THE INTERNATIONAL JOURNAL OF
DEVELOPMENTAL
BIOLOGY
www.intjdevbiol.com

Molecular signals that govern tuber development in potato

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ABSTRACT The potato serves as the fourth most important food crop on the planet after the three cereal crops. It is rich in starch, storage proteins and important vitamins, dietary antioxidants and minerals. Potato is a modified stem (stolon) that grows underground, at the base of the plant, under favourable conditions. Perception and processing of signals occur in leaves and the corresponding information is transported to the stolon-tip. The elongation of the stolon-tip ceases and the plane of cell division changes from transverse to longitudinal, causing swelling of the sub-apical region of the stolon. This is accompanied by synthesis of starch in leaves, followed by its transport to and accumulation in the stolon. The initiation of tuber developmental signals and the subsequent stolon-to-tuber transition (tuberization) is undoubtedly a dynamic process which involves integration of multiple molecular factors, environmental cues and crosstalk between various pathways, including phytohormones. Understanding the tuberization process has been an aim of many plant biologists across the globe. Recent discoveries have shown that apart from photoperiod and hormonal metabolism, there are crucial transcription factors, small RNAs, full-length mobile mRNAs and proteins that regulate tuberization in potato. Although we have gained significant knowledge about the tuberization process, many questions on the underlying mechanisms of tuber development remain to be answered. In this review, we summarize the crucial molecular signals that govern tuber formation and propose an updated tuberization network along with future research directions.

KEY WORDS: *small RNA, StBEL5, StSP6A, POTH1, mobile RNA*

Introduction

Potato (*Solanum tuberosum* L.), a member of the Solanaceae, is the fourth most important food crop in the world. The genus *S. tuberosum* is classified into two subspecies, andigena and tuberosum. Subspecies andigena forms tubers only under short-day photoperiod conditions (SD; 8 h light/16 h dark) and is mainly grown in the Andes, while the widely cultivated subspecies tuberosum is adapted to long-day conditions (LD; 16 h light/8 h dark) (Ewing and Struik, 1992). Under favourable conditions, starch synthesized in the compound leaves is mobilized to an underground modified stem (stolon) that finally develops into a mature tuber. Potato tubers accumulate large quantities of starch, protein and dietary fibre, and are low in fat. They are also enriched with micronutrients (vitamin C, niacin, thiamine, folate, pantothenic acid and riboflavin), dietary antioxidants, and minerals, such as, iron, potassium, phosphorus and magnesium. In food industry, tubers are used in chip and fries production, whereas their non-food usage includes the production of starch, fuel-grade ethanol and glue. Numerous potato varieties with diversity in tuber size, shape, colour, texture, cooking characteristics and taste are grown worldwide.

Tuber formation is a unique developmental process, where stolon originates from the base of the main stem and continues to grow horizontally, and if exposed to sufficient light, it becomes green and emerges from the soil to form a new shoot that behaves

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Abbreviations used in this paper: BEL, BEL1-like; BEL5/11/29, BEL1-like TFs; CDF1, cycling dof factor 1; CDPK, calcium-dependent protein kinase; CK, cytokinin; CO, constans; COL3, constans-like 3; FD, flowering locus D; KF1, flavin-binding kelch repeat F-box 1; FLC, flowering locus C; GA, gibberellin; *GA2ox*, gibberellin 2-oxidase; GI, gigentia; KAO, ent-kaurenoic acid oxidase; KNOX, knotted1-like; LD, long day; miR, microRNA; PcG, polycomb group; PEPB, phosphatidylethanolamine-binding; PhasiRNA, phased short interfering RNA; PHYA, phytochrome A; PTB, polypyrimidine binding protein; POTH1, potato homeobox 1; PRC, polycomb repressive complex; PTB, polypyrimidine tract-binding protein; RAP1, related to apetala2 1; RBP, RNA binding protein; SAM, shoot apical meristem; SD, short day; SP5G, repressor of SP6A; SP6A, Potato FT orthologue; SPL9, squamosa promoter binding protein-like 9; sRNA, small RNA; *St, Solanum tuberosum*; SUT4, sucrose transporter in potato; TAC, tuber activation complex; TF, transcription factor; TasiRNA, transacting RNA; UTR, untranslated region.

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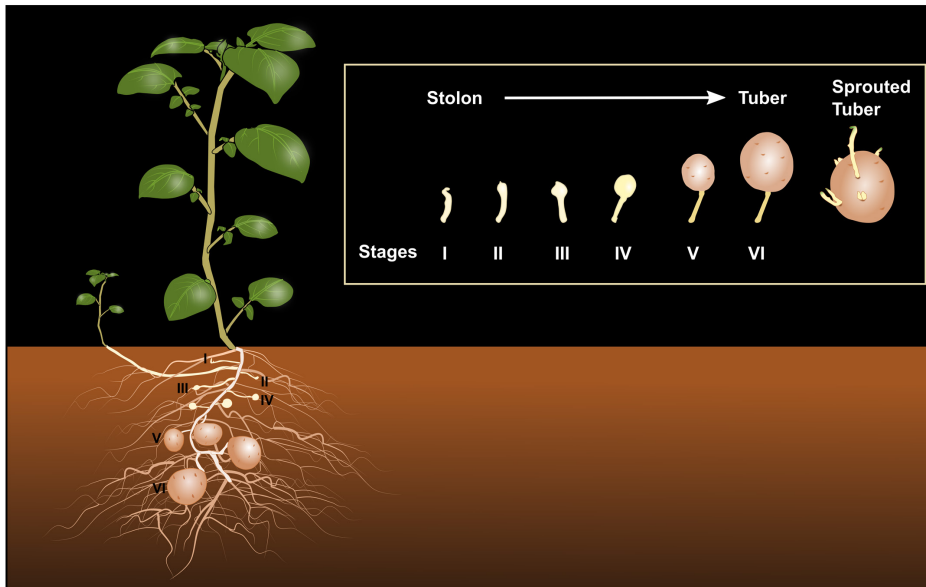
Fig. 1. Diagrammatic illustration of potato plant *and different stages of stolon-to-tuber transitions.*

like a mature plant (Fig. 1). While under inductive conditions, stolon elongation is arrested, radial swelling is triggered at the subapical region of the stolon, and eventually develops into a mature tuber (Xu *et al.*, 1998a; 1998b) (Fig. 1) with many dormant axillary buds known as tuber "eyes". After a period of dormancy, tuber eyes sprout (Fig. 1) and develop to a new plant. Hence, tubers serve the dual role of storage organ as well as vegetative propagation system.

At the onset of tuberization, growth of stolon apical meristem cells becomes determinate, and cell division ceases after a few rounds. However, growth is initiated in a specific layer of cells in the perimedullary zone just below the stolon apex and in close proximity to vascular tissue (Xu *et al.,* 1998b). In the stolon tip, the orientation of cell division changes from transverse to longitudinal leading to radial expansion. Most of the cell division and expansion occurs in the stolon sub-apical region between the cortex and pith of the developing tuber (Xu *et al.,* 1998b) and an alteration of hormone levels (gibberellin [GA], auxin and cytokinin [CK]) seem to be critical for such dynamic changes (Xu *et al.,* 1998a). As a result, the stolon sub-apical region acts as a strong sink, the mechanism of sucrose unloading shifts from apoplastic to symplastic, and it starts to accumulate a large amount of starch and storage proteins. Thus, it appears that in order to control the initiation of stolon-to-tuber transition, a simultaneous activation/suppression model needs to be functional in specific cell types during the development of a new tuber from the stolon tip (Ghate *et al.,* 2017).

Stolon-to-tuber transition in potato is an important developmental phase governed by many environmental, biochemical and hormonal cues. Over the years, many researchers have been investigating the molecular mechanism of tuber development in order to improve tuber yield. Potato genome sequencing (~850Mb) has further paved the way for the rapid progress in functional analysis of genes regulating tuber development. It is believed that tuber formation mainly involves three phases- induction, initiation and proliferation (Gregory, 1956). Tuber induction is associated with suppression of axillary branching, frequent abortion of flower buds, and enlargement of leaf size (Ewing and Struik, 1992). The factors involved in

the initiation and subsequent proliferation of growth at the stolon sub-apical region are explained in the later sections.

Tuberization mechanism has been studied for a number of decades. In past, it was believed that tuberization was controlled by specific stimuli produced in the terminal leaf cluster (Chapman, 1958), and also in old and young leaves (Hammes and Beyers, 1973) under inducing conditions of photoperiod and temperature (Nitsch, 1965; Menzel, 1985). The signal that causes the plant to develop potatoes has been illusive to potato biologists until the report of Chailakhyan *et al.,* (1981); where authors demonstrated that grafting of flowering tobacco (*Nicotiana tabacum*) shoots (scion) onto non-tuberizing potato stocks resulted in an induction of tuber formation, suggesting the presence of common regulatory factors between flowering and tuberization processes. In post-potato genome era, this report (Chailakhyan *et al.,* 1981) prompted researchers to search for potential mobile signals involved in potato development that are elaborated in the later sections of this review.

Photoperiod, temperature, light intensity, nitrogen supply and sucrose status decide tuber development

It is now established that short days, cool temperatures (Gregory, 1956), high light intensities (Bodlaender, 1964; Wheeler and Tibbitts, 1986), and low levels of nitrogen application (Gao *et al.,* 2014) stimulates tuber formation, whereas long days, high temperatures (Gregory, 1956), low light intensities (Ewing and Struik, 1992), and high nitrogen application (Gao *et al.,* 2014) delays tuberization in potato. Sucrose functions as an indispensable metabolic signal that regulates the demand of source-sink relationship during tuber development. Several studies have established that by increasing sugar accumulation or by exogenous sucrose application (Xu *et al.,* 1998a), tuber numbers can be increased, but tuber size remained smaller than wild-type. Moreover, sugars and phytohormones (especially GA, auxin and CK) regulate each other's metabolism, transport and signalling pathways, and their cross-talks play a crucial role in tuber formation (Jackson, 1999).

Role of plant hormones in tuber development

As explained above, the graft transmissible nature of tuberization signals and the accompanied morphological changes in the potato plant convinced researchers that plant hormones could be the stimulus for the tuber induction process. Different hormonal groups, such as GA (Xu *et al.,* 1998a; Xu *et al.,* 1998b; Martinez-Garcia *et al.,* 2001; Ewing and Struik, 1992; Kloosterman *et al.,* 2007; Rosin *et al.,* 2003), CK (Tao *et al.,* 2010; Eviatar-Ribak *et al.,* 2013), auxin (Xu *et al.,* 1998a; Roumeliotis *et al.,* 2012), abscisic acid (ABA) and strigolactone (SL) are implicated in tuber formation, and the changes in hormonal metabolism could be correlated with the onset of tuberization. It is likely that many of these hormones function in the stolon tissue and they regulate the stolon transitional stages.
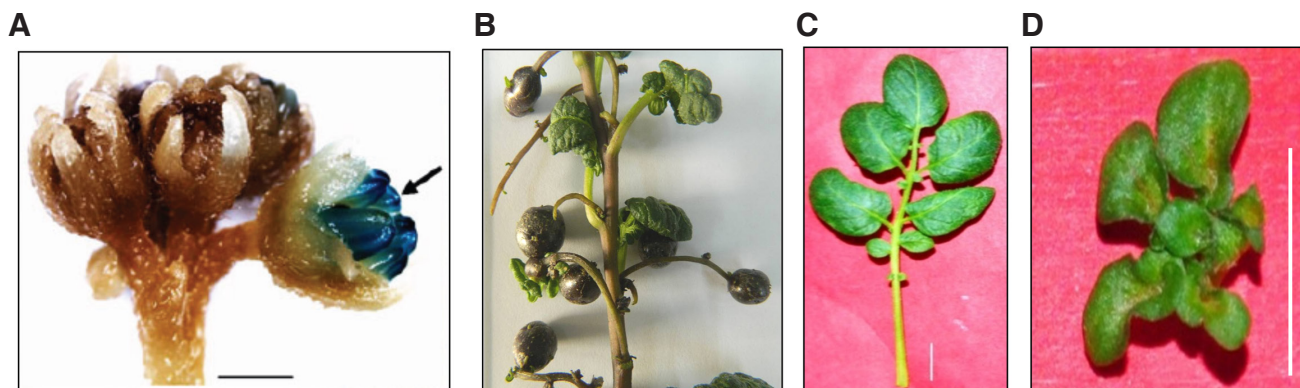
Fig. 2. *POTH1* promoter expression and pleiotropic effects of miR156 and POTH15 in potato. *Promoter activity of* POTH1 *(a class-I KNOX gene) in stamens of potato plant* **(A)**. *Aerial tubers in miR156 over-expression potato line under SD photoperiodic conditions* **(B)**. *Altered leaf development in over-expression line* **(D)** *of another class-I KNOX gene* POTH15 *compared to wild-type potato leaf* **(C)**. *Scale bar in (A) is 5 mm, whereas those in (C,D) are 1 cm each. Images adapted from studies of Mahajan* et al. *(2012), Bhogale* et al. *(2014) and Mahajan* et al. *(2016), respectively.*

However, there are no evidences yet to indicate if they associate with the production of leaf-derived induction stimuli for tuberization. At the initiation of stolon-to-tuber transition, the levels of GA go down (Xu *et al.*, 1998b), whereas that of auxin increase (Roumeliotis *et al.*, 2012) in the stolon subapical region resulting into a swollen stolon (Xu *et al.*, 1998b). A recent study demonstrated that over-expression lines of *TLOG1* (*Tomato Lonely Guy1*; a CK biosynthetic gene encoding an enzyme that converts inactive CK metabolites to its bioactive forms) exhibited production of aerial tubers from juvenile axillary meristems of tomato plant, which naturally does not form tubers (Eviatar-Ribak *et al.*, 2013). ABA application has been associated with earliness in *in vitro* tuberization and enhanced tuber yield (Menzel, 1980). However, the tuber promoting effect of ABA is thought to be because of its negative effect on GA levels (Xu *et al.*, 1998a). Recently, SL has been proposed as a tuber growth inhibitor, however, the mechanism is still unknown.

Mobile RNAs/proteins: signals for tuber development

Mobile RNAs of TALE superfamily regulate tuber development

KNOTTED1-LIKE (KNOX) and BEL1-LIKE (BEL) transcription factors (TFs) belong to the Three Amino Acid Loop Extension (TALE) superfamily of homeobox TFs and they are ubiquitous in plants. KNOX and BEL genes are implicated in diverse processes of plant growth and development (Reviewed in Hamant and Pautot, 2010; Hay and Tsiantis, 2010) and they interact with each other to form heterodimer that regulates the expression of target genes (Chen *et al.*, 2003; Hamant and Pautot, 2010). This BEL/KNOX interaction is selective and different heterodimers govern unique set of target genes in plants (Hay and Tsiantis, 2010). Based on expression patterns and sequence divergences, *KNOX* genes are grouped into two sub-classes; class-I and -II. Class-I KNOX TFs function as either transcriptional activators or repressors (Hay and Tsiantis, 2010) and are involved in shoot apical meristem (SAM) maintenance, leaf development, hormone homeostasis and tuberization (reviewed in Hay and Tsiantis 2010). Although functions of class-II *KNOX* genes are less explored than class-I *KNOX* genes, there are few reports that show their involvement in regulation of root development (Truernit and Haseloff, 2007) as well as in seed dormancy (Chai *et al.*, 2016).

Seven *KNOX* genes have been identified in potato, named as *Potato Homeobox 1* (*POTH1*), *POTH15, POTH20, StKn1, StHox1, StHox2* and *StPetroselinum* (Rosin *et al.*, 2003; Mahajan *et al.*, 2012; 2016). Of them, the role of *POTH1* and *POTH15* (class-I *KNOX* genes) in leaf development and tuberization has been characterised (Rosin *et al.*, 2003; Mahajan *et al.*, 2012; 2016), whereas the role of other *KNOX* genes in potato are not yet investigated. *POTH1* over-expression lines of andigena tuberized earlier and produced more tubers at a faster rate than controls under both SD and LD photoperiods in *in vitro* conditions (Rosin *et al.*, 2003). Later, Mahajan *et al.*, (2012) through a hetero-grafting experiment showed that transcript of *POTH1* moves basipetally through the phloem (Mahajan *et al.*, 2012). The promoter of *POTH1* was found to be active in the mid-vein of leaves, mature stamens (Fig. 2A), roots and stolon tip, and its promoter activity was also found to be light inducible (Mahajan *et al.*, 2012). In the stolon, POTH1 and its BEL partner (StBEL5) binds to a tandem TTGAC motif in the promoters of *GA20ox1* and *GA2ox*, and these interactions have been shown to reduce GA levels that is required for tuber formation (Chen *et al.*, 2003; 2004). *KNOX* genes also regulate levels of CK and auxin (reviewed in Hay and Tsiantis, 2010). Previous studies by Bolduc *et al.*, (2012) and Mahajan *et al.*, (2016) reported that *KNOX-I* genes target other homeobox TFs as well as hormone metabolism genes. Mahajan *et al.*, (2016) showed that POTH15 over-expression affected diverse developmental processes in potato (e.g. Fig. 2 C,D). In this study, authors further revealed that ~87% of randomly chosen POTH15 target genes had at least one tandem TGAC core motif in the 3.0 kb upstream sequence of the transcription start site, suggesting a possible BEL/KNOX interaction with the target genes (Mahajan *et al.*, 2016). Many questions still remain to be answered regarding the functions of *KNOX* genes in potato. For example, like *POTH1*, are the mRNAs of other class-I *KNOX* genes phloem mobile? Do other *KNOX* genes have a role to play in tuber formation?

BEL TFs contain two conserved domains essential for their functionality: the homeobox domain and the BEL domain. In potato, thirteen BEL TFs have been identified (Sharma *et al.*, 2014). Of them, *StBEL5, -11* and *-29* are the highly expressing BELs and they constitute nearly two-thirds of the total transcript values for the entire BEL family in potato (Sharma *et al.*, 2014). By yeast two-hybrid study,

authors further demonstrated that different BELs interact with KNOX proteins in potato (Sharma *et al.,* 2014). StBEL5 was the first BEL protein identified in potato and its full-length mRNA was shown to function as a long-distance mRNA signal that is transcribed in leaves and moves into roots and stolons to stimulate growth (Banerjee *et al.,* 2006, Lin *et al.,* 2013). It has also been demonstrated that both 3' and 5' Untranslated Regions (UTRs) affect the movement of *St-BEL5* mRNA (Banerjee *et al.,* 2009). StBEL5 over-expression plants exhibited a reduction in GA levels in stolons and leaves (Chen *et al.,* 2003; Rosin *et al.,* 2003), which was associated with the earliness in tuberization under *in vitro* conditions and an increased tuber yield in soil-grown plants under SD conditions. Moreover, *StBEL5* RNAi lines showed 30-40% reduction in tuber yield in soil-grown plants (Sharma *et al.,* 2016). All these studies established that StBEL5 acts as a positive regulator of tuberization. Similar to StBEL5, the transcripts of *StBEL11* and *-29* (two close homologs of StBEL5) are recently shown to be phloem mobile from leaf to root and stolon tissues, but they suppress tuber growth (Ghate *et al.,* 2017). It is also shown that all these three BELs regulate a common target gene *StSP6A* (Sharma *et al.,* 2016; Ghate *et al.,* 2017). Based on their RNA profiling in phloem cells, it appears that there is a crosstalk between StBEL5, -11 and -29 in the stolon and this tripartite module could govern the activation of the tuber development process.

In potato, a BEL/KNOX heterodimer (StBEL5/POTH1) has been shown to bind to the promoter of *GA20ox1* through a tandem TTGAC motif to repress its expression, resulting in reduced GA levels in the stolon (Chen *et al.,* 2003). Even single base pair mutation in this tandem TTGAC motif completely abolished the binding of StBEL5/POTH1 heterodimer to *GA20ox1* promoter (Chen *et al.,* 2004). These results established the importance of tandem TTGAC motifs for regulation of target genes by the BEL/KNOX complex.

Although the full-length mRNAs of *POTH1, StBEL5, -11* and *-29* were shown to be phloem mobile (Mahajan *et al.,* 2012; Banerjee *et al.,* 2006; 2009; Ghate *et al.,* 2017), what aids in their mobility was not known until 2014. Later, RNA binding proteins (RBPs), also known as polypyrimidine tract-binding proteins (PTBs), were identified as helpers in transport, stability and metabolism of mobile mRNAs (Lucas *et al.,* 2001). CmRBP50 from the pumpkin phloem was the first RBP protein to be identified as a long-distance transport carrier for six different full-length mRNA molecules (Ham *et al.,* 2009). CmRBP50 was found to bind specifically to 'Cytosine Uracil' (CU) motifs in the 5' and 3' UTRs of the target mRNAs. Six PTBs have been identified in potato: StPTB1, StPTB6, StPTB7, StPTB7.1, StPTB7.2 and StPTB7.3 (Cho *et al.,* 2015). StPTB1 and -6 are induced in leaves and RNA gel shift assays have reported that they bind preferentially to CU motifs in the 3' and 5' UTRs of *StBEL5* and mediate the movement of its mRNA in to the roots and stolons (Banerjee *et al.,* 2006; 2009; Lin *et al.,* 2013; Cho *et al.,* 2015). Moreover, *StPTB1* or *-6* mRNA suppression lines exhibited 50-80% reduction in tuber yield, whereas their individual over-expression lines showed enhanced tuber yield (Cho *et al.,* 2015), indicating that StPTBs act as positive regulators of tuber development possibly by enhancing the transport of *StBEL5* mRNA in to the stolons.

The phloem mobile FT protein StSP6A forms the tuberization activation complex (TAC) in the stolon to regulate tuber initiation

Flowering and tuberization pathways are known to share common molecular players, e.g. CONSTANS1/2 (StCO1/2) and a Flowering Locus T protein (StSP6A; Self-Prunning 6A) (Martinez-Garcia *et al.,* 2002; Navarro *et al.,* 2011), and are regulated by red light receptors, phytochrome A/B (PHYA/B) and blue light receptor, cryptochromes (CRY) (Endo *et al.,* 2007). PHYB operates upstream in the photoperiod-mediated tuberization pathway and PHYB silenced andigena lines showed increased RNA levels of *StSP6A, StBEL5* and miR172 in stolons, and tuberized even under non-inductive LD conditions (Martin *et al.,* 2009). Based on hetero-grafting experiments, it appeared that during light signalling, PHYB indirectly induces a tuberization repressor StCO1/2 (Jackson *et al.,* 1998; Martinez-Garcia *et al.,* 2002). It is also proposed that PHYB could exert its effects on tuber formation through GAs (Jackson *et al.,* 2000). PHYA is less studied as compared to PHYB for its role in tuber formation. A study by Yanovsky *et al.,* (2000) showed that PHYA resets the circadian clock, and its antisense lines tuberized early under non-inductive LD photoperiodic conditions, suggesting that like PHYB (Jackson *et al.,* 1998), PHYA also functions as an inhibitor of tuberization, however, the signalling network of PHYA remains to be investigated. Apart from PHYA/B, the role of other photoreceptors like CRY1/2, phototropins and ultraviolet-B UVR8 in tuberization is not known.

FT is a member of the phosphatidylethanolamine-binding (PEPB) family protein that interacts with the bZIP transcription factor FLOWERING LOCUS D (FD) at the shoot meristem to form the floral activation complex (FAC) that initiates flowering pathway in *Arabidopsis* (Abe *et al.,* 2005). Three FT orthologs StSP6A, StSP3D and StSP5G are present in potato (Navarro *et al.,* 2011). StSP6A and StSP5G are involved in tuberization, whereas StSP3D is involved in flowering (Navarro *et al.,* 2011). Hetero-grafting studies with StSP6A over-expression line as scion and wild-type as stock have demonstrated that StSP6A protein is mobile, and tuber yield was increased in hetero-grafted plants (Navarro *et al.,* 2011). StSP6A protein accumulates in leaves and stolons in response to SD conditions, which is in direct correlation with StBEL5 activity in leaves. It has been shown that the promoter sequence of *StSP6A* has five tandem TTGAC motifs, and StSP6A activity in leaves was completely abolished in TTGAC promotor mutant lines under SD conditions (Sharma *et al.,* 2016). Under SD conditions, StBEL5 induces *StSP6A* in leaves as well as in stolons. Over-expression lines of *StSP6A* tuberized even under LD conditions, whereas its suppression lines showed a decrease in tuber yield (Navarro *et al.,* 2011), confirming that StSP6A protein acts as a tuber activator. Moreover, a recent study by Abelenda *et al.,* (2019) identified a sugar transporter StSWEET11 as the potential interactor of StSP6A and this heterodimer in the stolon helps in establishing source-sink relationship during tuber swelling. Another study suggested that the tuber activation complex (TAC) is formed in the stolon tip *via* the heterodimeric interaction of StSP6A and FD-like protein (StFDL1) with St 14-3-3, which regulates the onset of tuberization (Teo *et al.,* 2017). However, if downstream tuber marker genes, such as *StGA20ox1* (Chen *et al.,* 2004), *StGA2ox1* (Kloosterman *et al.,* 2007) and *StIPT* (Lin *et al.,* 2013), are regulated by the TAC are not known.

StBEL5 functions upstream to *StCDF1* during photoperiodic pathway in potato

CYCLING DOF FACTORs (CDFs) are transcriptional regulators from the ZINC FINGER DOF family of proteins. Three allelic StCDF1 forms have been identified in different potato genotypes - StCDF1.1

(very late tuberizing variant), StCDF1.2 and StCDF1.3 (very early tuberizing variants) (Kloosterman *et al.,* 2013). StCDF1 acts as a central regulator between the circadian clock genes (GIGANTEA [StGI] and FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 [StFKF1]) and a mobile tuberization signal, StSP6A, to control plant maturity and tuberization (Navarro *et al.,* 2011; Kloosterman *et al.,* 2013). Precisely, StCDF1 stimulates tuberization by repressing the levels of *StCO1/2*, thereby increasing the levels of a positive regulator of tuber development StSP6A (Martinez-Garcia *et al.,* 2002; Navarro *et al.,* 2011; Kloosterman *et al.,* 2013). It is proposed that during LD conditions, the circadian clock proteins (StGI and StFKF1) bind to the StCDF1 protein and catalyses its degradation by the 26S proteasome (Sawa, 2007), resulting in inhibition of tuber formation. An interesting report by Sharma *et al.,* (2016) suggested that *StCDF1* could be a target of StBEL5. Preliminary observation revealed the presence of six tandem TGAC core motifs in the *StCDF1* promoter sequence. Using transgenic potato lines harboring the wild-type or the TGAC mutated promoter *StCDF1* fused to a reporter gene *GUS*, we showed that wild-type promoter *StCDF1::GUS* transgenic potato lines exhibit a widespread GUS activity, whereas mutated promoter *StCDF1::GUS* transgenic lines exhibited a complete abolishment of GUS activity. Moreover, yeast one-hybrid assay showed that StBEL5 protein interacts with the wild-type *StCDF1* promoter, but not with the TGAC mutated *StCDF1* promoter (Kondhare *et al.*, 2019). During different stolon-to-tuber transitional stages, the transcript levels of *StBEL5* and *StCDF1* were significantly higher under SD photoperiodic conditions compared to LD (Kondhare *et al.,* 2019). GUS activity was also seen in the tuber pith, tuber-stalk junction and 3-months old tuber sprouts of *StCDF1::GUS* promoter transgenic lines, suggesting that StBEL5-*StCDF1* module is functional during tuber development (Kondhare *et al.,* 2019). These results establish that StBEL5 functions upstream to *StCDF1* gene in potato.

Small RNAs and their targets function as crucial regulators of tuberization

Small RNAs (sRNAs), such as microRNAs (miRNAs) and short-interfering RNAs (siRNAs) (Xia *et al.,* 2017) function as crucial regulators of plant growth and development (Bartel, 2004). They are also involved in different abiotic and biotic stress responses (Axtell, 2013). miRNAs are generally 21 nt long single stranded endogenous sRNAs (Bartel, 2004), whereas siRNAs (20-22, 24 nt long) are produced from double stranded endogenous RNAs (Axtell, 2013). Specific siRNA groups, i.e. phased siRNAs (phasiRNAs) or trans-acting siRNAs (tasiRNAs), are mainly identified in plants (Xia *et al.,* 2017) and their mechanism of action is similar to miRNAs.

As miRNAs target multiple families of transcription factors to control developmental decisions of cell differentiation or organ patterning (Rhoades *et al.,* 2002), it was intriguing to hypothesize if sRNAs govern the early stages of stolon-to-tuber transitions in a photoperiod-dependent manner. Although two studies (Zhang *et al.,* 2013; Lakhotia *et al.,* 2014) were reported, they were limited to identification of miRNAs involved in overall tuber formation and did not emphasize the influence of photoperiod in governing the early stages of stolon transitions. Recently, our small RNA profiling revealed 7 conserved and 12 novel miRNAs to be differentially expressed in early stolon stages under SD vs. LD photoperiodic conditions (Kondhare *et al.,* 2018). qRT-PCR analysis showed that putative target genes of some of these miRNAs, such as *StGRAS, StTCP2/4*

and *StPTB6*, exhibited differential expression in early stolon stages under SD vs. LD photoperiodic conditions, suggesting that miRNAs and their putative targets could regulate the tuberization process.

miRNAs and tuber development

Although miRNAs have been implicated in regulation of diverse developmental and defence related processes in plants (Bartel, 2004; Islam *et al.,* 2018), so far only selective miRNAs are shown to be involved in tuber development. A cross-talk between two miRNAs, miR156 and miR172, controls the transition from juvenile to reproductive phases in *Arabidopsis* (Wu *et al.,* 2009). The levels of miR156 and miR172 were significantly higher in stolons and swollen stolons under SD conditions compared to LD conditions in wild-type andigena plants (Martin *et al.,* 2009; Bhogale *et al.,* 2014), suggesting the influence of photoperiod on differential expression of these miRNAs as well as their role in stolon-to-tuber transitions. In andigena background, the over-expression of miR172 promoted both flowering as well as tuberization under long days (Martin *et al.,* 2009), whereas that of miR156 over-expression lines repressed underground tuber yield but induced aerial tuber formation under SD (Fig. 2B) (Bhogale *et al.,* 2014). These studies also demonstrated that both miR156 and miR172 could function as phloem mobile signals involved in tuber development. Currently, a model for miRNA-mediated tuberization is apparent in the field. In this model, PHYB acts upstream to miR156-StSPL9 module. StSPL9 induces miR172. Potato RELATED TO APETALA2 1 (StRAP1) is a putative target gene of miR172, and it functions as a repressor of *StBEL5* gene (Martin *et al.,* 2009; Bhogale *et al.,* 2014). Under SD conditions, the induction of aerial tubers from the nodal-regions, but reduced underground tuber yield in miR156 over-expression lines poses an interesting question if miR156 functions as an inducer or inhibitor during tuberization pathway (Bhogale *et al.*, 2014). Authors proposed that the tissue-specific threshold level of miR156 could be required for underground tuber development, whereas over-expression of miR156 imposed every above ground nodes with the capacity to develop aerial tubers under short day conditions (Bhogale *et al.*, 2014).

Calcium signalling has been implicated in control of plant development, and Calcium Dependent Protein Kinases (CDPKs) transduce calcium signatures into specific responses including tuberization (Raices *et al.,* 2003). The protein activity of CDPK increases at the onset of tuber formation with *StCDPK1* expression showing a significant increase during stolon-to-tuber transitions in potato (Raices *et al.,* 2003). *In silico* analysis and *Agrobacterium* co-infiltration experiments have established that a conserved miRNA (miR390) cleaves *StCDPK1* mRNA in potato. StCDPK1 protein phosphorylates an auxin transporter, StPIN4, a potential downstream target involved in tuber development. These results suggest that the crosstalk between miR390 and StCDPK1 could serve as a regulator of tuber formation (Santin *et al.,* 2017).

Phased siRNAs and tuber development

So far, three studies reported the presence of phased siRNAs in non-tuber organs of potato plants, and they are predicted to be involved in defense responses (Shivaprasad *et al.,* 2012). Using sRNA profiling of early stolon stages under LD and SD photoperiodic conditions, we identified 830 *TAS-like* loci in potato (Kondhare *et al.,* 2018). Out of 830, 24 *TAS-like* loci were predicted to be cleaved by novel and conserved miRNAs and generated nearly 200 phased
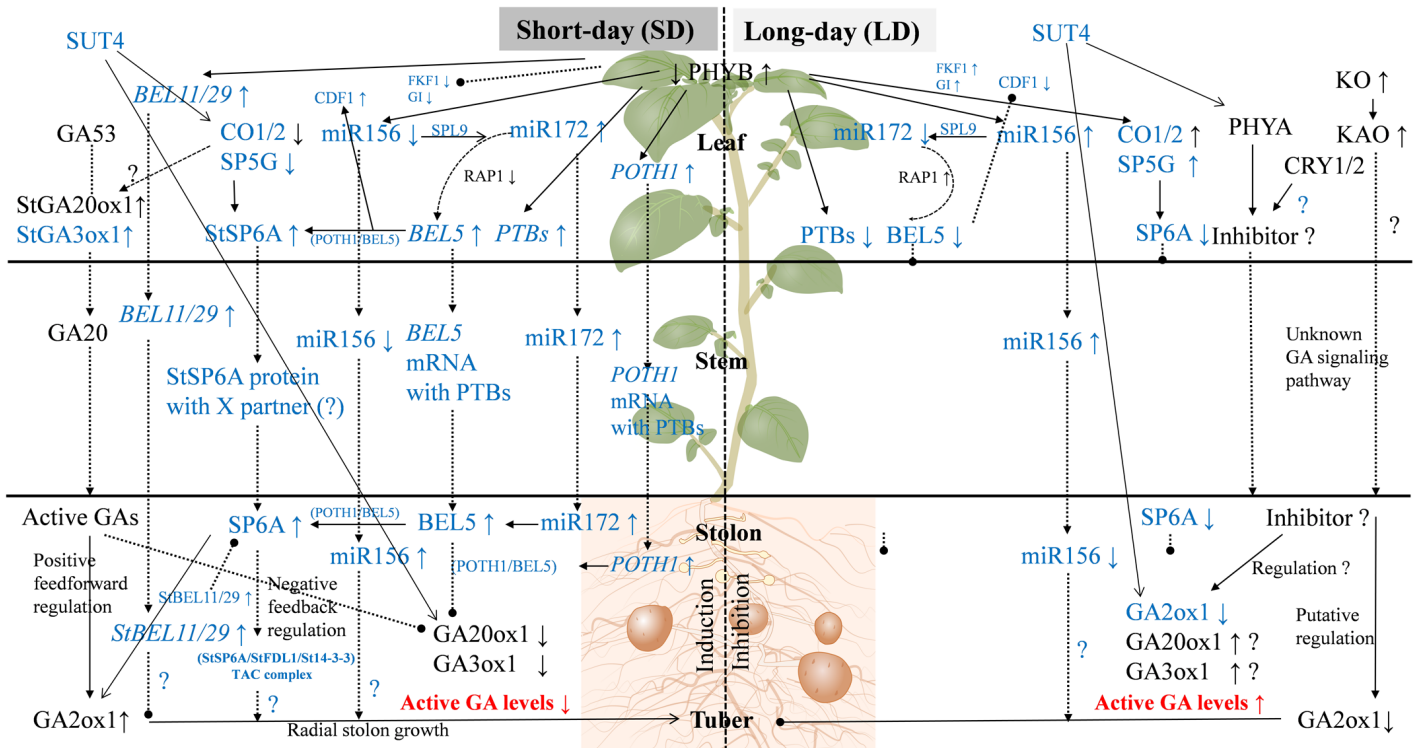
Fig. 3. Updated model for photoperiod-mediated regulation of tuberization in potato (*S. tuberosum* ssp. *andigena*). *Components represented in blue represent the recent additions to the pathway proposed earlier by Sarkar (2010). Dotted or thick line arrow (→) indicates induction, whereas dotted or thick line with closed end (–•) represents repression.*

siRNAs. Many of these siRNAs targeted crucial tuberization genes, such as *StGA2ox1*, *StGA3ox1*, *StPTB1, POTH1* and *StCDPKs* (Kondhare *et al.,* 2018). This study suggests a new layer of regulation for tuberization pathway by siRNAs and their putative targets. Further research is needed for their functional characterization in potato development.

Is the tuberization mechanism epigenetically regulated?

Polycomb Group (PcG) proteins are implicated in regulating the transition from juvenile-to-adult phase in plants. Pico *et al.,* (2015) reported that the suppression of one of the PRC1 (Polycomb Repressive Complex 1) members, AtBMI1, results in up-regulation of miR156 in *Arabidopsis*. Moreover, another PRC1 member, EMBRYONIC FLOWER (EMF1) participates in the regulation of SPL and miR172 genes (Pico *et al.,* 2015), and its mutants produce early flowering phenotype (Kim *et al.,* 2012). During photoperiodic and vernalization pathways, a PRC2 member MULTICOPY SUPRESSOR OF IRA (MSI1) governs the transition to flowering time, possibly by functioning upstream of crucial flowering genes, like FT, CO, Flowering Locus C (FLC; flowering repressor) and agamous-like 19 (AGL19) (Steinbach and Hennig, 2014). Hence, it would be worth investigating if PcG proteins mediate the regulation of miR156 and miR172 or any other tuber marker genes (*LOG, IPT, GA2ox1, GA20ox1, PIN1/4*) in potato.

An updated tuberization pathway

Considering the recent advancement in tuber development

pathway, we propose an updated model for tuberization (Fig. 3). As shown in this model, under SD photoperiod, PHYB indirectly regulates *StBEL5* gene expression in leaf *via* miR156-StSPL9 and miR172-StRAP1 modules. Simultaneously, *POTH1* mRNA and PTB proteins (StPTB1 and -6) are also induced in the leaves. The majority of *StBEL5* and *POTH1* mRNAs are transported from leaf to stolons via the RNP complex with StPTB1/6, and both RNAs get translated in the stolon. *StBEL5* mRNA seems to translate in the leaf also, where its protein possibly along with a KNOX partner induces *StSP6A* and *StCDF1* gene expression. Moreover, StCDF1 protein activity in leaf is regulated by the interaction with circadian clock proteins StGI and StFKF1 that indirectly induces StSP6A protein levels, by removing tuber repressors such as StCO1/2 and StSP5G. StSP6A protein as well as mRNAs of *StBEL11* and *-29* move from leaf to stolon. StPTB1/6's role as positive regulators of tuberization is demonstrated, however, it appears that they increase tuber yield indirectly by enhancing the accumulation of *StBEL5* and *POTH1* mRNAs in the stolon. StBEL5-POTH1 protein complex in the stolon is shown to regulate the expression of tuber marker genes, such as S*tGA20ox1*, *StGA3ox2* and *StGA2ox1*. StBEL11 and StBEL29 possibly along with a KNOX partner repress *StSP6A* gene expression to inhibit tuber formation. How this StBEL5/11/29 tripartite activator/repressor module works need further research. StSP6A protein has been shown to induce *StGA2ox1* in the stolon tip that reduces bioactive GA levels to stimulate tuber induction. Similar to the FAC complex at the SAM, StSP6A protein forms a TAC complex by interacting with StFDL1 and St 14-3-3 in the stolon. However, downstream components of TAC complex that regulate tuber development are not known. Silencing one of the

potato sucrose transporters, StSUT4, induced tuber formation, possibly by regulating StCO1/2, StSP6A and GA metabolism genes (*StGA20ox1*, *StGA3ox2* and *StGA2ox1*) (Chincinska *et al.*, 2013). GA metabolism seems to be regulated by both photoperiod-dependent as well as -independent pathways. Under LD conditions, the levels of *StBEL5* and *StSP6A* are reduced. As a result, GA levels increase causing inhibition of tuberization. Similar to PHYB, PHYA also functions as a repressor of tuberization. However, its downstream signalling pathway is less investigated. Blue light receptor's (CRY1/2) role in tuber development is also not known.

Although we have gained a significant knowledge about the majority of the molecular signals, there are still many questions that need to be answered in near future to shed more light on the tuber development pathways. Some of these are listed below.

- What is the protein partner(s) of StSP6A that helps in its phloem mobility from leaf to stolon? Does StBEL5 protein interact with StSP6A protein in the stolon?
- StBEL5 induces *StSP6A* gene in both leaf as well as in stolon/tuber. Does tissue specific activation have any direct correlation with the process of stolon to tuber transitions?
- Is *StCDF1* mRNA or protein phloem mobile?
- Do novel miRNAs/phased siRNAs and their target genes control stolon-to-tuber transitions?
- Are there any epigenetic modifiers, such as polycomb or trithorax group proteins, involved in SD-mediated induction of tuber activation pathways?
- Which molecular players distinguish photoperiod-sensitive potato cultivars from the day-neutral ones?
- A stolon develops into a tuber or a new shoot depending on whether it senses light or not. How does the fate of the stolon apical meristem (STAM) change?

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