博士論文番号：1181205

Analysis of florigen function in potato tuberization

（ジャガイモ塊茎におけるフロリゲン機能解析）

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Submitted on March 12, 2015

Abstract

Photoperiod-regulated flowering and potato tuber formation involve leaf-produced mobile signal, florigen and tuberigen respectively. The molecular nature of florigen has been revealed as FT/Hd3a protein in long-day (LD) plant Arabidopsis/short-day (SD) plant rice. In rice shoot apex, Florigen Activation Complex (FAC) composed of Hd3a, 14-3-3 and bZIP transcription factor OsFD1 induces transcription of floral meristem identity genes such as *OsMADS15*. Intriguingly, overexpression of *Hd3a* triggers strict SD potato to form tuber under LD conditions and the induction signal is graft-transmissible. Two different *FT*-like paralogues *StSP3D* and *StSP6A* that function in day-neutral flowering and in daylength-dependent tuberization respectively were found in potato plants. However, the molecular mechanism of how StSP6A triggers tuber formation at the stolons remains elusive. Shared common elements between rice flowering and potato tuberization has led to notion that FAC-like complex may be involved in potato tuberization. Hence, we hypothesized the presence of a FAC-like complex, termed Tuberigen Activation Complex (TAC) in triggering potato tuberization. Potential TAC members (StSP6A, StFDs and St 14-3-3s) were isolated from *Solanum tuberosum*. Interactions of StSP6A with 14-3-3s and potato FD homologs (StFD/ FD-like) were confirmed by Y2H analysis. The conserved key amino acid residues of StSP6A and phosphorylation of the TAP motif of StFD/FD-like were found essential for the binding to potato 14-3-3 proteins. Besides, the expression patterns of potential TAC members show positive correlation to tuberization. Early tuberization was observed when *StSP6A* was overexpressed. Mutation in StSP6A with impaired 14-3-3 binding affinity compromised the promotion of tuberization. Suppression of *StFDL2* delayed tuberization, but that of *StFD* did not. The molecular mechanism of how florigen regulates tuberization will be discussed.

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Acknowledgements

I would like to give thanks to the Al-mighty God Father for giving me strength and wisdom to end this 4-year journey in Japan. May His name be glorified and praised.

I wish to express my sincere appreciation to the late Professor Ko Shimamoto who entrusted me with the project. I am grateful for the wonderful opportunity given to have my PhD training in NAIST. His guidance, constructive criticisms and support will always be remembered. Very special thanks to Assistant Prof. Dr. Kenichiro Taoka, for his suggestions and advice throughout the study. This project will not be completed without his direct supervision and contribution. Not to forget my supervisory committee members, Professor Takashi Hashimoto, Professor Masaaki Umeda and Associate Prof. Mitsuhiro Aida for their valuable comments, advice and discussion that have helped to improve this research.

I would also like to extend my gratitude to my PhD comrades, especially Dr. Lai Pey Jiun and Dr. Abbie Yong, and church members from Nara International Christian Church for their love and emotional support. I would never been able to keep motivation without your prayers, encouragement and trust. Also, thanks to all my friends in Japan for making my life here so colorful.

Many thanks to the former and current lab members: Assistant Prof. Dr. Hiroyuki Tsuji, Assistant Prof. Dr. Yoji Kawano, Dr. Masayuki Higuchi, Dr. Minoru Nagano, Dr. Tadashi Fujiwara, Dr. Akira Akamatsu, Dr. Dang Thi Thu and Dr.-to-be Miho Suzuki for their kind help, sharing, brainstorming discussion and warm friendship. It is also my pleasure to acknowledge the funding sources from NAIST Global COE program and School of Biological Sciences that fully support my study life here.

Last but not least, I would like to dedicate this thesis to my beloved parents, sister and brothers for their endless love, support and trust to make this thesis a reality.

I. Introduction

I. 1. The potato crop

The cultivated potato, *Solanum tuberosum* L. originated from the Andean region of southern Peru is being grown worldwide. It is a highly heterozygous tetraploid species with 48 chromosomes (van Eck *et. al.*, 1994). Nowadays, potato has become the third most important food crop in the world, after rice and wheat (www. FAO.com). Easy cultivation and high nutritional value (Scott *et al.*, 2000) of potato have made this crop an excellent staple food to feed the growing world population.

Potato is a monocarpic perennials plant and in most cases is propagated vegetatively by using tubers (seed tubers). Potato tubers are shortened and thickened underground stems (stolons) that bear scale leaves subtending the dormant axillary buds or tuber "eyes" which will sprout and grow into new plant after undergo an endodormancy period (Ewing and Struik, 1992). The induction of tuber formation is a key developmental transition for the production of potatoes. This process is naturally favorable under short days (or long nights) and cool temperature conditions as would occur in autumn in order to ensure the survival of the plant during cool winter period. However, modern breeding and cultivation of potato has generated potato cultivars with considerable variation in response to day length due to iterative selection for early tuberization i.e. tuber formation in these cultivars can occur under the long days of midsummer and is relatively insensitive to day length as compared to wild potatoes such as *S. tuberosum* cv. andigena which is strictly short-day dependent (Kumar and Wareing, 1973).

I. 2. Development of potato tuber

Potato tuberization/tuber formation is a very complex developmental event that leads to the differentiation of underground stolon into a specialized storage organ and vegetative propagation system (Taylor *et. al.*, 1992; Visser *et. al*., 1994). The tuberization process occurs in sequential manner with the beginning of stolon initiation and stolon growth, followed by

cessation of stolon elongation, tuber induction and initiation (Fig 1). The initiated tubers undergo continual growth and reach maturity at the end of the plant cycle (Ewing and Struik, 1992).

Stolons are diageotropic lateral stems that arise from the underground nodes of the main stems, with long internodes and a hook-like shape of apical region. Under favorable conditions i.e. long days and high temperature, stolon elongation and branching occur (Booth, 1959). At the onset of tuber formation, longitudinal growth of stolons cease and induction and initiation of tuber formation take place with visible swelling of the sub-apical region of the stolon (Cutter, 1992). The swelling is due to longitudinal cell division and expansion in most parenchyma cells at the sub-apical region (Xu *et al*., 1998b). When this swelling reaches about twice the diameter of the subtending stolons, tuber initiation is considered to begin. The continuous increase in size and weight of tubers are the results of randomly oriented cell divisions and cell enlargement and starch and protein deposition (Xu *et al*., 1998b). The final size of tubers depends on the genotypes and environmental conditions. The tuber's skin will be set and suberized at maturity. Tubers undergo gradual increase in dormancy at the beginning of its initiation and reach maximum at the end of the plant cycle or at harvest after haulm removal, or shortly thereafter (Burton *et al*., 1992; reviewed by Claassens & Vreugdennil, 2000). The dormancy is released after few months of physiological rest and tubers will then sprout and serve as tuber seeds for new plant development.

I. 3. Regulation of potato tuber initiation

Potato tuber is a specialized vegetative structure which serves as asexual propagation organ and energy reservoir for the development of new plant. The transition of stolon to tuber at the correct timing is therefore crucial for plant regeneration and survival. The onset of tuberization is controlled by complex regulatory machinery involving various genes to perceive and respond to endogenous factors such as gibberellin levels and physiological age,

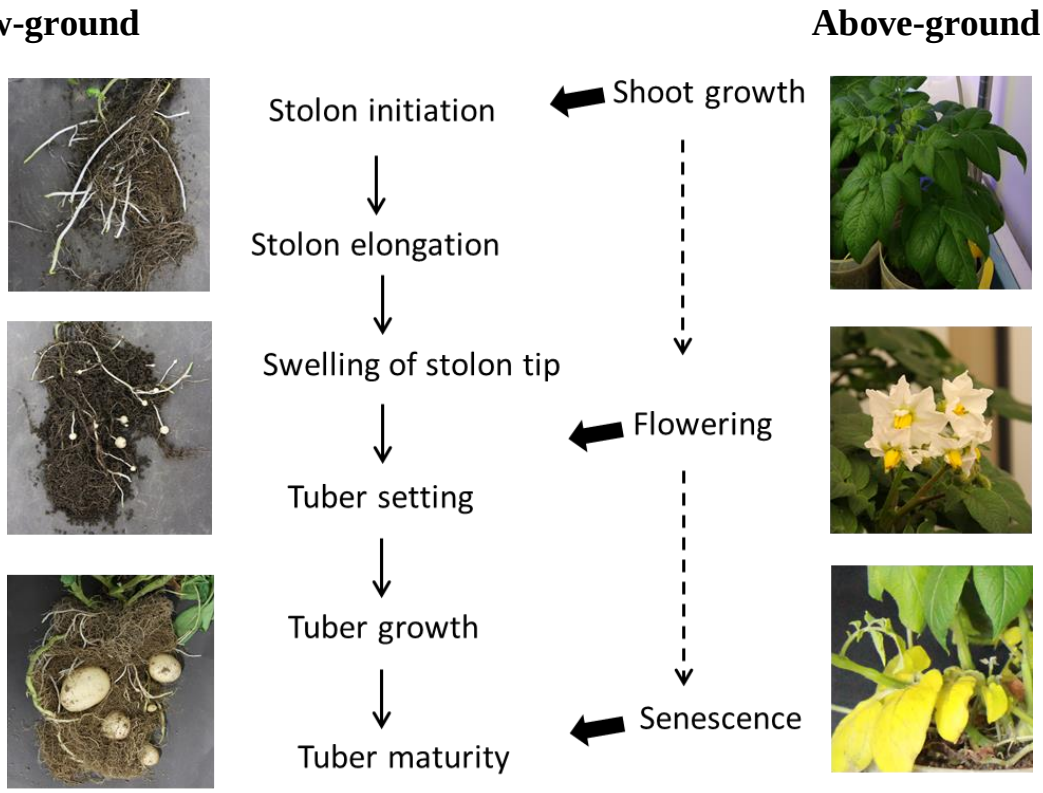


Fig. 1. Schematic representation of morphological events involved in potato plant development and tuber formation

as well as environmental cues such as temperature, nitrogen levels and daylength to ensure the occurrence of this developmental switch at optimal timing. High temperature and high nitrogen levels in soil delay tuberization (Menzel, 1983; Menzel, 1985; Simpson, 1962). Short days/long nights with low temperature promote tuber induction, although the strength of the photoperiodic response varies with different genotypes (Snyder and Ewing, 1989). Extensive studies have been undertaken to pinpoint the photoperiodic components and signaling pathways that may be involve in the regulation of tuber transition (reviewed by Abelenda *et al*., 2011, Hannapel, 2013, Suarez-Lopez, 2013 and Navarro *et al*., 2014).

I. 3. a. Photoperiodic regulation

Potato tuberization is induced under short day conditions (SD) and inhibited under long day conditions (LD) (Ewing, 1978; Ewing and Struik, 1992). Several components for photoperiodic regulation, such as CONSTANS (CO) (Martinez-Garcia *et al*., 2002; Martinez-Garcia *et al*., 2012; Gonzalez-Schain *et al*., 2012) and photoreceptor phytochrome B (PHY B) have been shown to involve in photoperiodic control of potato tuberization (Batutis and Ewing, 1982; Jackson *et al*., 1996). PHY B acts as negative regulator that inhibits tuberization under LD. Decreased levels of *PHY B* expression in the antisense potato plants led to loss of photoperiod dependence and these transgenic plants tuberize constitutively under both LD and SD conditions (Jackson *et al*., 1996; Jackson *et al*., 1998). This inhibitory effect is found to be resulted from the production and transmission of an inhibitor of tuberization in leaves controlled by PHY B under LD conditions (Jackson *et al*., 1998). Nevertheless, the identity of this inhibitor and detailed mechanism on how PHYB controls photoperiodic tuberization remains to be studied.

Grafting experiments in potatoes have provided evidence that leaves is the site of daylength perception in potato (Gregor, 1956; Chapman, 1958). Under inductive SD conditions, a graft-transmissible tuber inducing signal (tuberigen) is synthesized in the leaves and transported basipetally to the stolon tips, where tubers are induced (Ewing and Struik, 1992). Little is known about the identity of this leaf-produced transmissible tuberigen. Interspecific grafting experiments showed that grafting of flowering tobacco scions to non-inductive potato stocks were able to induce tuber formation (Fig. 2). This finding indicated that tuberization and flowering may be mediated by the same mobile signal (Chailakhyan *et al*., 1981) i.e. flowering-inducing signal; florigen may be functionally similar to tuberigen. Common signaling cascades are therefore possibly shared in daylength-induced flowering and potato tuberization. In *Arabidopsis*, CO promotes flowering in response to day length (Samach *et al*., 2000). Interestingly, ectopic expression of *AtCO* delays tuberization in potato (Martinez-Garcia *et al*., 2002) and silencing of endogenous potato *CO* induces tuber

formation under non-inductive LD conditions (Gonzalez-Schain *et al*., 2012). All these observations suggest that CO may functions as repressor in potato tuberization. CO-FT module which involves in flowering regulation may also participate in controlling photoperiodic potato tuberization (Gonzalez-Schain *et al*., 2012).

The identification of FLOWERING LOCUS T (FT) protein in *Arabidopsis* and its homolog Hd3a in rice as the mobile floral-inducing signal, florigen, (Corbersier *et al*., 2005; Tamaki *et al*., 2007) has provided useful clues and has much aided in the determination of the molecular nature of tuberigen in potato. Overexpression of rice *Hd3a* triggers strict short-day potato types to form tuber in long days and the induction signal is graft-transmissible (Navarro *et al*., 2011). This strongly suggested that tuberigen may be a FT-like protein in potato. With the fully sequenced potato and tomato genome, several FT homologs were isolated from potato. *StSP3D* gene, the ortholog of tomato *SFT*, plays major role in day-neutral flowering (Molinero *et al*., 2004; Lifschitz *et al*., 2006; Navarro *et al*., 2011) while StSP6A, another potato FT ortholog acts as the major tuber-inducing signal in potato. *StSP6A* is expressed in leaves under SD conditions. Overexpression of *StSP6A* leads to tuber formation under LD while suppression of *StSP6A* showed significant reduction in tuber number under SD. Moreover, the tuber-inducing effect of StSP6A is graft-transmissible. Notably, an autoregulatory loop exists in underground stolons regulating the local expression of *StSP6A* in stolons upon receiving StSP6A from aboveground parts of potato (Navarro *et al*., 2011). This phenomenon has not been found in flowering regulation of *Arabidopsis* and rice. Based on the experimental proofs obtained so far, it is very likely that StSP6A serves as the potato tuberigen that is responsible for stolon-tuber transition.

I. 3.b. Hormonal control of tuber formation

Plant hormones have great impacts in driving potato tuber transition, as reviewed by Rodriguez-Falcon *et al*. (2006) and Roumeliotis *et al.* (2012). Gibberellins (GAs) promote

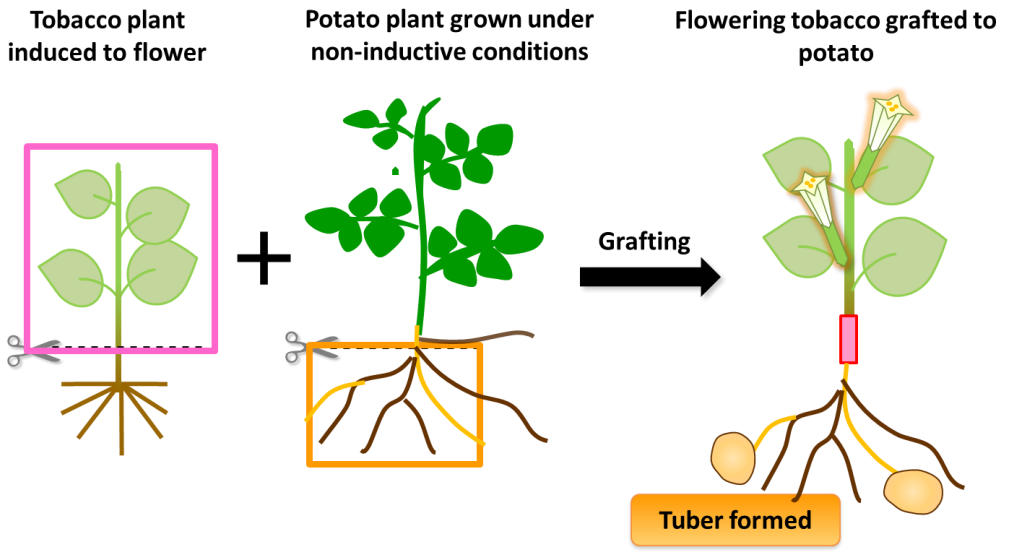


Fig. 2. Schematic representation of the interspecific grafting experiment between tobacco plants induced to flower and potato grown under non-inductive long day conditions (modified from Chailakhyan *et al*., 1981).

stolon growth but play an inhibitory role in tuberization onset (Xu *et al*., 1998a). Local induction of the active GA levels in the stolon is promotive for tuber transition (Kloosterman *et al*., 2007). Cytokinin (CK) stimulates tuber formation in many tuberiferous plant species (Ewing, 1995). Recent study showed that the ectopic expression of tomato *LONELY GUY 1* gene (*TLOG1*) encoding a cyctokinin-activating enzyme can induce aerial minitubers formation in the axillary meristems of tomato plants (Eviatar-Ribak *et al*., 2013). These findings imply that CK acts as general regulator of plant storage organ formation. On the other hand, the positive role of auxin in potato tuberization has been supported by numerous studies. It was demonstrated that auxin-related transcripts such as *StPIN-like* and *StacrA-like* expression were peaked upon tuber initiation. Besides, auxin content was strongly increased in the region proximal to the stolon swelling site and remaining high in the subsequent tuber growth (Faivre-Rampant *et al*., 2004; Kloosterman *et al*., 2005; Roumeliotis *et al*., 2012), indicating the involvement of auxin in tuber development. Strigolactones (SLs), a

phytohormone that controls shoot branching has also been implicated in tuberization. Silencing of strigolactones biosynthetic gene *StCCD8* in pototo leads to enhanced shoot branching, reduced stolon formation, loss of diageotropic growth nature of stolon, and aerial tubers formation (Pasare *et al*., 2013). The reduced dormancy and increased secondary growth of the new developing tubers in *StCCD8 RNAi* lines suggested that SLs play an important role and acts synergistically with auxin (Roumeliotis *et al*., 2012) to dictate the architecture of potato plants by inhibiting shoot branching and maintaining tuber bud dormancy.

Nevertheless, there is no clear role defined for any plant hormone in the direct signaling of tuber induction i.e. instead of triggering the onset of transition, it is thought that these hormones work in coordinated manner with tuberigen and is more likely involved in the process of transition upon tuber induction (reviewed by Aksenova *et al*., 2012; Roumeliotis *et al*., 2012; Navarro *et al*., 2014).

I. 4. Perception of the florigen and tuberigen signal

The regulatory mechanism driving *FT* gene activation and function in triggering floral transition in the shoot apex has been well elucidated. FT belongs to PEBP gene family and has been identified as a florigen which is transported from leaves to shoot apical meristem to induce floral evocation. In *Arabidopsis*, FT interacts with basic leucine zipper (bZIP) transcription factor FD and activates floral meristem identity genes, *APETALA1* (*AP1*), *SUPPRESSOR OF CONSTANS1* (*SOC1*) and *FRUITFUL* (*FUL*) to initiate floral transition (Wigge *et al*., 2005; Abe *et al*., 2005). In rice, Hd3a interact with rice FD OsFD1 via 14-3-3 protein to induce *OsMADS15* transcription (Taoka *et al*., 2011). 14-3-3 proteins are a family of conserved eukaryotic regulatory molecules that function as scaffold or adapter proteins. They bind to serine/ threonine-phosphorylated residue of target proteins such as kinases (Masters and Fu, 2001), transcription factors (Gampala *et al*., 2007), ion channels (Jahn *et al*., 1997) and pathogen defense-related proteins (reviewed by Robert *et al*., 2002) to modulate

their biological activities. This plethora of interacting proteins enables 14-3-3 to play critical roles in a wide range of plant physiological processes, such as biotic and abiotic stress response, primary metabolism, cell growth and division control, light response and hormone signaling pathways (reviewed by Denison *et al*., 2011). The seminal finding of 14-3-3 proteins as a florigen receptor in rice flowering has unraveled the molecular mechanism on how Hd3a induces rice floral evocation in the rice shoot apical meristem. By forming a binary complex with Hd3a in the cytoplasm, 14-3-3s facilitate the translocation of Hd3a into the nucleus to bind to OsFD1. The ternary protein complex comprised of Hd3a, 14-3-3 protein and OsFD1 is thus formed in the nucleus and induces transcription of floral meristem identity genes that lead to flowering (Taoka *et al*., 2011). The crystal structure of this complex, named the Florigen Activation Complex (FAC) has been determined. The binding of 14-3-3 proteins is essential in controlling the activity of florigen by regulating its transport to the nucleus and mediating its binding to OsFD1 (Taoka *et al*., 2011). Despite floral transition, FT participate in various plant growth and development events such as stomata opening (Kinoshita *et al*., 2011), bud set and cessation in tree (Bohlenius *et al*., 2006) and onion bulb formation (Lee *et al*., 2013). It is thus thought that FT acts as a mobile and universal signal for plant development. Undoubtedly, FAC ternary complex has presented a complex module that may help to explain the wide array of FT functions in different plant developmental events (reviewed by Pin and Nilsson, 2012 & Taoka *et al*., 2013). The different biological activities conferred by FT may be determined by the transcription factors integrated into the ternary complex (Taoka *et al*., 2013; Tsuji *et al*., 2013).

StSP6A, one of the FT members in potato, was identified as tuberigen in potato (Navarro *et al*., 2011). The mechanism of StSP6A perception in potato stolons is still largely unknown. Based on the modularity provided by FAC ternary complex, it is probable that StSP6A forms complex with FD-like transcription factors via 14-3-3 proteins to triggers tuber-specific genes expression that ultimately lead to tuber induction (Fig. 3). Nevertheless,

vigorous experimental testing is needed to validate the presence and function of this complex in potato tuberization.

I. 5. Objectives of the study

Shared common elements between rice flowering and potato tuberization and the identification of potato FT, StSP6A as the tuber-inducing signal has led to notion that an FAC-like complex may be involved in potato tuber induction (Fig. 3). The FAC-like complex, termed Tuberigen Activation Complex (TAC) was hypothesized to trigger potato tuberization. In this study, potential TAC components (*StSP6A*, *StFD* and *St14-3-3*) were isolated from potato. Expression, interaction and functional analysis of these candidates were also carried out to understand the mechanism of how tuberigen is perceived in potato tuber induction.

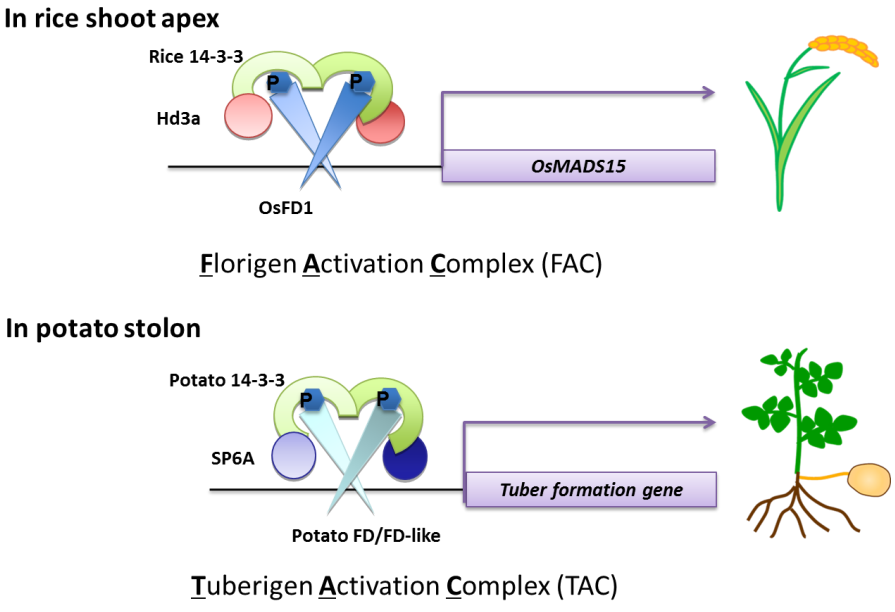


Fig. 3. Model of Tuberigen Activation Complex (TAC) in regulating potato tuberization. Potato FT, StSP6A forms protein complex with potato FD/FD-like via 14-3-3 proteins to trigger potato tuber induction.

II. Materials and Methods

II. 1. Plant materials and growth conditions

Potato (*Solanum tuberosum* group tuberosum *ssp* Sayaka), a commercial potato variety was used as wild-type. For potato tissue culture and *in vitro* tuberization assay, *in vitro* cultured plants were grown in growth chambers (Sanyo MLR-351H) at 22˚C with a light/dark cycle of 16 h/ 8 h (LD) or 8 h/ 16 h (SD). Light was provided by fluorescent white light tubes with intensity of 80 μmol $m^{-2}s^{-1}$. For soil tuberization analysis, *in vitro* cultured plants were grown for 3 weeks before transferred to 2 L soil filled pots. Soil plants were grown in climate chamber under LD/ SD conditions with daily cycle of 16 h/ 8 h (LD) or 8 h/ 16 h (SD) at 20˚C. Light was provided by white LED illumination for LD and fluorescent white light tubes for SD with intensity of about 100-120 μmol $m^{-2}s^{-1}$.

II. 2. Isolation of *StSP6A, StSP3D, StFD, StFDL1, StFDL2* and *St14-3-3s*

Based on potato genome sequence information obtained from Potato Genome Sequencing Consortium (PGSC) database (http://potatogenomics.plantbiology.msu.edu), full length cDNAs for *StSP6A*, *StSP3D*, *StFD(L)s* and *St14-3-3s* homologs in potato were cloned by RT-PCR with KOD Neo PCR polymerase (Toyobo, Japan). cDNAs from leaves, stems or shoot apex of potato were used as templates. To obtain the correct ORF regions, RT-PCR cloning was first performed with primer sets which can anneal with predicted 5' and 3'-untranslated regions. After amplified fragments were sequenced, primers for ORF were designed and ORF regions were PCR-amplified and cloned into pENTR™/D-TOPO vector (Invitrogen, USA) to obtain entry clones. Site-directed mutagenesis was performed with primers listed in Table 1.

II. 3. Plasmid construction and Plant transformation

II. 3. a. *StSP6A*, *StSP6A* mutants, *StFD*, *StFDL1* and *StFDL2* overexpression potato lines

To construct plasmids for overexpression of *StSP6A* and m*StSP6A* under the control of *CaMV 35S* promoter, their coding regions were cloned into pGWB5 (Nakagawa *et al*., 2007) using LR clonase™ II (Invitrogen, USA). To construct plasmid for overexpression of *StFD*, *StFDL1*and *StFDL2* under the control of CaMV35S promoter, their coding regions were cloned into pGWB21 (Nakagawa *et al*., 2007)

II. 3. b. *StSP6A*, *StSP3D*, *StFD* and *StFDL2 RNAi* suppression potato lines

Full or part of coding regions of *StSP6A*, *StSP3D*, *StFD* and *StFDL2* were cloned into *RNAi* triggered vector pANDA35HK (Miki and Shimamoto, 2004) by using LR clonase™ II. Transformation into *Agrobacterium tumefaciens* GV3101 was carried out via electroporation.

II. 3. c. Generation of transgenic potato

Generation of transgenic potato was performed according to Yamamizo *et al*. (2006). Internodal segments (5-10 mm) collected from 3- to 4-weeks old potato plants were incubated in a saturated culture of *A. tumefaciens* GV3101 carrying the expression binary vector for 3 minutes. The internodal segments were then dried on sterile blotting paper and placed in 3C5ZR media (MS basal medium, with 3% (w/v) sucrose, 0.53 mg/L IAA, 1.75 mg/L zeatin riboside and 0.2% (w/v) phytagel) for 3-4 days at 22˚C under long day conditions. After 3-4 days of co-cultivation with GV3101, the explants were transferred to fresh 3C5ZR medium added with 0.3 g/L cefotaxime and 0.1 g/L kanamycin and sub-cultured in every 7 days for about 4 months. Initiated shoot buds were excised and transferred to S1 media (S1 basal medium, with 1.5% (w/v) sucrose, 0.3% (w/v) phytagel, Fe-EDTA and V2 vitamine) added with 0.3g/L cefotaxime and 0.03 g/L hygromycin. Candidates of putatively transformed shoots were selected for further analysis. Transgene expression or suppression was confirmed

by semi-quantitative RT-PCR and these transgenic lines were maintained in S1 media and subjected to tuberization analysis.

II. 4. Yeast 2-hybrid interaction assay

Yeast 2-hybrid assay was performed essentially according to Taoka *et al*., (2011). Gateway destination vectors pBTM116-GW (Bartel *et al*., 1993) and pVP16-GW (Hollenberg *et al*., 1995) were used to construct the bait and prey vectors by LR clonase II. Yeast cells were grown on SC medium with or without various concentration of 3-amino-1, 2, 4-triazole (3-AT) at 30℃ for five days. The concentration of 3-AT was determined by the bait-prey combination.

II. 5. *In vitro* pull down assay

In vitro pull down assay was done essentially according to Purwestri *et al*. (2009). *StSP6A, St14a* and *St14f* were cloned into pDEST15 and pDEST17 vectors respectively via LR recombination reactions (Invitrogen, USA). GST (driven by pGEX-6P-1 vector), GST-StSP6A, His-St14a and His-St14f were expressed in *Escherichia coli* BL21 Rosetta 2 (DE3) pLysS (Novagen, Germany) and purified with Glutathinoe-Sepharose 4B beads (GS4B) (GE Healthcare, USA) and HisTrap™ FF crude columns (GE Healthcare, USA) according to manufacturer's instructions. In vitro binding assay was performed as below. The concentration of each fusion proteins was determined by Coomasie blue staining. Equal amount of GST-StSP6A bound to GS4B and purified His-St14a or His-St14f were incubated in 1x PBS added with 1% Triton X overnight on rotator at 4°C, respectively. The beads were then washed with ice cold 1xPBS added with 1% Triton X for four times. Bound proteins were eluted in 1x SDS sample buffer by boiling for 10 minutes, separated by 10% SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) membrane (Milipore, Japan) and subjected to immunoblotting with anti-His antibody. After washing with Tris-buffered saline (TBS)

containing 0.1% Tween (TBST), the membrane was incubated for 2 h with anti-mouse IgG conjugated to horseradish peroxidase (GE Healthcare, USA). Detection was performed using enhanced chemiluminescence (ECL) protein gel blot detection reagents (GE Heathcare, USA) and visualized using LAS-4000 Imager (Fujifilm, Japan).

II. 6. Semi-quantitative RT-PCR analysis

Potato leaves, stems, developing stolons, roots and tubers of 2-, 4-, 6- and 8-weeks old *in vitro* cultured and soil-grown plants under LD and SD conditions were collected, respectively. Total RNAs were extracted by using the RNeasy Plant Mini Kit (Qiagen, USA) and treated with DNase I (Invitrogen, USA) to eliminate trace amounts of genomic DNA. For total RNA extraction from potato tubers, Trizol Reagent (Invitrogen, USA) and Fruitmate™ for RNA purification (Takara, Japan) were used to eliminate contamination of starch. After treatment of total RNA with DNase I, first-strand cDNA was synthesized from 1 μg of total RNA using SuperScript II reverse transcriptase (Invitrogen, USA). The cDNA were subjected to RT-PCR by using specific primers for each gene (Table 2) with exponential phase determined on 30-35 cycles with KOD FX Neo polymerase (Toyobo, Japan). Gel image captured using Gel Doc (Vilber Lourmat, Germany).

II. 7. Analysis of tuberization and flowering

Both *in vitro* cultured wild type and transgenic plants were grown in 22 ℃ under LD conditions in growth chamber for 3 weeks before transfer to 2 L soil filled pots. These plants were then shifted to either LD or SD conditions. For tuberization analysis, underground parts of soil-grown plants were checked at 4-, 6-, and 8-weeks after transferred from *in vitro* culture media to soil. Tuber number and tuber size (cm) were scored from at least 5 independent transgenic lines for each gene constructs. The data were collected from three independent experiments and subjected to statistical analysis.

To analyze the flowering time, the shoot apex of LD-grown potato was carefully checked for visible signs of flowering every 7 days. The flowering time was measured as the number of days from transferred to soil until the appearance of the floral bud.

II. 8. *In vitro* tuberization assay

In vitro tuberization assay was done essentially as described in Fixen *et al*. (2012) with small modifications. Stem segments including one node from 3-4 weeks old *in vitro* cultured plants were placed in S1 media under 16 hours of fluorescent white light (80 μmol $m^{-2}s^{-1}$) for 2 weeks. Subsequently, the *in vitro* cultured plants were transferred to S1 media containing 8% (w/v) sucrose and placed under SD, LD or dark conditions for 3-4 weeks. Tuberization conditions were checked and the number of lines with tuber induction was scored. Data from three experimental replicates with at least 5 independent lines included were collected and subjected to statistical analysis.

Table 1: Primer sets used for gene isolation and cloning

Primer Name	**Sequence (5' to 3')**	**Purpose**
StGF14-1 5'	TAATTCAATTCAACAAAAGAAAATAGGCAAAG	Isolation of *St14a* ORF
StGF14-1 3'	ACAATGATTCGCCGACACCTCTTGAACTTCAA	Isolation of *St14a* ORF
StGF14-2 5'	CTCCATTTTTTTCAACTCAGAGAGAAGATCGG	Isolation of *St14b* ORF
StGF14-2 3'	GAGCCCATTCCTAAATAGAGACACCATGCCAA	Isolation of *St14b* ORF
StGF14-3 5'	CTCTTTCGCCTAAATTTTCTCTCTACTTCATCC	Isolation of *St14c* ORF
StGF14-3 3'	GAGCCCATTCCTAAATAGAGACACCATGCCAA	Isolation of *St14c* ORF
StGF14-4 5'	TTTCTCCGGCGGAAATTACCGGCGATCGACG	Isolation of *St14d* ORF
StGF14-4 3'	TGAGCTCACCCTCCTGGAGGAGCGGTATCATG	Isolation of *St14d* ORF
StGF14-5 5'	CGAAAAACTCTCACTTTCTCTCTCTAATATCA	Isolation of *St14e* ORF
StGF14-5 3'	CCTAAAGAAATTTCACCAGTTTTTACTGCTGC	Isolation of *St14e* ORF
StGF14-6 5'	AACAAAAAGAGATCCCAAATACTGAATCCATT	Isolation of *St14f* ORF
StGF14-6 3'	AAGAAGTCAAATGATAATCTGAGGACCAGTTC	Isolation of *St14f* ORF
StGF14-7 5'	TGTTGAACCAGGTAACTTTACAGACAACAAAC	Isolation of *St14g* ORF
StGF14-7 3'	AATAAGGAGTACAAGACTAGGCTACTTAGGTA	Isolation of *St14g* ORF
StGF14-8 5'	AATCTTTGATTTCAATTTGAGAGAGATCGGAA	Isolation of *St14h* ORF
StGF14-8 3'	AGACCAGCAATTATCTAAAGACTGAAACAAC	Isolation of *St14h* ORF
StGF14-9 5'	CCTCTTTCTCTCTCTAGAACACAGAACCATCA	Isolation of *St14i* ORF
StGF14-9 3'	TCCTAAGCAAAGAGGTTTCACTCACGTTGTTA	Isolation of *St14i* ORF
StGF14-10 5'	TTTGTTGAATCAGGTGTAATTTCACATACAAC	Isolation of *St14j* ORF
StGF14-10 3'	GATAAAAACTACAGGACTATGCTACTATCTCA	Isolation of *St14j* ORF
StGF14-11 5'	ATTCAGATCTCAAAAAACTTATTTCCGAGCAC	Isolation of *St14k* ORF
StGF14-11 3'	GTGAAAAGCAAATTAGAGGGTTTCAATTCCCC	Isolation of *St14k* ORF

Table 1: Continued

Primer Name	**Sequence (5' to 3')**	**Purpose**
StFD 5'-RACE	AGAATTGTCCTCTGTTTCAGGTACAACC	*StFD* full length cDNA isolation
StFD 3'-RACE	CACTTCAAGATCACACTACTAATTACTC	*StFD* full length cDNA isolation
StFDL1 5'	TCTTTACTCTTAAAGAGTTGCACTACTTTCAC	*StFDL1* full length cDNA isolation
StFDL1 3'	AAAATATTAGACATTTCATTGTTGGAAATTCC	*StFDL1* full length cDNA isolation
StFDL2 5'	TCTTTACTCTTAAAGAGTTGCACTACTTTCAC	*StFDL2* full length cDNA isolation
StFDL2 3'	AAAATATTAGACATTTCATTGTTGGAAATTCC	*StFDL2* full length cDNA isolation
StSP6A 5'-RACE	GCAATTGAAGTAAACAGCTGCAACAGGC	*StSP6A* full length cDNA isolation
StSP6A 3'-RACE	ACTCTGATTATGGTGGATCCTGATGCTCC	*StSP6A* full length cDNA isolation
StSP3D 5'	CACCAGTTTTATTTTGTTTGTTTATCGTGAACCATCATC	*StSP3D* full length cDNA isolation
StSP3D 3'	CGTAATTAATAAGTAGTAGTAGAGTTATAGATATATA	*StSP3D* full length cDNA isolation
StFT R60K-U	AAGATCGTCCCCTCCAATATGAACC	PCR-directed mutagenesis for *StSP6A R60K*

Table 1: Continued

Primer Name	Sequence (5' to 3')	Purpose
StFT R60K-D	ATATTGGAGGGGACGATCTTAAAAACTTTTACA	PCR-directed mutagenesis for *StSP6A R60K*
StFT R128K-U	TCTTGATTGTCGAAATAAAACCAA	PCR-directed mutagenesis for *StSP6A R128K*
StFT R128K-D	TTTTATTTCGACAATCAAGAAAAGAAACAGTGTA	PCR-directed mutagenesis for *StSP6A R128K*
StFT P92L-U	GATATCTGTGACCAGCCAATGTAG	PCR-directed mutagenesis for *StSP6A P92L*
StFT P92L-D	ATTGGCTGGTCACAGATATCTTGGCAACTACAAA	PCR-directed mutagenesis for *StSP6A P92L*
StFT-F99A-U	GCTTGTATTTGTAGTTGCTGGGAT	PCR-directed mutagenesis for *StSP6A F99A*
StFT-F99A-D	CAGCAACTACAAATACAAGCGCGGGAAATGAAG	PCR-directed mutagenesis for *StSP6A F99A*
StFDmE GWF	CACCATGTGGTCATCAAGCAGGTCTTC	PCR-directed mutagenesis for *StFD T206E*
StFDmE GWR	TCAAAATGGAGCGGCTGACGTCCGA	PCR-directed mutagenesis for *StFD T206E*
StFDL1mE GWF	CACCATGTGGTCATCAAGTAATGAAGAAC	PCR-directed mutagenesis for *StFDL1 T221E*
StFDL1mE GWR	TCAAAATGGGGCCTCTGATGTTCT	PCR-directed mutagenesis for *StFDL1 T221E*

Table 1: Continued

Primer Name	Sequence (5' to 3')	Purpose
StFDL2mE GWF	CACCATGTGGTCATCAAGTAATGAAGAAC	PCR-directed mutagenesis for *StFDL2 T228E*
StFDL2mE GWR	TCAAAATGGGGCCTCTGATGTTCT	PCR-directed mutagenesis for *StFDL2 T228E*
StFDmA GWF	CACCATGTGGTCATCAAGCAGGTCTTC	PCR-directed mutagenesis for *StFD T206A*
StFDmA GWR	TCAAAATGGAGCGGCTGACGTCCGA	PCR-directed mutagenesis for *StFD T206A*
StFDL1mA GWF	CACCATGTGGTCATCAAGTAATGAAGAAC	PCR-directed mutagenesis for *StFDL1 T221A*
StFDL1mA GWR	TCAAAATGGGGCCGCTGATGTTCT	PCR-directed mutagenesis for *StFDL1 T221A*
StFDL2mA GWF	CACCATGTGGTCATCAAGTAATGAAGAAC	PCR-directed mutagenesis for *StFDL2 T228A*
StFDL2mAGWR	TCAAAATGGGGCCGCTGATGTTCT	PCR-directed mutagenesis for *StFDL2 T228A*
StSP6A-GWF	CACCATGCCTAGAGTTGATCCATTGATAG	ORF cloning for overexpression and *RNAi*
StSP6A-GWR	TTATGCGCGACGTCCTCCAGTGCCAC	ORF cloning for overexpression and *RNAi*
StSP3D-GWF	CACCATGCCTAGAGAACGCGATCCTCTC	ORF cloning for *RNAi*
StSP3D-GWR	TCAATCAGCAGACCTTCTACGTCCACC	ORF cloning for *RNAi*
GUS link F out	CGTAAGTCCGCATCTTCATG	*RNAi* clones verification

Table 1: Continued

Primer Name	**Sequence (5' to 3')**	**Purpose**
GUS link R out	CCGAATACGGCGTGGAT	*RNAi* clones verification
23897-GWF	CACCATGTGGTCATCAAGTAATGAAGAAC	*StFDL2* cloning for *RNAi*
FDL2-Ri-GWR	GTCACCAGAATTGTTATCTGATTC	*StFDL2* cloning for *RNAi*
StFD-GWF	CACCATGTGGTCATCAAGCAGGTCTTC	*StFD* cloning for *RNAi*
FDFDL2-RiU	ATGACCACATGTCCCCTGTAGAATTGTCCTCTG	*StFD* cloning for *RNAi*

Table 2: Primer sets used in semi-quantitative RT-PCR analysis

Primer Name	**Target Gene**	**Sequence (5' to 3')**
StGAPDH-A	*StGAPDH*	TTCACTGTTGTCGTACC
StGAPDH-S	*StGAPDH*	CAAGGACTGGAGAGGTGG
StSP6A-F	*StSP6A*	ACTGGAGGACGTCGCGCATAA
StSP6A-R	*StSP6A*	CGCTCCTGAATCATGTTATAGATCTC
23897-5	*StFDLs*	TCTTTACTCTTAAAGAGTTGCACTACTTTCAC
23897-3	*StFDLs*	AAAATATTAGACATTTCATTGTTGGAAATTCC
03652-5'	*StFD*	TGTTCTCTATATATTTTTTTTCAAGAATCCAA
FDFDL2 Ri-U	*StFD*	ATGACCACATGTCCCCTGTAGAATTGTCCTCTG
06415-GWF	*St14a*	CACCATGGAGAAGGAAAGAGAGAAACAG
06415-GWR	*St14a*	CTAGTTCTCTCCCTGGCGCTCATC
07807-GWF	*St14b*	CACCATGGCTTCATCCAAAGAACGTGAA
07807-GWR	*St14b*	TCACTCTGCATCCTCGCCCACTTTG
12899-GWF	*St14c*	CACCATGGCGGCTCCAATCCCTGAAAATC
12899-GWR	*St14c*	TCAAGATTCATCCAACTGATCCTGAG
16141-GWF	*St14d*	CACCATGGCCTTGCCTGAAAATTTAACC
16141-GWR	*St14d*	TCAAGCCTCGTCCATCTGCTCCTG
16221-GWF	*St14e*	CACCATGGCGTCGCCACGCGAGGAAAAC
16221-GWR	*St14e*	TCATTCATTATTATCTGGTTTAGG
17753-GWF	*St14f*	CACCATGGCGCGTGAGGAGAATGTGTAC
17753-GWR	*St14f*	TCACTGTTGTTCATTGTCGGGTTTG
19587a-GWF	*St14g*	CACCATGGCCGATTCACGTGAAGAAAATG
19587a-GWR	*St14g*	TCACTGCTGTCCATCACCCGACTC

Table 2: Continued

Primer Name	**Target Gene**	**Sequence (5' to 3')**
20425-GWF	*St14h*	CACCATGGCTTCTTCCAAAGAACGTGAG
20425-GWR	*St14h*	TCACTCTGCATCTTCACCTCCACC
23590-GWF	*St14i*	CACCATGGCGTCTCCACGTGAAGAGAAC
23590-GWR	*St14i*	CTACTCATTATCAGCTTTTGATGG
24187-GWF	*St14j*	CACCATGGCTGACTCTTCGCGTGAAGAA
24187-GWR	*St14j*	TCACTGCTGCCTCTCGCCTGACTC
30814-GWF	*St14k*	CACCATGGCGGTGGCACCGACGGCGCG
30814-GWR	*St14k*	TCAAATTTTTTCTTCAGGTTTGGG
StSP3D-GWF	*StSP3D*	CACCATGCCTAGAGAACGCGATCCTCTC
StSP3D-3'	*StSP3D*	CGTAATTAATAAGTAGTAGTAGAGTTATAGATATATA

III. Results

III. 1. Determination of tuberization time of potato

Commercial potato cultivar Sayaka was used in this study because an efficient transformation protocol is available (Yamamizo *et al.,* 2006) and maintenance and growth of *in vitro* cultured potato are relatively easy. However, as in most of the cultivated potato plants, the short-day requirements for tuberization have been weakened in potato Sayaka i.e. tuber formation can eventually occur under LD conditions. To examine day length requirement of potato in this study, a total number of 15 potato plants were planted in soil under SD and LD conditions, respectively, and the underground parts of these plants were observed every two weeks. It was found that tuber induction occurred more rapidly under SD conditions (8h light/16h dark), in which tuber formation was observed at 4 weeks after planting (WAP) whereas under LD conditions (16h light/8h dark), tubers were induced at 6 WAP (Supplementary Fig. 1). This suggested that potato Sayaka still retains some degrees of SD-dependency for tuber induction. These time points were used to determine early or delayed tuberization in subsequent transgenic analysis.

III. 2. Isolation of *StSP3D, StSP6A, StFD(L)s* and *St14-3-3s*

An FAC-like complex, Tuberigen Activation Complex (TAC), was hypothesized to regulate potato tuberization. TAC consists of three members: FT homolog, 14-3-3 protein and FD homolog. In *Solanum phureja* genome (The Potato Genome Sequencing Consortium, 2011), two *FT-like* genes, two *FD-like* genes, and eleven *14-3-3* genes were identified. By using PCR cloning strategy based on expected sequence conservation between potato phureja and potato Sayaka, intact ORF regions of these genes were cloned (Table 3 and Figs. 4-6). The two *FT* homologs isolated encoded for previously identified *StSP6A* and *StSP3D* (Navarro *et al.*, 2011, Table 3, Fig. 5). The key amino acid residues required for 14-3-3 binding in rice Hd3a were conserved in both potato StSP6A and StSP3D (Fig. 5b).

Table 3: FT, FD and 14-3-3 homologs in *Solanum tuberosum* cv. Sayaka

Gene name	Accession number	Locus ID[a]
StSP6A	LC011886	PGSC0003DMG400023365
StSP3D	LC011887	PGSC0003DMB400000142
StFD	LC011883	PGSC0003DMG400003652
StFDL1	LC011884	PGSC0003DMG400023897
StFDL2	LC011885	PGSC0003DMG400023897
St14a	LC011872	PGSC0003DMG400006415
St14b	LC011873	PGSC0003DMG400007807
St14c	LC011874	PGSC0003DMG400012899
St14d	LC011875	PGSC0003DMG401016141
St14e	LC011876	PGSC0003DMG400016221
St14f	LC011877	PGSC0003DMG400017753
St14g	LC011878	PGSC0003DMG400019587
St14h	LC011879	PGSC0003DMG400020425
St14i	LC011880	PGSC0003DMG400023590
St14j	LC011881	PGSC0003DMG400024187
St14k	LC011882	PGSC0003DMG400030814

[a]Corresponding locus in *Solanum phureja* genome

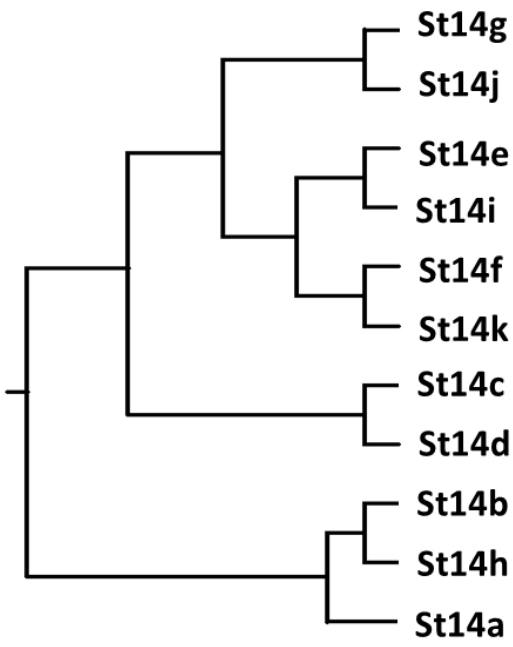


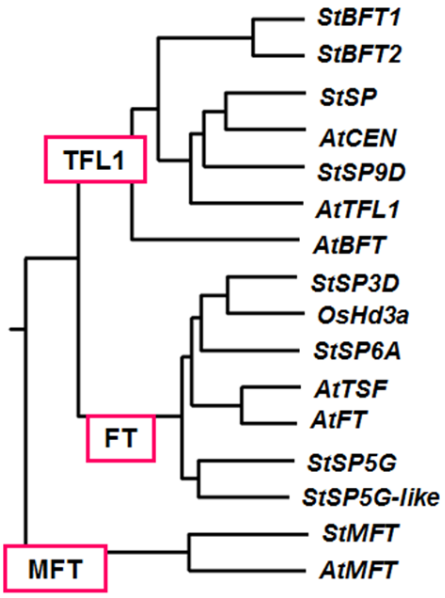
Fig. 4. Phylogenetic tree of 14-3-3 isoforms in potato.

On the other hand, three potato *FD-like* clones encoding predicted proteins with bZIP and S/TAP motif were obtained from PCR cloning. One was a closest homolog to tomato *SPGB* and named as *StFD*. The others were two different but highly similar between each other and named as *StFD-like 1* (*StFDL1*) and *StFD-like 2* (*StFDL2*) (Fig. 6). *StFDL1* and *StFDL2* are probably representing syntenic genes or different alleles of the same *StFD-like* gene in potato Sayaka, because only one corresponding gene can be found in phureja genome sequence. Sequence alignment analysis revealed that the StFD, StFDL1 and StFDL2 share homology with the bZIP motif and C-terminal phosphorylation motif (S/TAP motif) of Arabidopsis FD and tomato SPGB (Fig. 6a). The eleven potato 14-3-3 isoforms were named as *St14a-St14k* (Table 3 and Fig. 4).

III. 3. Expression analysis of *StSP6A, StFD(L)s* and *St14-3-3s*

Spatio-temporal expression patterns of *StSP6A*, *StSP3D*, *St14-3-3s*, *StFD* and *StFD-like* (*FDLs*) were examined by semi-quantitative RT-PCR. Preliminary expression analysis of *St14-3-3s* revealed that the eleven St14-3-3 isoforms are most likely expressed constitutively in potato plants (Supplementary Fig. 2). Therefore, *St14a* and *St14f* were selected as representatives of *St14-3-3s*. In *in vitro* cultured potato, *St14-3-3s* were expressed ubiquitously in leaves, stems and roots under both SD and LD conditions (Fig. 7). *StSP6A* was specifically expressed in leaves under SD conditions. On the other hand, *StSP3D* expression was not detected in all the tissues under both SD and LD conditions. Meanwhile *StFD* was expressed in leaves and stems and *StFD-like (StFDLs)* was expressed in stems and roots of *in vitro*-cultured potato under SD and LD conditions. Both potato *FD* homologs showed higher expression under LD condition as compared to SD condition in *in vitro*-cultured potato. In soil grown potato, *St14-3-3s* were constitutively expressed in all tissues under both SD and LD conditions. Under SD conditions, *StSP6A* expression was detected in leaves, stems and developing stolons (Fig. 8). While *StSP3D* expression was

A



B

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Hd3a      1 MAGSGRDRDPLVVGRVVGDVLDAFVRSTNLKVTYGSKTVSNGCELKPSMVTHQPRVEVGG 60
StSP3D    1 -MSSIRGRDTLELGGVISDVLDPFTRSISLSVVYNHREVINGTNLRPSQITNQPRVEIGG 59
StSP6A    1 ----MPRVDPLIVGRVIGDVLDPFTRSVDLRVVYNNKDVNNACVLKPSQVVMQPRVHIGG 56

Hd3a     61 NDMRTFYTLVMVDPDAPSPSDPNLREYLHWLVTDIPGTTAASFGQEVMCYESPRPTMGIH 120
StSP3D   60 NGLSTFYTLIVVDPDAPSPSNPNLREYLHWLVTDIPATTGVTFGNEVICYESPRPSMGIH 119
StSP6A   57 DDLRNFYTLIMVDPDAPSPSDPNLREYLHWLVTDIPATTNTSFGNEVVCYENPTPTMGIH 116

Hd3a    121 RLVFVLFQQLGRQTVYAPGWRQNFNTKDFAELYNLGSPVAAVYFNCQREAGSGGRRVYP 179
StSP3D  120 RIVFSLFRQLGRETVYAPNWRQNFNTRQFAELYNLGSPVAAVYFNCQRENGTGGRRCE- 177
StSP6A  117 RFVLVLFRQSRRETVYAPGWRQNFNTRDFAELYNLGLPVAAVYFNSHRESGTGGRRA-- 173
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Fig. 5. FT homologs in potato. (A) Phylogenetic tree of the potato and Arabidopsis PEBP genes. They are classified into three subfamilies- FLOWERING LOCUS T (FT), MOTHER OF FT AND TFL1 (MFT), and TERMINAL FLOWER1 like (TFL1). Rice Hd3a is included for reference. (B) Alignment of Hd3a, StSP6A and StSP3D. Note that the key amino acid residues required for 14-3-3 binding are conserved among them (highlighted in red box).

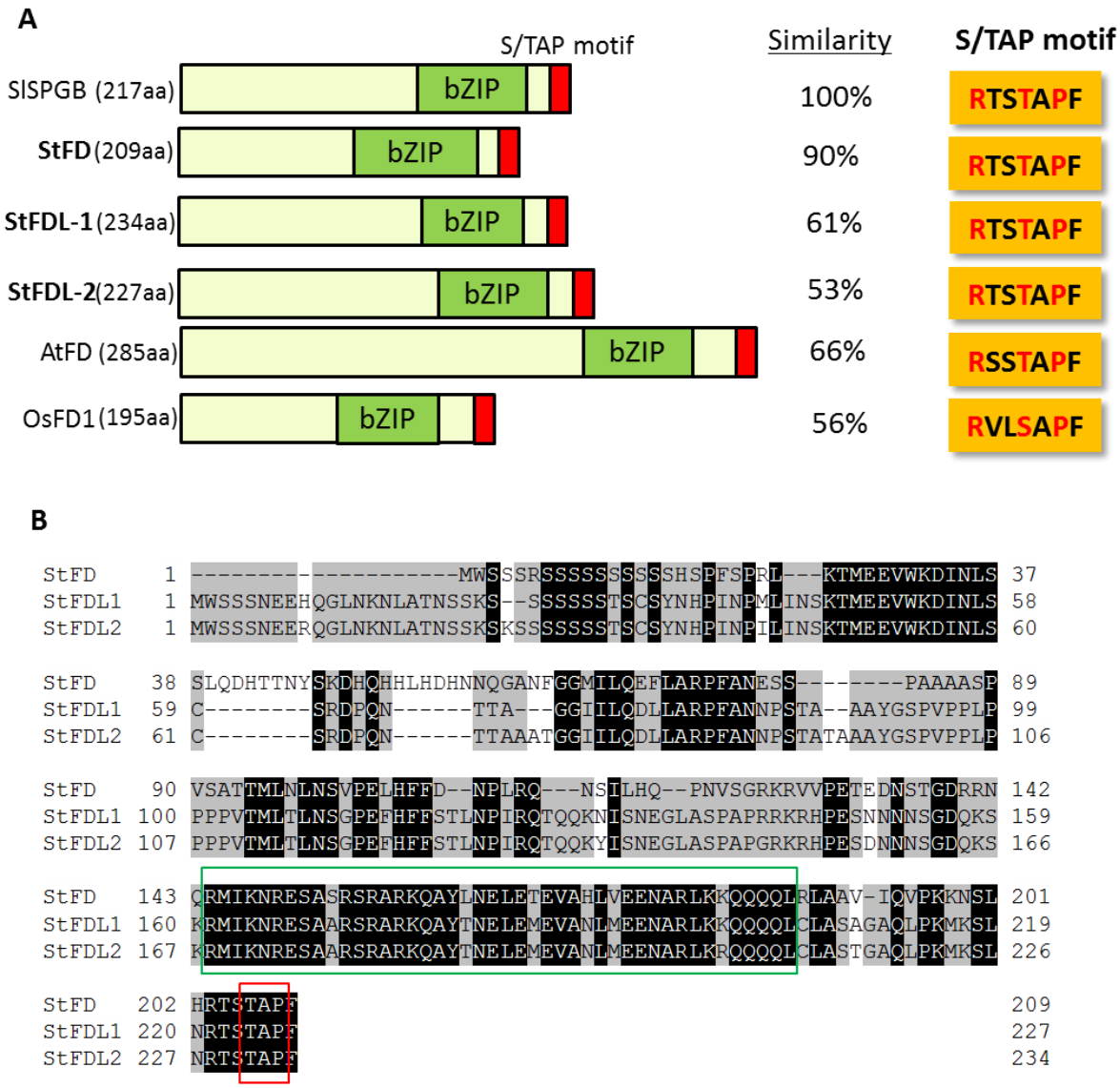


Fig. 6. FD homologs in potato. (A) Schematic representation of protein sequence alignment among tomato SPGB, potato StFD, StFDL1 and StFDL2 and Arabidopsis FD. (B) Alignment of StFD, StFDL1 and StFDL2. The conserved bZIP region and TAP motif are highlighted in green and red box, respectively.

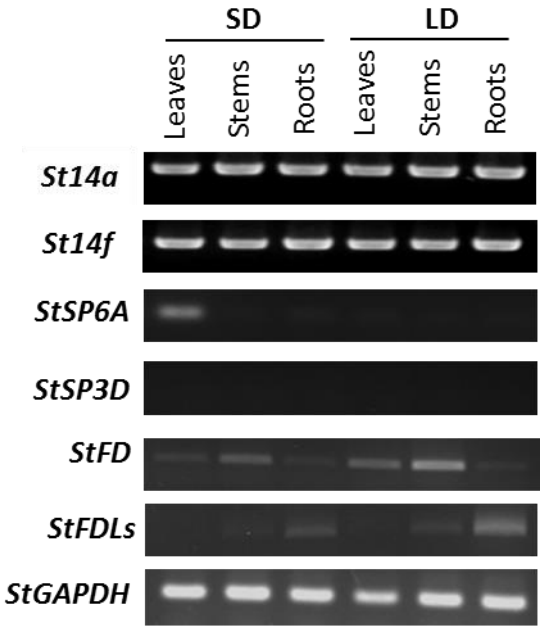
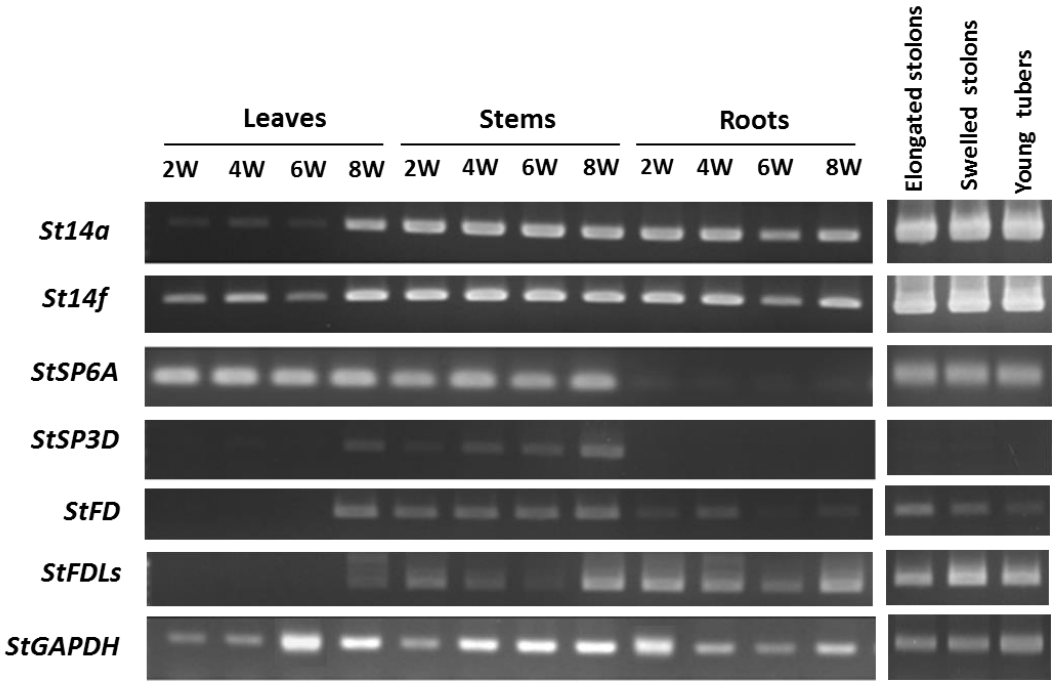


Fig. 7. Semi-quantitative RT-PCR analysis of *St14a*, *St14f*, *StSP6A*, *StSP3D*, *StFD* and *StFDLs* in leaves, stems and roots of 3-weeks old potato grown *in vitro* under short days (SD) and long days (LD) conditions.

A



B

Fig. 8. Spatio-temporal expression pattern of *St14a*, *St14f*, *StSP6A*, *StSP3D*, *StFD* and *StFDLs*. (A) Semi-quantitative RT-PCR was performed by using cDNAs from leaves, stems, roots and developing stolons of soil-grown potato under short days conditions. (B) Morphology of underground elongated stolon, swelled stolon and young tuber. Stolon tips were used for expression analysis. Scale bars= 0.4 cm

detected in 8-weeks old leaves and developing stems. *StFD* and *StFDLs* expression were both detected in leaves, roots and developing stolons. Under LD conditions, *StSP6A* expression was detected in leaves and developing stolons (Fig. 9). Another potato *FT*, *StSP3D* expression was induced in leaves at 4W and expressed constitutively at 6W and 8W. No expression of *StSP3D* was detected in developing stolons. As for potato FDs, both *StFD* and *StFDL*s were expressed in stems, roots and developing stolons.

The expression analysis that showed constitutive expression pattern of *St14-3-3s*, accumulation of *StSP6A* mRNAs in leaves and developing stolons and expression of both *StFD* and *StFDLs* in developing stolons suggested that these candidate genes are the potential components of TAC that controls potato tuberization.

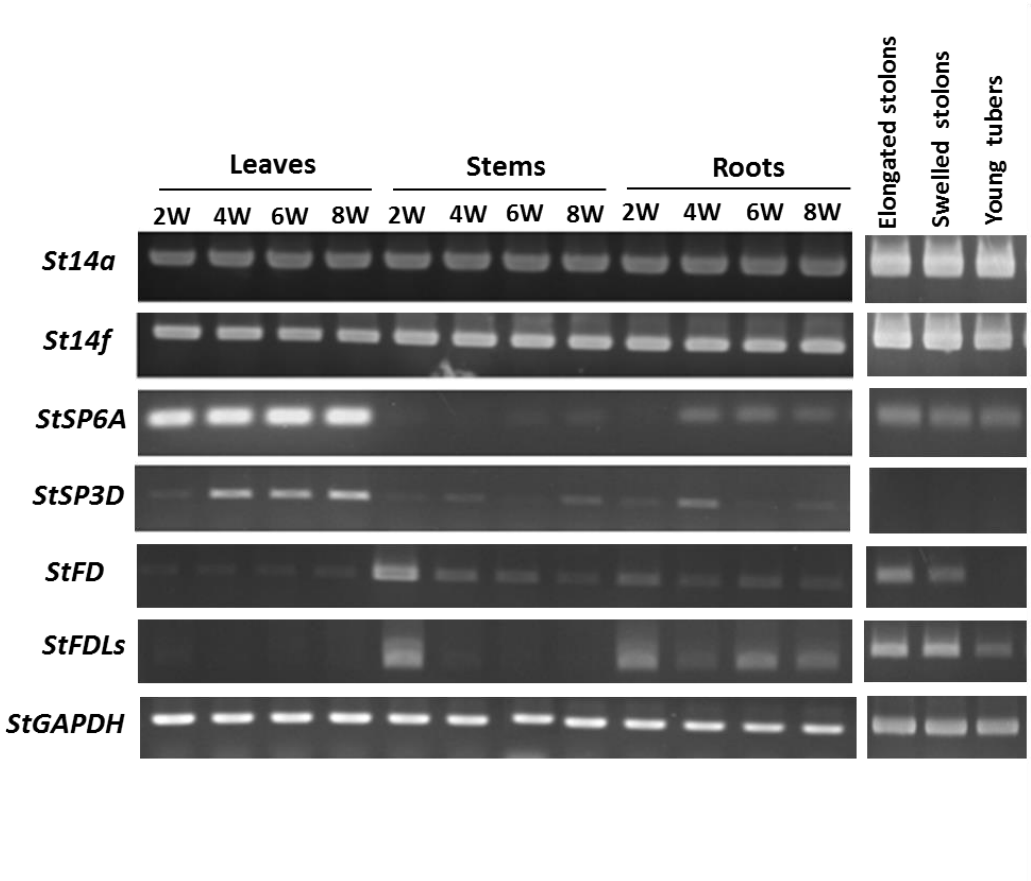


Fig. 9. Spatio-temporal expression pattern of *St14a*, *St14f*, *StSP6A*, *StSP3D*, *StFD* and *StFDLs*. Semi-quantitative RT-PCR was performed by using cDNAs from leaves, stems, roots and developing stolons of soil-grown potato under long days conditions.

III. 4. StSP6A and StFD(L)s interact with St14-3-3s

In rice flowering, both Hd3a and bZIP transcription factor OsFD1 interact directly with 14-3-3 proteins in order to form Florigen Activation Complex. Hence the interaction of StSP6A, StFD(L)s with St14-3-3s was analyzed by yeast two-hybrid and *in vitro* glutathione S-transferase (GST) pull down assay. As shown in Figure 10, StSP6A interacted with potato 14-3-3s in yeast. When the conserved key amino acid residues required for 14-3-3s binding in StSP6A were mutated, the interaction between StSP6A and St14-3-3s was lost, indicating the importance of these amino acids in their interaction. The direct interaction between StSP6A and St14-3-3s was further confirmed by the GST pull down assay.

Interaction of StFD(L)s with St14-3-3s were also confirmed in yeast (Fig. 11a). As phosphorylation of the S/TAP motif in FDs is essential for 14-3-3s binding (Taoka *et al*., 2011), threonine in the TAP motif of StFD, StFDL1 and StFDL2 were substituted with alanine or glutamate. As shown in Figure 11b, the alanine substitution disrupted the interaction and phosphomimic glutamate replacement retrieved it. These results suggest that the phosphorylation of TAP motif of StFD, StFDL1 and StFDL2 is necessary for interaction with St14-3-3s as shown in OsFD1-Os14-3-3s interaction.

III. 5. StSP6A interacts with StFD(L)s in 14-3-3 dependent manner

In rice, 14-3-3 proteins mediate the interaction between Hd3a and OsFD1 (Taoka *et al*., 2011). To explore whether 14-3-3 proteins also mediate the interaction between StSP6A and StFDs, yeast two-hybrid assay were performed. In the presence of endogenous yeast 14-3-3 proteins, StSP6A showed interaction with StFD(L)s. It was found that the mutation of key amino acid residues in StSP6A weakened the 14-3-3 proteins binding affinity and compromised the interaction of StSP6A with StFD(L)s. All two StSP6A mutant proteins showed weak or no interaction with StFD, StFDL1 and StFDL2 (Fig. 12). On the other hand, alanine substitution in the TAP motif of StFD(L)s disrupted StSP6A-StFD(L)s interaction and

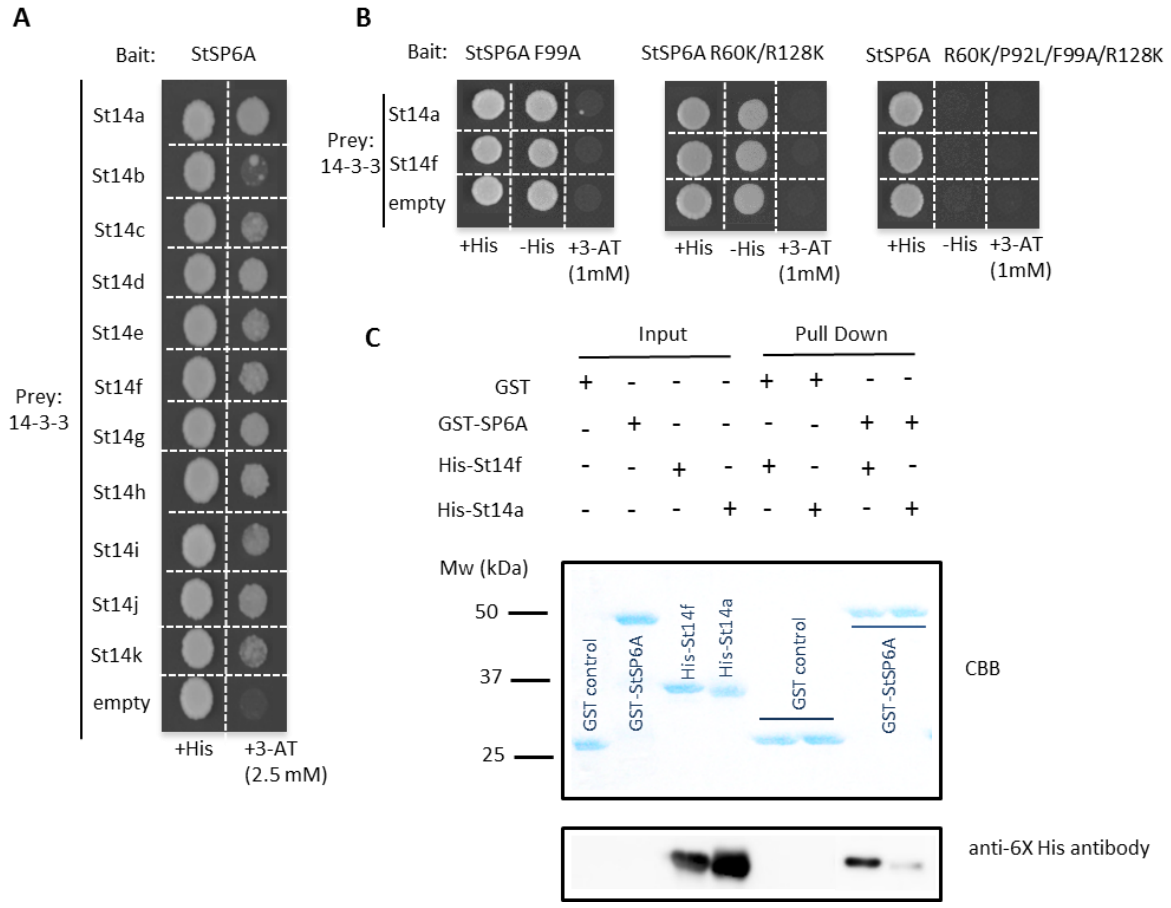


Fig. 10. Interaction between StSP6A and St14-3-3s. (A) Yeast two-hybrid interaction assay of StSP6A and St14-3-3s. StSP6A interacts with all eleven St14-3-3s. (B) Mutation analysis of StSP6A in St14-3-3 interaction. (C) *In vitro* pull down assay demonstrating the direct interaction between StSP6A and St14-3-3s.

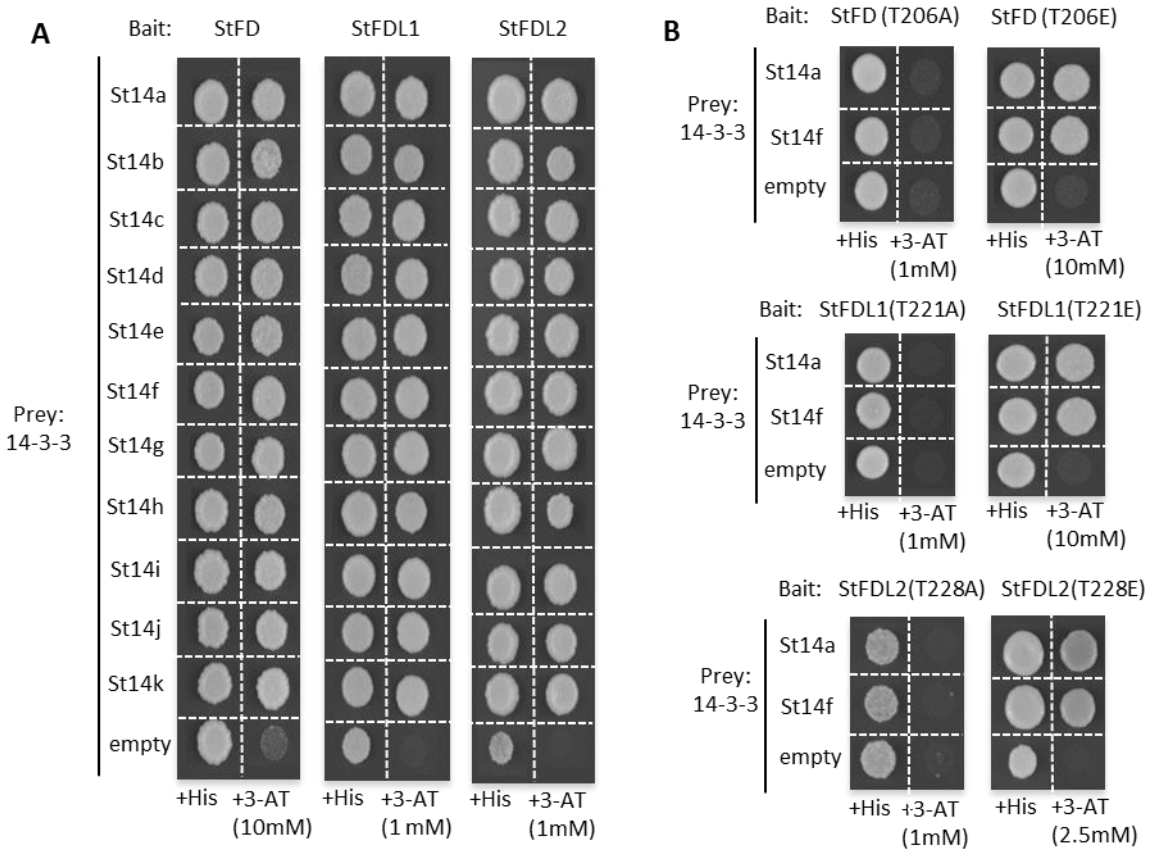


Fig. 11. Interaction between StFD(L)s and St14-3-3s. (A) Yeast two-hybrid interaction assay of StFD(L)s and St14-3-3s. StFD, StFDL1 and StFDL2 can interact with all eleven St14-3-3s. (B) Alanine substitution lost the interaction and phosphomimic replacement retained the binding.

phosphomimic glutamate substitution retained the binding. This further supported the mediator role of St14-3-3s in StSP6A-StFDs interaction.

III. 6. Overexpression of *StSP6A* in *in vitro* cultured potato plants promotes tuber formation

In order to gain insight into the role of StSP6A in promotion of tuber formation and to clarify the impacts of the interaction between StSP6A and St14-3-3s in potato tuberization, transgenic plants overexpressing *StSP6A* or *StSP6A* mutants with single, double or quadruple mutation of the key amino acid residues for 14-3-3s binding were generated. Overexpression of the transgenes was driven by *CaMV 35S* promoter and transgene expression of each transgenic line used for phenotypic analysis was confirmed by semi-quantitative RT-PCR (Supplementary Fig. 3).

Non-transformed control and transgenic plants were cultured *in vitro* under normal culture media for two weeks before transferred to high sucrose culture media. This is to ensure differentiation of axillary bud into leafy shoot and to avoid the undesirable development of aerial tuber that may inhibit normal growth of *in vitro* cultured plants. After transfer to high sucrose media, stolon-borne tubers are formed, depending on the influence of the transgenes. As shown in Fig. 13, 26% of the non-transformed control plants formed tuber after three weeks cultured under LD conditions. *StSP6A* mutant transgenic plants with impaired 14-3-3 binding either showed similar tuber induction rate (24% in *SP6A F99A*; 31% in *SP6A R60K/R128K*) with the control plants or showed no tuber induction at all (0% in *StSP6A R60K/ P92L/F99A/ R128K*). However, under the same conditions, a significantly higher percentage (80%) of *StSP6Aox in vitro* cultured plants induced tuber. The promoting effect of StSP6A in inducing tuber formation was clear and thus, this *in vitro* tuberization data supported the positive role of *StSP6A* in tuber induction and illustrated the impact of 14-3-3 binding in potato tuberization.

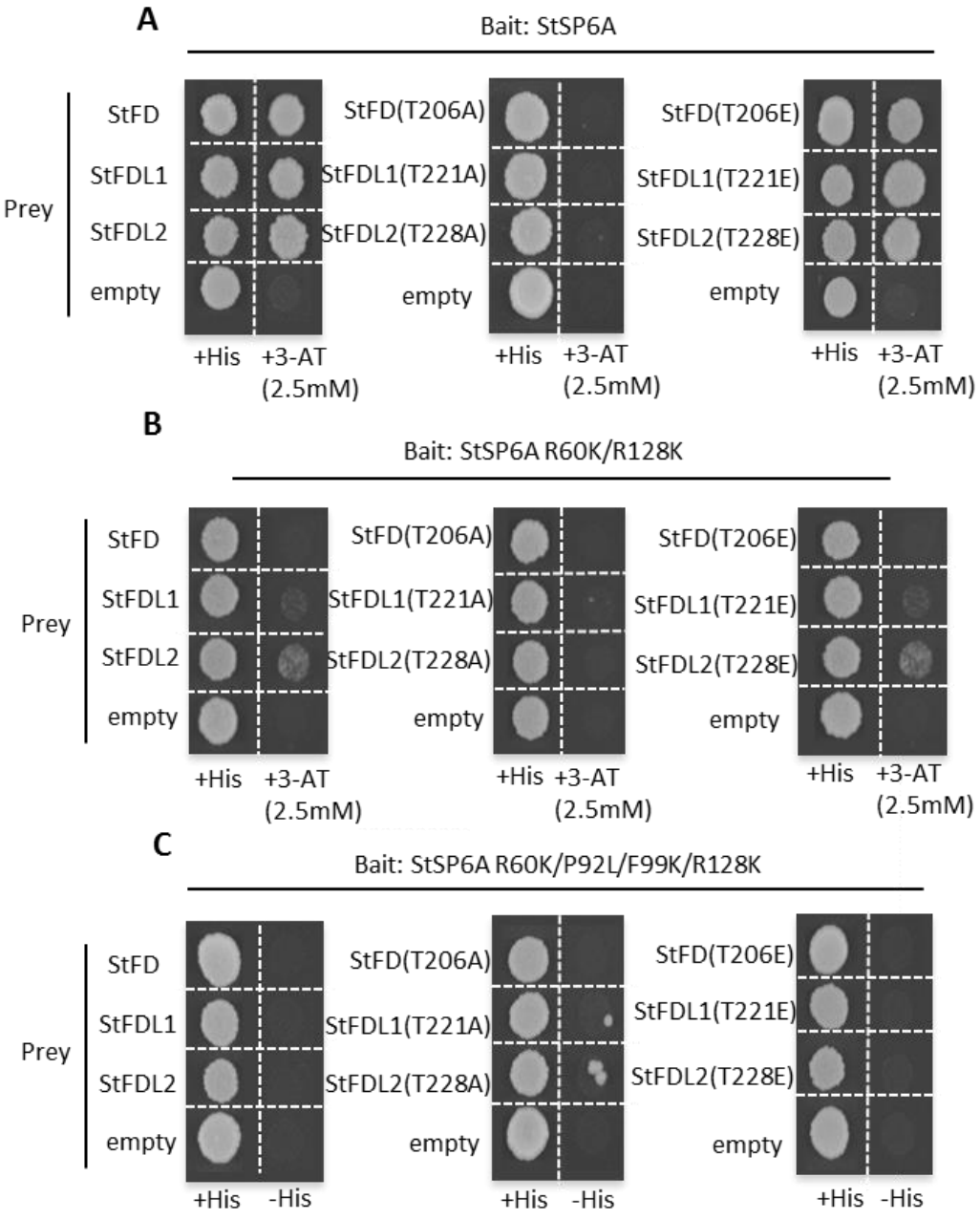


Fig. 12. 14-3-3 binding sites are essential for StSP6A-StFD(L)s interaction. (A) Yeast two-hybrid analysis of protein interaction of StSP6A with StFD, StFDL1 and StFDL2. Alanine substitution of StFD, StFDL1 and StFDL2 lost the binding while phosphomimic replacement retained it. StSP6A mutants R60K/R128K (B) and R60K/P92L/F99A/R128K (C) do not interact with StFD, StFDL1 and StFDL2. Note that these mutations lost the interaction with St14-3-3 (Fig. 8).

A

B

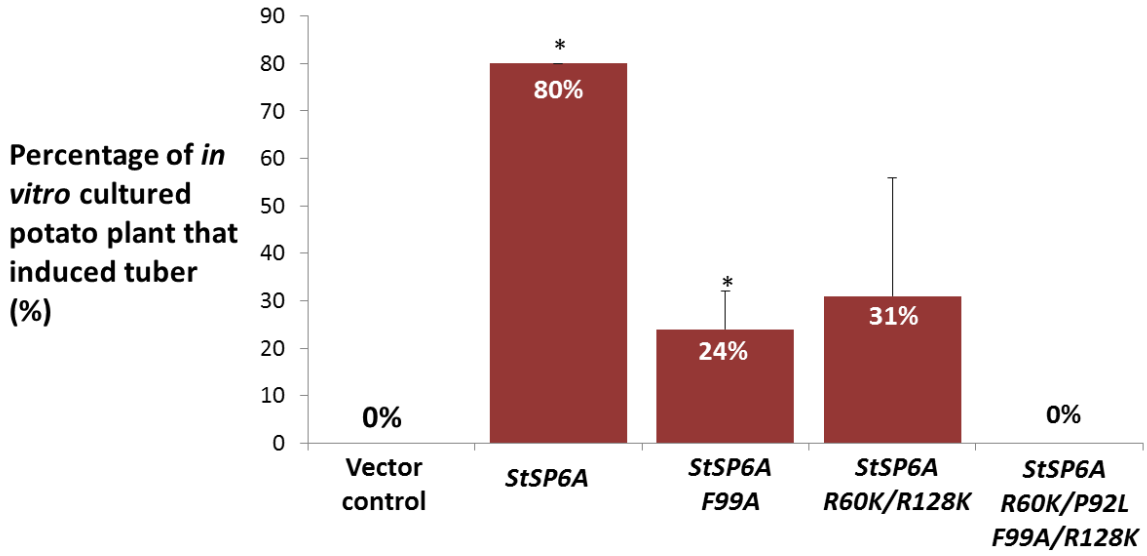


Fig. 13. *In vitro* tuberization of *StSP6A* overexpression plants. (A) Phenotypes of transgenic plants cultured in high sucrose media for 3 weeks under LD conditions. Scale bars = 1 cm. NT, non-transformed control. (B) Average percentage of plant cultured *in vitro* with induced tuber. Data were obtained from triplicate plantings with 5 independent lines included for each gene construct. Error bars represent standard deviation. Statistically difference relative to NT:* $p \leq 0.01$(T-test)

III. 7. Overexpression of *StSP6A* induces early tuber formation under LD conditions

In vitro cultured transgenic plants were transferred to soil under SD and LD conditions for analysis. The tuberization was checked at 4, 6, and 8 WAP. Under SD conditions, there was no difference in the timing of tuber induction between the non-transformed control and transgenic plants. Both non-transformed control and transgenic plants induced tubers at 4 WAP under SD conditions. However, under LD conditions, early tuber induction was observed in *StSP6A* overexpression plants (Fig. 14a). Potato tubers were induced at 4 WAP in the transgenic plants whereas in non-transformed control and vector control plants, tuber formation normally occurred at 6 WAP under LD conditions. Up to 95% of *StSP6A ox* plants induced tuber 2 weeks earlier than control plants under LD conditions (Fig. 14c), whereas in *StSP6A* mutants overexpression plants, percentage of plants with early tuber induction were reduced corresponding with their decreased 14-3-3 binding affinity in yeast. It was about 80% of single mutant (*StSP6A F99A*) plants and 64% of double mutant (*StSP6A R60K/R128K*) plants induced tubers, and none (0%) of the quadruple mutant (*StSP6A R60K/P92L/F99A/R128K*) plants induced tuber at 4 WAP under LD conditions (Fig. 14c). Although more than 50% of *StSP6A F99Aox* plants and *StSP6A R60K/R128Kox* plants induced tubers at 4 WAP, however, the tubers observed are much smaller than those induced from *StSP6Aox* plants (Fig. 14b). The different tuber size indicates different developmental stages of the tubers i.e. the larger tubers in *StSP6Aox* plants could have been induced earlier than those in *StSP6A mutants ox* plants. The smaller tubers from *StSP6A mutants ox* plants could be either just initiated or yet to be induced at 4 WAP. The lower percentage of plants with induced tubers and smaller tuber size in *StSP6A* mutant plants suggest the importance of StSP6A-St14-3-3s interaction and the complex formation in triggering potato tuber induction.

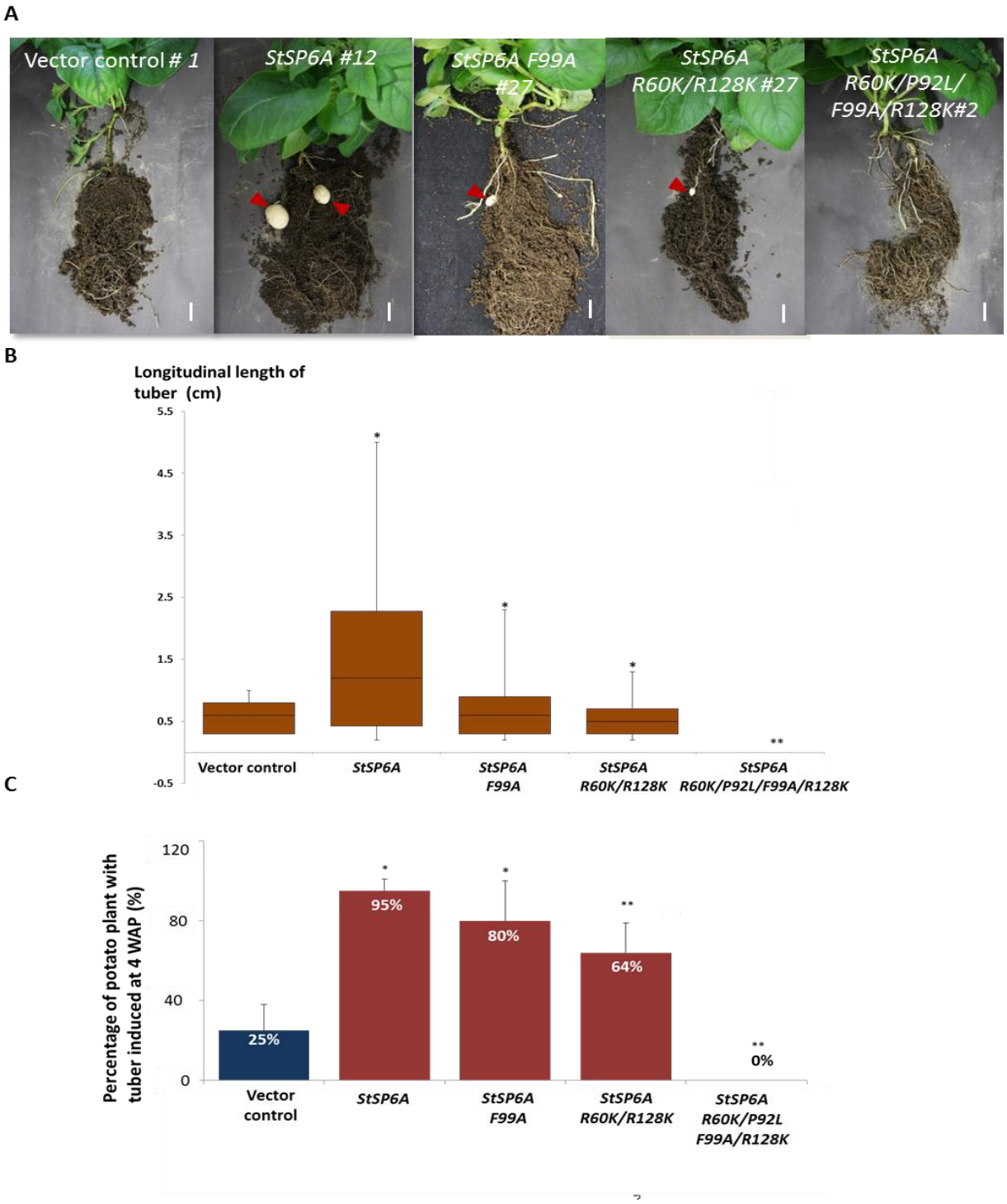


Fig. 14. Analysis of soil-grown *StSP6A* overexpression plants. (A) Representative pictures of soil-grown transgenic plants at 4 weeks after planting under LD conditions. Scale bars= 2.5cm. NT, non-transformed control. (B) Boxplot of potato tuber size distributions. (C) Percentage of soil-grown transgenic plants with tuber at 4 weeks after planting. Data were obtained from triplicate plantings consists of 5 independent lines for each gene construct. Errors bars represent standard deviation. Statistically difference relative to vector control:* $p \leq 0.01$ ** $p \leq 0.05$

III. 8. Suppression of *StSP6A* delays tuber induction under SD and LD conditions

To further confirm the functional role of *StSP6A* as the tuber-inducing signal in potato tuberization, *StSP6A-RNAi* suppression plants were generated and planted in soil under SD and LD conditions, respectively. In addition, *StSP3D-RNAi* suppression plants were also generated to provide evidence for its non-functional role in potato tuberization as inferred by *StSP3D* expression profile. Tuberization conditions were checked at 4, 6, and 8 WAP. As shown in Figure 15, *StSP3D* suppression did not affect tuberization under both SD and LD conditions.

In contrast, delayed tuberization was observed in *StSP6A-RNAi* plants (Fig. 16). Under SD conditions, no tuber was observed in all of the suppression lines at 6 WAP whereas in non-suppression lines and non-transformed control plants, tuber formation was observed at 4 WAP. Under LD conditions, no tuber was observed at 8 WAP in suppression lines whereas tuber formed in non-suppression lines and non-transformed control plants at 6 WAP. As flowering time and stolon growth were unaffected by *StSP6A* silencing (Table 4), it is clear that StSP6A plays a key role in tuber induction and is likely the tuberigen *in vivo* that participates in the TAC formation.

III. 9. *StFD(L)s* overexpression does not affect tuber formation

To study the functional role of *StFD, StFDL1 and StFDL2* in potato tuberization, the phosphomimic form of *StFD* (*StFD T206E*), *StFDL1* (*StFDL1 T221E*) and *StFDL2* (*StFDL2 T228E*) was used to generate the overexpression plants. Based on the phenotypic and statistical analysis, there is no significant difference between *StFD(L)s ox* plants and non-transformed control plants in tuber formation at 4 WAP (Fig. 17).

Fig. 15. Tuberization of *StSP3D RNAi* suppression plants. (A) Semi-quantitative RT-PCR analysis of *StSP3D* expression in *StSP3D-RNAi* suppression plants. NT, non-transformed control. (B) *StSP3D RNAi* suppression plant at 4 weeks after planting under inductive short days (upper panel) and at 6 weeks after planting under long days (lower panel) conditions. Scale bars= 4cm.

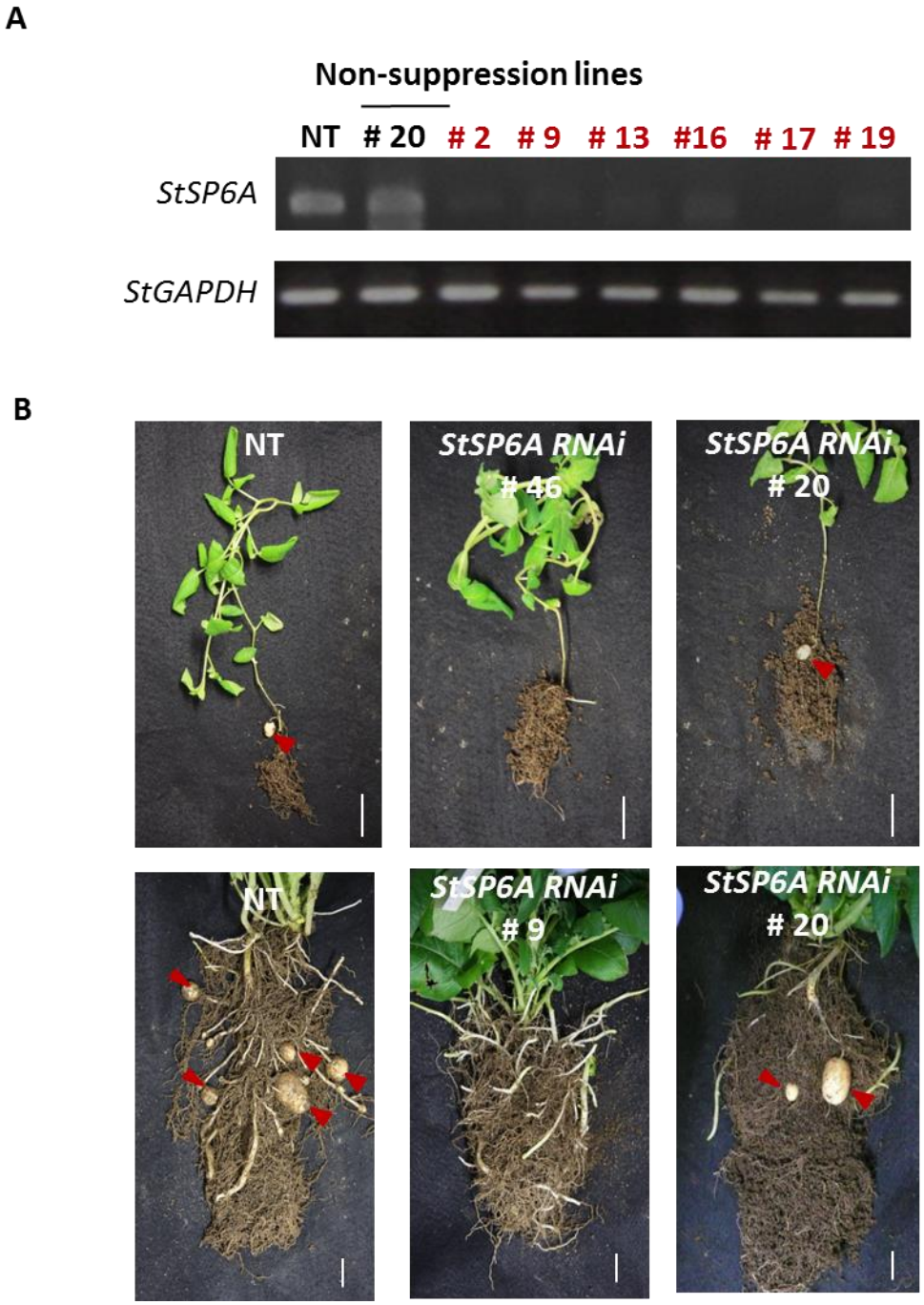


Fig. 16. Tuberization of *StSP6A RNAi* suppression plants. (A) Semi-quantitative RT-PCR analysis of *StSP6A* expression in leaves of *StSP6A-RNAi* suppression plants. (B) Delayed tuberization of *StSP6A* suppressed lines. Pictures showed NT, non-transformed control, *StSP6A RNAi* suppressed lines and *StSP6A* non-suppression line (from left to right) at 6 weeks after planting under short days (upper panel) and at 8 weeks after planting under long day conditions (lower panel). Scale bars= 4 cm.

Table 4: Flowering time and number of stolons in *StSP6A RNAi* plants. Data were collected from triplicate plantings with 5 independent lines used.

Genotype	**Flowering time under LD (n=15)**	**Stolon number scored at 6 WAP under SD (n=15)**
StSP6A RNAi	6-8 weeks	1.0 ±0.5
Non-transformed control	6-8 weeks	1.1 ±0.3

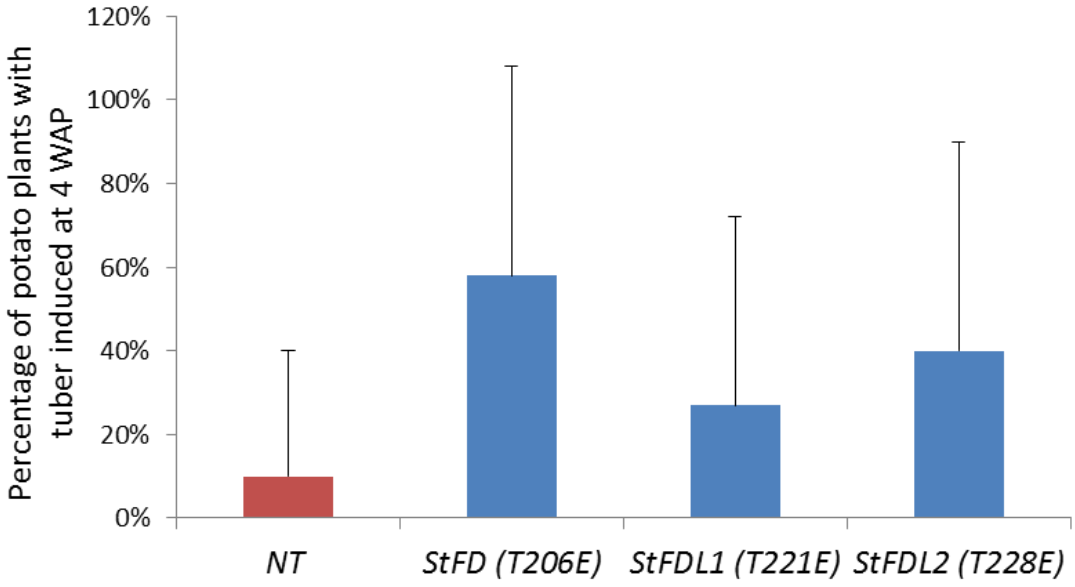


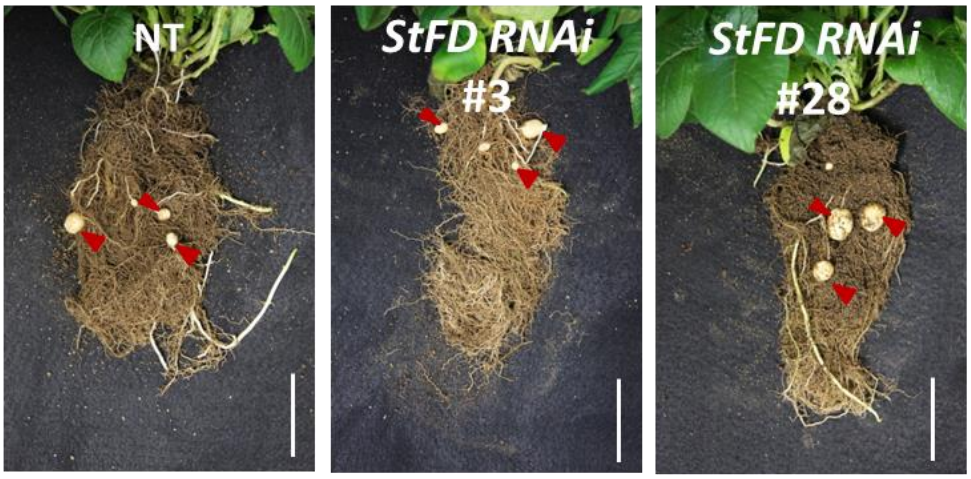
Fig. 17. Tuberization analysis of *StFD(L)s* overexpression plants. The graph shows percentage of plants with tuber induced at 4 WAP compared to non-transformed control plants, NT. Data were obtained from triplicate plantings with 5 independent lines included for each gene construct. T-test showed no significant difference from NT.

III. 10. *StFD* suppression has no effects in potato tuberization but *StFDL2* suppression delays potato tuberization

There was no significant difference in tuber induction time between the *StFD-RNAi* plants and non-transformed control plants (Fig. 18). However, when *StFDL* was suppressed, tuberization was delayed for 2 weeks and this phenotype was consistently observed in all the 4 suppression lines under study (Figure 19). In contrast, the non-suppression line #11 induced tuber the same time as in non-transformed control plants. This showed that potato tuber induction was impeded by the absence of StFDL2 in stolons. Taken together, StFDLs may play vital role in potato tuberization and is likely the potential interacting-partner of StSP6A in potato tuber induction.

A

B

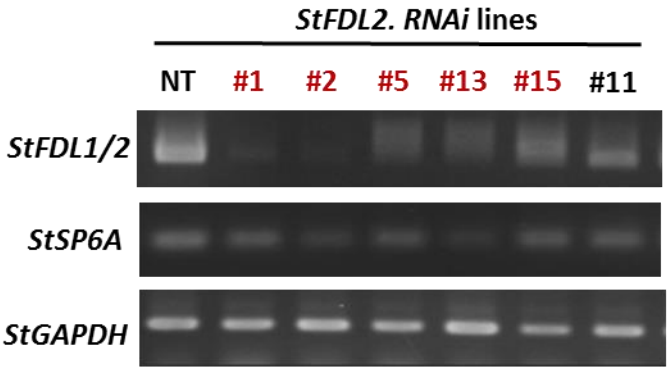


C

Genotype	% of plants with tuber induced at 6 W under LD	n
NT	100%	9
StFD RNAi	100%	15
**StFD RNAi* # 28	100%	3

Fig. 18. Tuberization analysis of *StFD RNAi* suppression plants. (A) Semi-quantitative RT-PCR analysis of *StFD* in *StFD RNAi* lines. Suppression lines were highlighted in red. Note that line #3 with confirmed *StFD* suppression formed tubers as non-suppression line # 28 (B)Underground parts of *StFD RNAi* suppression plants at 6 weeks after planting (WAP) under long day conditions. (C) Percentage of potato plants with tuber induced at 6 WAP under LD conditions. NT, non-transformed control plants. Scale bars= 5 cm. Data were obtained from triplicate plantings with 5 independent lines included.

A



B

C

Genotype	% of plants with tuber induced at 6 W under LD	n
NT	100%	9
StFDL2 RNAi	0%	15
**StFDL2 RNAi* # 11	100%	3

Fig. 19. Tuberization analysis of *StFDL2 RNAi* suppression plants. (A) Semi-quantitative RT-PCR analysis of *StFDL1/2* in *StFDL2 RNAi* lines. Note that *StFDL1/2* is not suppressed in line # 11. (B) Underground parts of *StFDL2. RNAi* suppression plants at 6 weeks after planting (WAP) under long day conditions. Note that only line # 11 formed tubers. NT, non-transformed control plants. Scale bars= 4 cm. (C) Percentage of plants with tuber induced at 6 WAP under long days conditions. Data obtained from three independent replicates of planting (n= 3)

IV. Discussion

Flowering and tuberization are two different reproductive strategies adopted by plants and are both under photoperiodic regulation (Jackson, 2009). Several molecular components such as phytochrome B (Jackson *et al.*, 1998) and CONSTANS (Martinez-Garcia *et al*., 2002) have been implicated in these two developmental processes. Intriguingly, FT, the floral inducing signal, has also been identified as the tuber inducing signal/ tuberigen in potato (Navarro *et al*., 2011). However, the detailed mechanism on how FT regulates potato tuber formation remains elusive. In this study, we investigated if potato FT forms Tuberigen Activation complex (TAC) with potato bZIP transcription factor FD via 14-3-3 proteins to induce potato tuber formation. Candidate members of TAC- potato *FT StSP6A, StSP3D,* eleven *St14-3-3s, StFD* and two *StFD-like* genes (*StFDL1* and *StFDL2*) were isolated from the genome of a commercial potato cultivar (*S. tuberosum* cv. Sayaka) for analysis.

IV. 1. Conserved gene structure and function of *StSP3D* and *StSP6A*

Both sequence alignment and functional analysis in the present study support the existence and functional conservation of potato FT homologs, *StSP3D* and *StSP6A* in commercial potato cultivar Sayaka. The expression profiling and transgenic analysis supported the floral-inducing role of *StSP3D* and tuber-inducing role of *StSP6A* in potato Sayaka, as indicated previously in wild potato andigena (Navarro *et al*., 2011). *StSP3D* expression was not detected in stolons and its expression in *in vitro* cultured leaves was irresponsive to inductive SD conditions. Besides, *StSP3D* expression was induced in leaves of soil grown plants under non-inductive long day conditions, much lower than *StSP6A* (Fig. 7, 8 & 9). In addition, RNA interference (RNAi)-mediated knockdown of *StSP3D* did not affect tuber induction under SD and LD conditions (Fig. 15). We therefore concluded that *StSP3D* is not involved in potato tuberization. In contrast, *StSP6A* expression was detected in leaves and

stolons undergoing transition to tuberization fate. Under *in vitro* conditions, *StSP6A* was expressed exclusively in leaves of SD-grown plants that exhibit high tendency of minituber formation (Supplementary. Fig. 4). Under soil conditions, expression of *StSP6A* was detected in leaves and stolons of both SD- and LD-grown plants. Potato Sayaka is not a strict SD type cultivar and has acclimated for LD tuberization (Supplementary. Fig. 1). It has been shown that a potato accession with permissive tuberization under LD evades long day inhibition of *StSP6A* expression (Morris *et al*., 2014), it is thus sensible to detect *StSP6A* expression under LD conditions. Interestingly, *StSP6A* expressed in stems of SD-grown plants, which was not found in plants grown under LD conditions (Fig. 8). It is thought that this enhanced *StSP6A* expression in stems may contribute to the rapid tuberization response observed in the SD-grown plants. Moreover, overexpression of *StSP6A* promotes early tuber induction and *RNAi* suppression delays tuber formation under both SD and LD conditions (Fig. 12 & 16). This is consistent with the identity of StSP6A as tuberigen that induces potato stolon-tuber transition, as shown by Navarro *et al.* (2011). StSP6A is likely one of the main components of TAC.

IV. 2. StSP6A is likely to form protein complex with StFD(L)s via St14-3-3s

14-3-3 proteins bind and regulate key proteins involved in various physiological processes. More than 60 different proteins have been reported to associate with 14-3-3 proteins *in vivo* (Chung *et al.*, 1999; Finnie *et al.*, 1999; Fu *et al.*, 2000; Roberts, 2000; Skoulakis and Davis, 1998). 14-3-3 can bridge two proteins together by serving as a phosphorylation dependent scaffold protein (Luo *et al.*, 1996; Marshall, 1996; Xiao *et al*., 1995) that bind to specific phosphoserine/threonine motifs on the target protein. This mode of action of 14-3-3 has been demonstrated in rice that leads to the formation of Florigen Activation Complex (FAC). 14-3-3 form a binary complex with Hd3a and facilitates Hd3a binding to the 14-3-3 target proteins OsFD1 or OsFD2 that are either promotes rice floral

transition or controls rice leaf development (Taoka *et al*., 2011; Tsuji *et al*., 2013). In potato, both StSP6A and StFD(L)s can bind to St14-3-3s *in vitro* (Fig. 10 & 11). Mutation of putative 14-3-3-binding sites of StSP6A or phosphorylation site of StFD(L)s abolished StSP6A-StFD(L)s interaction in yeast, supported the mediator role of St14-3-3s in StSP6A-StFD(L)s interaction (Fig. 12). In addition, *StSP6A*, *St14-3-3s* and *StFD(L)s* expression were detected in developing stolons (Fig. 8 & 9), the site where potato tuber transition occur (Ewing and Struik, 1992). These findings support the possibility of TAC formation in stolons during tuber formation.

However, direct evidence of ternary complex formation in stolon is still lacking. Technical complications accompanied by the non-synchronous tuberization of stolons in a single potato plant and difficulty in protein extraction from these underground stems make it difficult to perform *in planta* protein interaction analysis.

IV. 3. StSP6A-St14-3-3 binding is important for potato tuber induction

In plants, interaction between 14-3-3 proteins and various receptor kinases, transcription factors, structural proteins, ion channels and signaling molecules have been implicated in diverse physiological roles in plants (reviewed by van Heusden, 2005; Schoonheim *et al*., 2007; Zhang *et al*., 2010). In the present study, the protein interaction between StSP6A and St14-3-3s was confirmed by *in vitro* interaction assay. Our yeast 2-hybrid assay showed that StSP6A with single (F99A), double (R60K/R128K) or quadruple mutations (R60K/P92L/F99A/R128K) are defective in St14-3-3 binding at different levels, with the weakest binding being observed in StSP6A of quadruple mutation (Fig. 10b). To assess the physiological impacts of St14-3-3 binding to StSP6A in potato tuberization, tuberization analysis of transgenic plants overexpressed with these *StSP6A* mutants were done in *in vitro* and in soil conditions, respectively. When compared with plants overexpressing *StSP6A*, plants overexpressing *StSP6A* mutants with decreasing affinity for St14-3-3s lost the

accelerated tuberization. Early tuber induction observed in the *StSP6Aox* plants was compromised when StSP6A binding to St14-3-3 was reduced, particularly in StSP6A quadruple mutant of the weakest 14-3-3 affinity. It was observed that none of the quadruple mutant plants promote early tuberization under *in vitro*-cultured and soil-grown conditions (Fig. 13 & 14). On the other hand, early tuberization was still observed in plants expressing *StSP6A* with single or double mutation on the putative 14-3-3 binding sites, although to a lesser extent. This phenomenon may be attributed to the residual activity of St14-3-3 binding in these mutants. As shown in rice flowering, Hd3a R64G mutant that showed no apparent interaction with 14-3-3 in yeast could still induce *OsMADS15* activation and early flowering in rice (Taoka *et al.*, 2011). Likewise, StSP6A F99A and StSP6A R60K/R128K mutants may still possibly interact with St14-3-3s *in planta* to promote potato tuberization. Nevertheless, the size of tubers induced in *StSP6A* mutants plants were significantly smaller, suggesting that these tubers were induced later than those from *StSP6Aox* plants (Fig. 14b). These results indicate that the binding of 14-3-3 proteins is essential for StSP6A function in tuberization. It is clear that the binding of StSP6A to St14-3-3s is indispensable for potato tuber induction.

In the present study, eleven potato 14-3-3 isoforms have been isolated. All these eleven isoforms expressed ubiquitously in potato leaves, stems, roots and stolons (Fig. 8, 9 & Suppl. Fig. 3). In plants, 14-3-3 proteins are highly abundant proteins with large number of isoforms (reviewed by Chung *et al.*, 1999). Different isoforms may functions redundantly or specifically in certain developmental events (Rosenquist *et al.*, 2000; Zhang *et al.*, 2010; reviewed by de Boer *et al.*, 2013). One of the rice 14-3-3 isoforms, GF14e functions specifically in regulating cell death and disease resistance in plant defense mechanism (Manosalva *et al.*, 2011) while four rice 14-3-3 isoforms, GF14b, c, e and f could strongly interact with Hd3a and potentially participate in regulation of flowering in rice (Taoka *et al.*, 2011). Detailed investigation is necessary to clarify if these potato 14-3-3 isoforms work redundantly or specific isoforms are involved in TAC formation.

IV. 4. Function of FDs in potato tuberization and TAC formation

FD is a basic leucine zipper (bZIP)-containing transcription factor that was first identified in Arabidopsis as transcriptional regulator of flowering (Abe *et al*., 2005). A SAP motif targeted by calcium-dependent protein kinases (CDPKs) is located at its C-terminal and is essential for 14-3-3s binding (reviewed by Fu *et al*., 2000). Alanine substitution of a serine/threonine residue within this motif disrupts FD function and 14-3-3 interaction (Abe *et al*., 2005; Wigge *et al*., 2005; Taoka *et al*., 2011). FD function in flowering seems to be conserved in higher plants. Rice OsFD1, tomato SPGB, maize DLF1 and wheat FDL2/FDL6 which are homologs of Arabidopsis FD, can interact with rice Hd3a, tomato SFT, maize ZCN8 and wheat TaFT florigen proteins that are implicated in flowering, respectively (Lifschitz *et al*., 2006; Muszynski *et al*., 2006; Li and Dubcovsky 2008; Meng *et al*., 2011; Taoka *et al*., 2011). Based on genome sequence information derived from doubled monoploid *S. tuberosum* group Phureja DM1-3 516 R44 in the genome database (The Potato Genome Consortium Sequencing, 2011), the presence of two potato *FD* genes were initially predicted. However, three potato *FD* homologs (*StFD, StFDL1* and *StFDL2)* were isolated from tetraploid *S. tuberosum* group tuberosum *ssp* Sayaka in this study. StFD showed higher sequence similarity to tomato SPGB and Arabidopsis FD and is thought to involve in flowering, whereas StFDL1 and StFDL2 sequences are more divergent from tomato SPGB (Fig. 6a) and may possibly confer other biological functions in potato. The difference in ploidy state between diploid Phureja and tetraploid Sayaka may explains the presence of two *StFD-like* genes in potato Sayaka, i.e. the underlying gene duplication events might give rise to these two *StFD-like* genes during breeding and cultivation. The extremely high sequence similarity between StFDL1 and StFDL2 also supported this idea (Fig. 6b).

All three potato FD homologs-StFD, StFDL1 and StFDL2 showed interaction with StSP6A, a tuberigen protein found to induce potato tuberization via 14-3-3 proteins (Fig. 14). This finding raised the possibility of involvement of FD in potato tuberization through

formation of a ternary protein complex, TAC. It is noteworthy that StFD, StFDL1 and StFDL2 interact with 14-3-3 proteins through a canonical mode of 14-3-3–phosphoserine interaction, as similar as in OsFD1-14-3-3 binding (Taoka *et al*., 2011). Hence, transgenic potato overexpressed with the phosphorylated form of StFD, StFDL1 and StFDL2 were generated. Overexpression of *StFD(L)s* did not show significant promotion of tuberization (Fig.17), suggesting that the amount of StFD(L)s is not the rate-limiting factor for tuber induction. In line with this, strong expression of *StFD(L)s* was observed in stolons (Fig. 9). However, *RNAi* suppression analysis revealed the positive role of StFDLs in tuber induction i.e. *StFDL2* suppression significantly delayed potato tuberization (Fig. 19b). As *StSP6A* expression was unaffected in the stolons of these suppression plants (Fig. 19a), it is thought that the tuberization delay is mainly attributed to *StFDL* suppression. Due to the extremely high sequence similarity of *StFDL1* and *StFDL2*, gene-specific primers that could distinguish these two FD homologs in RT-PCR were unable to be designed. Sequencing analysis showed that the apparent single PCR band contained two sequences corresponding to *StFDL1* and *StFDL2* (data not shown). Therefore *StFDL1* could also be suppressed in the *StFDL2* suppression plants. It is plausible that both *StFDL1* and *StFDL2* are associated with potato tuber induction. Our results suggest that both StFDL1 and StFDL2 may be components of Tuberigen Activation Complex.

VI. 5. Potential downstream target genes of TAC

In flowering, Arabidopsis *AP1*/rice *OsMADS15* act as direct downstream genes of FT/Hd3a in triggering floral meristem development (Wigge et al., 2005; Taoka *et al.*, 2011). While in potato tuberization, direct downstream genes of StSP6A/TAC still remain to be identified. Our attempts to profile the expression patterns of *AP1* homologs in potato did not find any correlation with tuberization (data not shown). Unlike flowering, tuber transition does not involve development of new organs; rather, it involves reorientation of the plane of

cell division and cell expansion (Xu *et al*., 1998b) that leads to swelling at the subapical region of stolons. Stolon swelling is more likely a hormonal response and thus, it is conceivable that hormone signaling genes may be the candidates that act downstream of TAC to initiate stolon-tuber transition. Kloosterman *et al*. (2008) has identified few highly up-regulated genes prior to visible stolon swelling during tuber development. These genes are mainly involved in gibberellins degradation (*GA2-oxidase*), auxin response that implicated in cell division (*arcA*) and others. The rapid induction of *StGA2-oxidase* after chemical induction of *StSP6A* in stolons strongly suggested this gene for GA catabolic enzyme as the direct target of StSP6A protein (Navarro *et al*., 2011). In addition, Roumeliotis *et al*. (2012) identified StPIN proteins in potato and suggest their possible role in redistributing auxin in the swelling stolon. Moreover, ectopic expression of a tomato cytokinin biosynthesis gene, *TLOG1* induces tuber formation from the basal axillary meristem in tomato plants (Eviatar-Ribak *et al*., 2013). These findings strengthen the notion that hormonal genes may be the candidates of TAC downstream genes. Nevertheless, vigorous experimentation is needed to reveal the identity of this tuber-identity gene and how it is regulated by TAC.

VI. 6. Concluding remarks

The discovery of FAC in modulating rice floral evocation has provided an excellent framework for our understanding on how FT works in triggering reproductive transition in plants. Shared common regulatory elements between flowering and tuberization offer insights into the participation of a FAC-like complex, named as Tuberigen Activation Complex (TAC) in potato tuberization. Our results confirm the identity of potato FT ortholog StSP6A as tuberigen protein and indicate that StSP6A binding to St14-3-3s play a pivotal role in potato tuber induction. Delay in tuberization by *StFDLs* suppression suggests the essential role of *StFDLs* in potato tuberization. Besides, StSP6A interacts with potato FD homologs in 14-3-3-dependent manner. Taken together, it is likely that StSP6A interacts with StFDLs via St14-3-3 protein to form TAC in regulating potato tuberization. Nonetheless, it remains challenging to elucidate how TAC is formed in potato stolons and how this ternary complex regulates potato tuberization i.e. the direct downstream tuber identity genes induced by TAC to trigger stolon-tuber transition. Given the potential of TAC formation in triggering potato tuber induction, the molecular basis of how *FT* genes regulate storage organ formation will be uncovered and leads to understanding of florigen function in tuberization.

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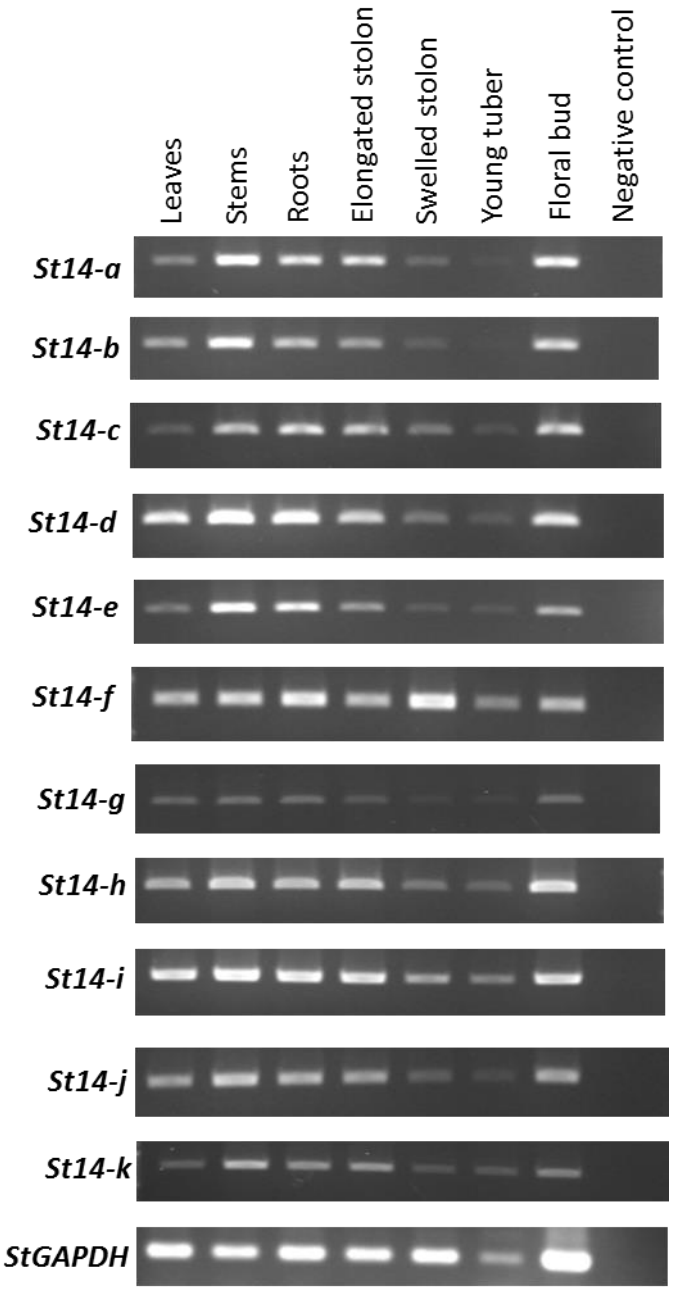
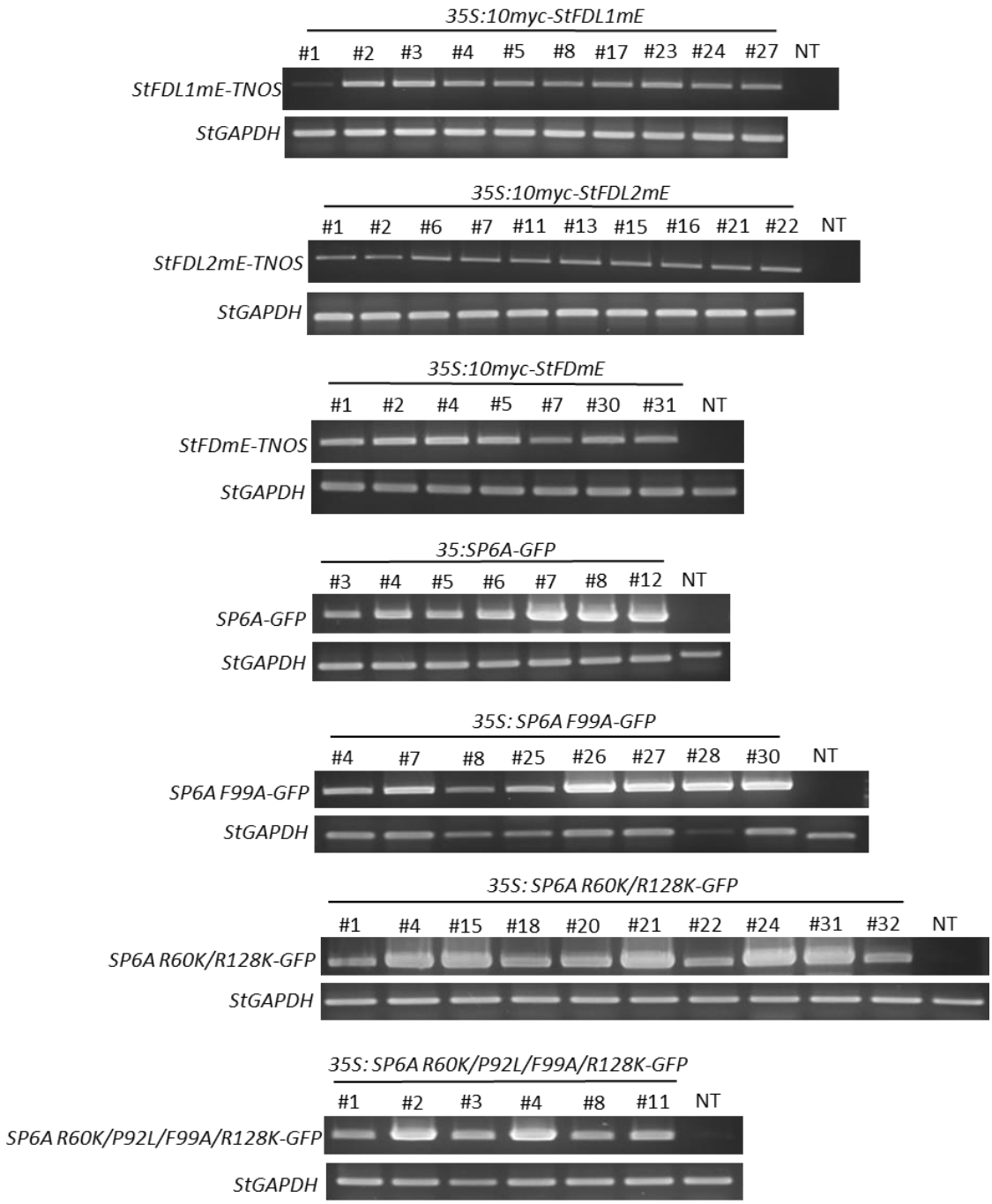
Supplementary Figures

Supplementary Fig. 1. Tuberization of soil-grown potato Sayaka under short-day (A) and long day conditions (LD). Scale bars= 3cm. n= 15



Supplementary Fig. 2. Semi-quantitative RT-PCR analysis (25 cycles) of eleven potato *St14-3-3s* in leaves, stems, roots and developing stolons and floral buds under LD conditions.



Supplementary Fig. 3. Transgene expression in leaves was confirmed by semi-quantitative RT-PCR analysis

A

Daylength	Minituber formation rate (n=24)
SD	50% ±10%
LD	0 %

B

Supplementary Fig. 4. Microtuber induction of *in-vitro* cultured wild type plants under SD. (A) Percentage of plants with microtuber induced under SD and LD conditions.(B) Phenotypes of *in vitro*-cultured potato plants under SD (right) and LD (left) conditions. Red arrows indicate microtuber. Scale bars= 3 cm