As discussed earlier, genes/proteins that are positive regulators of the tuberization process, include StSP6A, StTFL1, StPOTH1, StBEL5, StmiR172, StPOTM1, StCDF, StPA2Ac, StTUB19, StTUB7, StABF2 and StABF4 (Figure 5). StSP6A (a FT-like paralog) has been shown to be positively regulating tuberization transition in potato, this may be an important gene for its overexpression for inducing tuberization (Figure 6). As overexpression of StTFL1, another FT member of potato, has been suggested to increase the number of tuber produced, it can also be a target for improving tuberization in potato through its overexpression. Two other proteins, StBEL5 and POTH1 (transcription factors belonging to TALE superclass), have been proven to be positive regulators of the tuberization process in potato. Through tandem interactions, these two proteins positively regulate the tuberization process by inhibiting GA synthesis. Hence, these two genes StBEL5 and POTH1 may also be prominent candidates for improving tuberization through their simultaneous overexpression. StmiR172 has been shown to accelerate tuberization in potato. Hence, StmiR172 is also a prominent candidate gene for genetic engineering of tuberization through its overexpression. StCDF, which acts as an inhibitor of StCO expression, may also be a candidate gene for overexpression. Other genes/proteins that are suggested to be positively associated with tuberization include POTM1, StPA2Ac, StTUB19, StTUB7, StABF2, StABF4, etc. These may also be utilized for genetic engineering of tuberization of potato through their overexpression.

Similarly, the negative regulators of tuberization in potato include StPHYB, StCO, StSUT4, StSP5G, StRAP1, etc. (Figure 5). StPHYB has been shown to have the inhibitory effect on tuberization under long-day conditions. Hence, it is a prominent candidate gene for improving tuberization through suppression/inhibition of its expression (Figure 6). Similarly, StCO TF has been found to inhibit tuberization. Its silencing has already been shown to promote tuberization. Hence, it is also an important candidate gene for improving tuberization through its suppression. The StSUT4-, a sucrose transporter, has been proven to be a negative regulator of tuberization in potato. Hence, its suppression may be utilized for promoting tuberization. The expression status of StSP5G (a member FT family of potato) has also been found to be negatively correlated with the tuberization process, and this gene may be a target for repressing/inhibiting its expression to improve tuberization in potato. StRAP1 (AP2-like gene of potato) that contains a StmiR172 target site may also be a probable candidate for improving tuberization through its suppression (Figure 6).

Through genetic engineering, it is now possible to simultaneously overexpress/suppress more than one gene, it might therefore be a more effective approach to utilize these target genes simultaneously (in different combinations) for improving tuberization in potato through genetic engineering.

**Conclusion**

The formation and growth of a potato tuber is a complex process regulated by different environmental signals and plant hormones. Complete understanding of the signal transduction pathways leading to tuber induction still remains an elusive target though the relevant literature and has been updated from time to time. Researchers around the globe have been working for the last several decades to decipher the molecular mechanisms of tuberization in potato with the aim to identify and exploit the key triggers and molecular switches of tuberization in potato. This indeed may lead to making potato tuberize and cultivate under a broader range of most limiting and crucial environmental factors such as temperature and photoperiod. High
temperature and long days have been shown to impede or inhibit tuberization in potato. Also, various growth regulators, especially GA, have been shown to be an inhibitor of tuberization. On the other hand, cytokinins, ABA and sucrose have been found to be positive modulators of tuberization. R & D interventions to have better insights into the molecular mechanisms associated with tuberization in potato have led to the identification of various genes and proteins. Also, some signaling molecules involved in transferring the perceived signals from aerial tissues to the stolon have been identified and are being analyzed further. Although the availability of sequence of the potato genome [108] may be of great help to have the better and clearer picture of the tuberization process, functional genomics studies will essentially be required to delineate the precise role of each components associated with tuberization process. Understanding and exploitation of tuberization becomes even more relevant and is essential in the face of changing the global climate and expectations from the potato for it to be a main crop to meet the food and nutritional demands of a growing human population.

Various biotechnological and genomics tools as discussed recently [109] may be used for manipulating this tuberization, regulating biomolecules and mechanisms with the aim of increasing the productivity of potato. Detailed physiological, biochemical and molecular biological studies for the last several decades have lead to the identification of the key molecular targets (genes and proteins) that can be utilized for genetic engineering mediated manipulation of tuberization process in potato (Figures 5 and 6). Genes acting as positive regulators of the tuberization (StSP6A, StTFL1, StPOTH1, StBEL5, miR172, POTH1, StCDF, StPA2Ac, StTUB19, StTUB7, StABF2 and StABF4) process are candidates for overexpression whereas genes that are negative regulators (StPHYB, StCO, StSUT4, StSP5G, StRAP1) are targets for suppression/inhibition. Therefore, these genes and the associated mechanisms can be utilized for improving tuberization and thereby the productivity of potato through genetic engineering.

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