Volume 15(6). Published March, 01, 2015
www.jnsciences.org
ISSN 2286-5314

Recent Genomic and Proteomic Profile of tuberization in potato (Solanum tuberosum L.)

F. MANI * AND C. HANNACHI

High Agronomic Institute Chott Mariem. Laboratory of Hortical and in Vitro Sciences, Chott Mariem , Sousse, 4042, Tunisie

* Corresponding author: ferdaousmani78@yahoo.fr

Abstract - Tuberization is a critical stage of potato growth. Some studies reported that many molecules act as signalling potato tuberization. Identification of proteomic and genomic profile of potato during tuberization give some new signalling genes and proteins regulatig potato tuberization. Besides, new studies pointed the critical role of transcription factors in tuberization induction. This review present some genes, transcription factors and proteins implicated in hormonal regulation of tuber induction and repression under different environmental factors.

Keywords: Potato / genes, proteins / transcription factors / tuberization.

1. Introduction

Potato (*Solanum* spp.), mainly *S. tuberosum* L. (2n=48), Solanaceae *Solanum* is a large genus of ca. 1,250 species having worldwide distribution. Only about 180 species produce tubers. They are found in the Americas along the Andean mountains from south Chile to southwest USA. About 30 tuber-bearing species may be edible, but only 5 - 6 of them are of any economic importance. Their chromosome numbers are 2n=24, 48, 60, 72. Tubers of most species contain some neurotoxins like solasidine. Hence, the tubers need to be detoxified by various processing procedures before they are consumed (Nayar, 2014). Potato is grown and eaten in greater countries more than some other crops (Jackson, 1999). It is a crop that grows mainly in climates with cool temperate and full sunlight, moderate daily temperatures and cool nights. Generally, short days generally induce tubers in potatoes, although many modern cultivars can initiate tuberization in the long days of north temperate regions. Among the most important crops in the world (Fernie and Willmitzer, 2001) and Iran (FAO, 2011), potato is ranked in fourth grade in annual production after the cereal species rice, wheat and barley. Iran is the world's 12th potato producer and the third biggest producer in Asia, after China and India's mentioned above (FAO, 2011). Increase in global numerical population especially in developing nations has gradually led to food shortage and hence increase in poverty. Addressing and tackling the issue and causes of poverty in the developing nations is one major challenge to breeders (Fu and Somers, 2009). Genetic diversity is a pre-requisite equipment to select the suitable parents which may produce new important recombinant lines (Agahi *et al.*, 2011). Genetic diversity studies therefore, is a step wise process through which existing variations in the nature of individual or group of individual crop genotypes are identified using specific statistical method or combination of methods. It is expected that the identified variations would form a pattern of genetic relationship usable in grouping genotypes (Aremu, 2012).The reason that potato plants form tubers, or the process of tuberization, has long puzzled both farmers and scientists. Of course we can say that potato contains the genes both to form the tubers and to regulate when these tubers form, but that begs the question as to the exact nature of the controls on the process (Davis, 2009). Here we present recent proteomic and genomic profile of potato tuberization.

2. Genomic profile of tuberization

The FT genes is a primary signaling of potato tuberization

The concept of florigen, postulated in the early 1930s, has taken form after the identification of the FLOWERING LOCUS T (FT) protein as the flowering-inducing signal. Besides their role in flowering, FT genes were subsequently reported to play additional functions in other biological processes. This is particularly relevant in the nightshades, where the FT genes appear to have undergone considerable expansion at the functional level and gained a new role in the control of storage organ formation in potato (*Solanum tuberosum*). FT homologs in the nightshades identifies these proteins as a new class of primary signaling components that modulate tuberization in potato crop (Abelenda *et al.*, 2014). The identification of FLOWERING LOCUS T (FT) and several FT homologs as phloem-mobile proteins that regulate flowering has sparked the search for additional homologs involved in the long-distance regulation of other developmental processes. Given that flowering and tuber induction share regulatory pathways, the quest for long-distance tuberization signals has been further stimulated. Several tuberization regulators have been proposed as mobile molecules, including the FT family protein StSP6A, the plant growth regulators gibberellins and the microRNA miR172. Although some of these hypotheses are attractive and plausible, some evidence that these molecules are transmissible in potato plant has yet to be obtained. Two mRNAs encoding transcription factors, StBEL5 and POTATO HOMEOBOX 1 (POTH1), are mobile and correlate with tuberization induction. Otherwise, evidence that StBEL5 or POTH1 are required for tuberization is not available yet (Rommens *et al.,* 2006). Thus, there are several good candidates for long-distance molecules in the tuberization process. Further research should test their role as systemic tuberization (Suárez-López,2013).

Phloem-mobile signals that are regulated by day length activate both flowering and tuber formation. Both signaling processes have numerous elements in common. In this review, FLOWERING LOCUS T and the three signals currently implicated in controlling tuberization, SP6A, miR172, and the StBEL5 complex, are discussed with a focus on their functional roles, their mechanisms of long-distance transport, and their possible interactions (Hannapel, 2013).

StGA3ox2, StGA20ox1, genes encoding a GA 3-oxidase, contributes to the control of photoperiod-mediated tuberization in potato

Some potato species require a short-day (SD) photoperiod for tuberization, a process that is negatively affected by gibberellins (GAs). The isolation of StGA3ox2, a gene encoding a GA 3-oxidase, whose expression is increased in the aerial parts and is repressed in the stolons after transfer of photoperiod-dependent potato plants to SD conditions.In addition, Over-expression of StGA3ox2 under control of constitutive or leaf-specific promoters results in taller plants which, in contrast to StGA20ox1 over-expressers previously reported, tuberize earlier under SD conditions than the controls. Otherwise, StGA3ox2 tuber-specific over-expression results in non-elongated plants with slightly delayed tuber induction (Hamernik *et al.,* 2009). Together these findings support that StGA3ox2 expression and gibberellin metabolism significantly contribute to the tuberization time in strictly photoperiod-dependent potato plants (Bou-Torrent, 2011).

ArabidopsisABF4 gene in potato enhances tuberization

Potato (*Solanum tuberosum* L.) tuberization is regulated by many signals, such as abscisic acid (ABA), sucrose and gibberellic acid (GA). ABA and sucrose are positive modulators, while GA is an inhibitor of the process. ABF (ABRE-binding factor) proteins are transcription factors involved in ABA and stress signaling. Previously, i twas reported that *S. tuberosum* StABF1 could mediate the ABA effects on tuberization. To evaluate the potential use of ABF genes to enhance tuberization and to determine the molecular mechanism involved transgenic potato plants expressing the Arabidopsis *ABF4* or *ABF2* genes were generated, and their tuberization capacity and response to tuberization-related signals were analyzed in vitro. The results indicate that both ABF4 and ABF2 proteins positively regulate potato tuber induction; while, only *ABF4* expression significantly increases the number and weight of the tubers obtained, without stunting growth. ABF4 and ABF2 transgenic plants exhibit ABA hypersensitivity

during tuberization, accompanied by a GA-deficient phenotype (Bhaskar *et al.*, 2010). *ABF4* expression triggers a significant rise in ABA levels in stolons under tuber-inducing conditions as compared with wild-type plants and a transcriptional deregulation of GA metabolism genes. These findings demonstrate that Arabidopsis ABF4 functions in potato ABA-GA signaling crosstalk during tuberization by regulating the expression of ABA- and GA-metabolism genes. *ABF4* gene might be a potential tool to increase tuber production, since its heterologous expression in potato enhances tuber induction without affecting plant growth (Noelia *et al.*, 2014).

The interaction between flowering locus T (StSP6A) and the cycling dof factor (StCDF1) to enhance tuberization

Recent advances have defined some of the components of photoperiodic signalling that lead to tuberization in potato including orthologues of flowering locus T (StSP6A) and cycling dof factor (StCDF1). The Neo-Tuberosum accession, but not the Andigena, contains alleles that encode StCDF1 proteins modified in the C-terminal region, likely to evade long day inhibition of StSP6A expression. An allele of StSP6A from the Neo-Tuberosum accession, absent in the Andigena, which is expressed under long days was also identified. Other leaf transcripts and metabolites that show different abundances in tuberizing and non-tuberizing samples were identified adding detail to tuberization-associated processes. Overall, the data presented highlight the subtle interplay between components of the clock-CONSTANS-StSP6A axis which collectively may interact to fine-tune the timing of tuberization (Morris *et al.*, 2014).

The effect of StCO gene on tuber induction

T (FT), which is a key component of systemic flowering signals in other species. We also found that StFT/StSP6A transcript levels correlate with the induction of tuber formation in wild-type plants. These results show that StCO plays an important role in photoperiodic tuberization and, together with the recent demonstration that StFT/StSP6A promotes tuberization, indicate that the CO/FT module participates in controlling this process. Moreover, they support the notion that StCO is involved in the expression of long-distance regulatory signals in potato, as CO does in other species. CONSTANS (CO) is involved in the photoperiodic control of plant developmental processes, including flowering in several species and seasonal growth cessation and bud set in trees. It has been proposed that CO could also affect the day-length regulation of tuber induction in *Solanum tuberosum* (potato), a plant of great agricultural relevance (Bradshow and Bonierbale, 2010). A potato CO-like gene, StCO, was identified and found to be highly similar to a previously reported potato gene of unknown function. Potato plants overexpressing StCO tuberized later than wild-type plants under a weakly inductive photoperiod. StCO silencing promoted tuberization under both repressive and weakly inductive photoperiods, but did not have any effect under strongly inductive short days, demonstrating that StCO represses tuberization in a photoperiod-dependent manner. The effect of StCO on tuber induction was transmitted through grafts. Furthermore, StCO affected the mRNA levels of StBEL5 - a tuberization promoter, the mRNA of which moves long distances in potato plants - and StFT/StSP6A, a protein highly similar to flowering locus (González-Schain *et al.*, 2012).

Overexpression of a bacterial 1-deoxy-D-xylulose 5-phosphate synthase gene perturbs potato tuberization

Potato tubers were engineered to express a bacterial gene encoding 1-deoxy-D-xylulose 5-phosphate synthase (DXS) in order to investigate the effects of perturbation of isoprenoid biosynthesis. Twenty-four independent transgenic lines out of 38 generated produced tubers with significantly elongated shape that also exhibited an early tuber sprouting phenotype. Expression analysis of nine transgenic lines (four exhibiting the phenotype and five showing a wild-type phenotype) demonstrated that the phenotype was strongly associated with dxs expression. At harvest, apical bud growth had already commenced in dxs-expressing tubers whereas in control lines no bud growth was evident until dormancy was released after 56-70 d of storage. The primordial phase of bud growth in dxs tubers was followed by a lag period of approximately 56 d, before more elongation of the developing sprouts could be detected. Thus dxs expression results in the separation of distinct phases in the dormancy and sprouting processes. The levels

of plastid-derived isoprenoid growth regulators were measured in transgenic and control tubers. The major difference measured was an increase in the level of trans-zeatin riboside in tubers at harvest expressing dxs. Furthermore, compared with controls, in some dxs-expressing lines, tuber carotenoid content increased approximately 2-fold, with most of the increase accounted for by a 6-7-fold increase in phytoene (Morris *et al.*, 2006).

The role of CCD8 gene in potato tuberization

Strigolactones (SLs) are a class of phytohormones controlling shoot branching. In potato (Solanum tuberosum), tubers develop from underground stolons, diageotropic stems which originate from basal stem nodes. As the degree of stolon branching influences the number and size distribution of tubers, it was considered timely to investigate the effects of SL production on potato development and tuber life cycle. Transgenic potato plants were generated in which the CAROTENOID CLEAVAGE DIOXYGENASE8 (CCD8) gene, key in the SL biosynthetic pathway, was silenced by RNA interference (RNAi). Consequently, the resulting CCD8-RNAi potato plants showed significantly more lateral and main branches than control plants, reduced stolon formation, together with a dwarfing phenotype and a lack of flowering in the most severely affected lines. New tubers were formed from sessile buds of the mother tubers. The apical buds of newly formed transgenic tubers grew out as shoots when exposed to light. In addition, we found that CCD8 transcript levels were rapidly downregulated in tuber buds by the application of sprout-inducing treatments. These findings indicates that SLs could have an effect, solely or in combination with other phytohormones, in the morphology of potato plants and also in controlling stolon development and maintaining tuber dormancy (Pasare *et al.,* 2013).

SnRK1 gene in transgenic potato tubers alters responsiveness to hormones during tuberization

Trehalose-6-phosphate (T6P) is a signaling metabolite that regulates carbon metabolism, developmental processes, and growth in plants. In Arabidopsis (Arabidopsis thaliana), T6P signaling is, at least in part, mediated through inhibition of the SNF1-related protein kinase SnRK1. To investigate the role of T6P signaling in a heterotrophic, starch-accumulating storage organ, transgenic potato (*Solanum tuberosum*) plants with altered T6P levels specifically in their tubers were generated. Transgenic lines with elevated T6P levels (B33-TPS, expressing Escherichia coli osmoregulatory trehalose synthesis A [OtsA], which encodes a T6P synthase) displayed reduced starch content, decreased ATP contents, and increased respiration rate diagnostic for high metabolic activity. On the other hand, lines with significantly reduced T6P (B33-TPP, expressing E. coli OtsB, which encodes a T6P phosphatase) showed accumulation of soluble carbohydrates, hexose phosphates, and ATP, no change in starch when calculated on a fresh weight basis, and a strongly reduced tuber yield. [^{14}C]glucose feeding to transgenic tubers indicated that carbon partitioning between starch and soluble carbohydrates was not altered. Transcriptional profiling of B33-TPP tubers revealed that target genes of SnRK1 were strongly up-regulated and that T6P inhibited potato tuber SnRK1 activity in vitro. Among the SnRK1 target genes in B33-TPP tubers, those involved in the promotion of cell proliferation and growth were down-regulated, while an inhibitor of cell cycle progression was up-regulated. T6P-accumulating tubers were strongly delayed in sprouting, while those with reduced T6P sprouted earlier than the wild type. Early sprouting of B33-TPP tubers correlated with a reduced abscisic acid content. Collectively, our data indicate that T6P plays an important role for potato tuber growth (Debast *et al.,* 2011).

Expression of auxin synthesis gene tms1 enhances potato tuberization

Phytohormones, auxins in particular, play an important role in plant development and productivity. Earlier data showed positive impact of exogenous auxin on potato (Solanum tuberosum) tuberization. To generate potato plants with increased auxin level predominantly in tubers, end pBinB33-tms1 vector was constructed harboring the Agrobacterium auxin biosynthesis gene tms1 fused to tuber-specific promoter of the class I patatin gene (B33-promoter) of potato. Among numerous independently generated B33:tms1-lines those without visible differences from control were selected for detailed studies. In the majority of transgenic lines tms1 gene transcription was detected, mostly in tubers rather than in shoots.

IAA content in tubers and the auxin tuber-to-shoot ratio were increased in tms1-expressing transformants. The organ-specific increase in auxin synthesis in B33:tms1-transformants accelerated and intensified the process of tuber formation, reduced the dosage of carbohydrate supply required for in vitro tuberization and decreased the photoperiodic dependence of tuber initiation. Overall a positive correlation was observed between tms1 expression, IAA content in tubers and stimulation of tuber formation. The revealed properties of B33:tms1-transformants imply an important role for auxin in potato tuberization and offer prospects to magnify potato productivity by a moderate organ-specific enhancement of auxin content (Kolachevskaya *et al.*, 2014).

Three potato CYP707A genes involved in tuberization

The effects of azole-type P450 inhibitors and two metabolism-resistant abscisic acid (ABA) analogues on in vitro ABA-8'-hydroxylase activity, in planta ABA metabolism, endogenous ABA content, and tuber meristem dormancy duration were examined in potato (Solanum tuberosum L. cv. Russet Burbank). Three potato CYP707A genes were demonstrated to encode enzymatically active ABA-8'-hydroxylases with micromolar affinities for (+)-ABA. The in vitro activity of the three enzymes was inhibited by the P450 azole-type inhibitors ancymidol, paclobutrazol, diniconazole, and tetcyclasis, and by the 8'-acetylene- and 8'-methylene-ABA analogues, with diniconazole and tetcyclasis being the most potent inhibitors. The in planta metabolism of [(3)H](±)-ABA to phaseic acid and dihydrophaseic acid in tuber meristems was inhibited by diniconazole, tetcyclasis, and to a lesser extent by 8'-acetylene- and 8'-methylene-ABA. Continuous exposure of in vitro generated microtubers to diniconazole resulted in a 2-fold increase in endogenous ABA content and a decline in dihydrophaseic acid content after 9 weeks of development. Similar treatment with 8'-acetylene-ABA had no effects on the endogenous contents of ABA or phaseic acid but reduced the content of dihydrophaseic acid. Tuber meristem dormancy progression was determined ex vitro in control, diniconazole-, and 8'-acetylene-ABA-treated microtubers following harvest. Continuous exposure to diniconazole during microtuber development had no effects on subsequent sprouting at any time point. Continuous exposure to 8'-acetylene-ABA significantly increased the rate of microtuber sprouting. The results indicate that, although a decrease in ABA content is a hallmark of tuber dormancy progression, the decline in ABA levels is not a prerequisite for dormancy exit and the onset of tuber sprouting (Suttle *et al.*, 2012).

Modifying expression of genes involved in regulating ABA synthesis and metabolism during tuberization.

Abscisic acid (ABA) has been shown to play a critical role in tuber dormancy control but the mechanisms regulating ABA content during dormancy, as well as the sites of ABA synthesis, and catabolism are unknown. A temporal correlation between changes in ABA content and certain ABA biosynthetic and catabolic genes has been reported in stored field tubers during physiological dormancy progression. Otherwise, the protracted length of natural dormancy progression complicated interpretation. To address this issue, the synthetic dormancy-terminating agent bromoethane (BE) was used to induce rapid and highly synchronous sprouting of dormant tubers. The endogenous ABA content of tuber meristems increased 2-fold 24 h after BE treatment and then declined dramatically. By 7 d post-treatment, meristem ABA content had declined by >80%. Exogenous [(3)H]ABA was readily metabolized by isolated meristems to phaseic and dihydrophaseic acids. BE treatment resulted in an almost 2-fold increase in the rate of ABA metabolism. A differential expression of both the StNCED and StCYP707A gene family members in meristems of BE-treated tubers is consistent with a regulatory role for StNCED2 and the StCYP707A1 and StCYP707A2 genes. These results show that the changes in ABA content observed during tuber dormancy progression under tuberization are the result of a dynamic equilibrium of ABA biosynthesis and degradation that increasingly favours catabolism as dormancy progresses (Destefano-Beltrán *et al.*, 2006).

New genes involved on tuber anthocyanin accumulation during potato tuberization

Anthocyanin content of potato tubers is a trait that is attracting increasing attention as the potential nutritional benefits of this class of compound become apparent. The advanced potato selections, CO97216-3P/PW and CO97227-2P/PW, developed by conventional breeding procedures, produced tubers with incomplete expression of tuber flesh pigmentation. This feature permits sampling pigmented and non-pigmented tissues from the same tubers, in essence, isolating the factors responsible for pigmentation from confounding genetic, environmental, and developmental effects. An examination of the transcriptome, coupled with metabolite data from purple pigmented sectors and from non-pigmented sectors of the same tuber, was undertaken to identify these genes whose expression correlated with elevated or altered polyphenol composition. As a similar study using eight other conventional cultivars and advanced selections with different pigmentation, it was possible to produce a refined list of only 27 genes that were consistently differentially expressed in purple tuber tissues compared with white (Stushnoff *et al.*, 2010).

Gene expression in potato leaf chloroplasts and tuber amyloplasts during tuberization

To compare gene expression in potato (*Solanum tuberosum*) tuber amyloplasts and leaf chloroplasts, amounts of transcripts of all plastid genes were determined by hybridization to plastome arrays. Except for a few genes, transcript accumulation was much lower in tubers compared with leaves. Transcripts of photosynthesis-related genes showed a greater reduction in tubers compared with leaves than transcripts of genes for the genetic system. Plastid genome copy number in tubers was 2- to 3-fold lower than in leaves and thus cannot account for the observed reduction of transcript accumulation in amyloplasts. Both the plastid-encoded and the nucleus-encoded RNA polymerases were active in potato amyloplasts. Transcription initiation sites were identical in chloroplasts and amyloplasts, although some differences in promoter utilization between the two organelles were evident. For some intron-containing genes, RNA splicing was less efficient in tubers than in leaves. In addition, tissue-specific differences in editing of ndh transcripts were detected. Furthermore, hybridization of the plastome arrays with RNA extracted from polysomes indicated that, in tubers, ribosome association of transcripts was generally low. Nonetheless, some mRNAs, such as the transcript of the fatty acid biosynthesis gene accD, displayed relatively high ribosome association. Selected nuclear genes involved in plastid gene expression were generally significantly less expressed in tubers than in leaves. Hence, compared with leaf chloroplasts, gene expression in tuber amyloplasts is much lower, with control occurring at the transcriptional, posttranscriptional, and translational levels. Candidate regulatory sequences that potentially can improve plastid (trans)gene expression in amyloplasts have been identified (Valkov *et al.*, 2009).

Regulation of hypoxia-responsive ERF (StHRE) genes during potato tuberization

Internal oxygen concentrations decrease inside growing potato tubers, due to their active metabolism and increased resistance to gas diffusion as tubers grow. Three hypoxia-responsive ERF (StHRE) genes whose expression is regulated by the gradual decrease in oxygen tensions that occur when potato tubers grow larger. Increasing the external oxygen concentration counteracted the modification of StHRE expression during tuber growth, supporting the idea that the actual oxygen levels inside the organs, rather than development itself, are responsible for the regulation of StHRE genes. Several sugar metabolism-related genes co-regulated with StHRE genes during tuber development and possibly involved in starch accumulation were identified. Taken together, these data suggest a possible role for low oxygen in the regulation of sugar metabolism in the potato tuberization, similar to what happens in storage tissues during seed development (Licausi *et al.*, 2011).

A major QTL on chromosome V associated with in vitro tuberization in a potato

The complexity of tetrasomic inheritance and the lack of pure lines in the cultivated potato (Solanum tuberosum L.) (a autotetraploid specie) increase the difficulty of genetic analysis of the inherited characteristics. Tuberization is the determinant step for economic yield of potato. To understand the complex genetic basis of tuberization of the cultivated potato, linkage maps for a tetraploid population (F1) of 237 genotypes and mapped QTLs for the percent of in vitro tuberized plantlets (% IVT) were deveolped. The paternal map for E108 (well tuberized) covered 948 cM and included 12 linkage groups,

all of which contained all four homologous chromosomes. The maternal map for E20 (nontuberized) covered 1,286 cM and included 14 linkage groups, 12 of which contained all four homologous chromosomes. All 12 chromosomes of potato were tagged using the SSR markers. A major QTL (MT05) with additive effect was detected on chromosome V of E108 which explained 16.23 % of the variation for % IVT, and two minor QTLs (mt05 and mt09) displaying simplex dominant effects were located on chromosome V and chromosome IX of E20 which explained 5.33 and 4.59 % of the variation for % IVT, respectively. Based on the additive model of MT05, the segregation ratio of the gametic genotypes (Q-: qq = 5:1) matched the ratio of the tuberized genotypes to the nontuberized genotypes in the population suggesting that the segregation of in vitro tuberization in this population is controlled by a major-effect gene or genes. The mapping results of three important candidate genes indicated that the QTL causal genes detected this study are new. In this study, almost complete linkage maps of a tetraploid population, identified a major QTL on chromosome V affecting in vitro tuberization, suggested a major-effect gene with minor modifiers model controlling this trait and found that the QTLs identified here correspond to new tuberization genes were developed. This provides new and useful information about the genetic basis for tuberization of this autotetraploid crop (Zhou *et al.,* 2014).

3. Proteomic profile of potato tuberization

Potato tuberization is a complicated biochemical process, which is dependent on external environmental factors. Tuber development in potato consists of a series of biochemical and morphological processes at the stolon tip. Signal transduction proteins are involved in the source-sink transition during potato tuberization. A total of 251 proteins were identified and classified into 9 groups according to distinctive expression patterns during the tuberization stage. Stolon stage-specific proteins were primarily involved in the photosynthetic machinery. Proteins specific to the initial tuber stage included patatin. Proteins specific to the developing tuber stage included 6-fructokinase, phytoalexin-deficient 4-1, metallothionein II-like protein, and malate dehydrogenase. Novel stage-specific proteins identified during in vitro tuberization were ferredoxin-NADP reductase, 34 kDa porin, aquaporin, calmodulin, ripening-regulated protein, and starch synthase. Superoxide dismutase, dehydroascorbate reductase, and catalase I were most abundantly expressed in the stolon; however, the enzyme activities of these proteins were most activated at the initial tuber. Proteomic study provides insights into the proteins that show altered expression during in vitro potato (Yu *et al.,* 2012).

Comparative Proteomics of Tuber Induction

To understand the molecular basis of tuberization in potato, a comparative proteomic approach has been applied to monitor differentially expressed proteins at different development stages using two-dimensional gel electrophoresis (2-DE). The differentially displayed proteomes revealed 219 protein spots that change their intensities more than 2.5-fold. The LC-ES-MS/MS analyses led to the identification of 97 differentially regulated proteins that include predicted and novel tuber-specific proteins (Brummel *et al.,* 2011). Nonhierarchical clustering revealed coexpression patterns of functionally similar proteins. The expression of reactive oxygen species catabolizing enzymes, viz., superoxide dismutase, ascorbate peroxidase and catalase, were induced by more than 2-fold indicating their possible role during the developmental transition from stolons into tubers. In addition, nearly 100 proteins, associated with tuber cell differentiation, regulate diverse functions like protein biogenesis and storage in potato tuber, bioenergy and metabolism, and cell defense and rescue impinge on the complexity of tuber development in potato (Agrawal *et al.,* 2008).

Manganese-stabilizing protein associated with early tuberization and increased tuberization frequency

Manganese-stabilizing protein (MSP) represents a key component of the oxygen-evolving complex (OEC). Transgenic potato plants with both enhanced (sense) and reduced (anti-sense) MSP expression levels were generated to investigate the possible physiological role of MSP in overall plant growth, particularly in tuberization. MSP antisense plants exhibited both higher tuberization frequency and higher tuber yield with increased total soluble carbohydrates. The photosynthetic efficiencies of the plants were examined using the OJIP kinetics; MSP-antisense plants were photosynthetically more active than the

MSP-sense and UT (untransformed) control plants. In addition, the oxygen measurements indicated that the relative oxygen evolution was directly proportional to the MSP expression, as MSP-antisense plants showed much lower oxygen evolution compared to MSP-sense as well as UT plants. MSP-sense plants behaved like the UT plants with respect to morphology, tuber yield, and photosynthetic performance. Furthermore, Chlorophyll a fluorescence analyses indicate a possible lack of intact Oxygen Evolving Complexes (OECs) in MSP antisense plants, which allow access to internal non-water electron donors (e.g., ascorbate and proline) and consequently increase the Photosystem II (PSII) activity of those plants. These findings further indicate that this altered photosynthetic machinery may be associated with early tuberization and increased tuberization frequency (Gururani, 2012).

The BEL1-like family of transcription factors in potato.

BEL1-type proteins are ubiquitous plant transcription factors in the three-amino-acid-loop-extension superfamily. They interact with KNOTTED1-like proteins, and function as heterodimers in both floral and vegetative development. Using the yeast two-hybrid system with POTATO HOMEOBOX1 (POTH1) as the bait, seven BEL1-type proteins were originally identified. One of these genes, designated StBEL5, has transcripts that move long distances in the plant and enhance tuberization and root growth. Using the potato genome database, 13 active BEL1-like genes were identified that contain the conserved homeobox domain and the BELL domain, both of which are essential for the function of BEL1-type proteins. Phylogenetic analysis of the StBEL family demonstrated a degree of orthology with the 13 BEL1-like genes of Arabidopsis. A profile of the gene structure of the family revealed conservation of the length and splicing patterns of internal exons that encode key functional domains. Yeast two-hybrid experiments with KNOTTED1-like proteins and the new StBELs confirmed the interactive network between these two families. Analyses of RNA abundance patterns clearly showed that three StBEL genes, BEL5, -11, and -29, make up approximately two-thirds of the total transcript values for the entire family. Among the 10 organs evaluated here, these three genes exhibited the 12 greatest transcript abundance values. Using a phloem-transport induction system and gel-shift assays, transcriptional cross-regulation within the StBEL family was confirmed (Sharma *et al.*, 2014).

Signal transduction proteins

Signal transduction proteins are involved in the source-sink transition during potato tuberization. A total of 251 proteins were identified and classified into 9 groups according to distinctive expression patterns during the tuberization stage. Stolon stage-specific proteins were primarily involved in the photosynthetic machinery. Proteins specific to the initial tuber stage included patatin. Proteins specific to the developing tuber stage included 6-fructokinase, phytoalexin-deficient 4-1, metallothionein II-like protein, and malate dehydrogenase. Novel stage-specific proteins identified during in vitro tuberization were ferredoxin-NADP reductase, 34 kDa porin, aquaporin, calmodulin, ripening-regulated protein, and starch synthase. Superoxide dismutase, dehydroascorbate reductase, and catalase I were most abundantly expressed in the stolon; otherwise, the enzyme activities of these proteins were most activated at the initial tuber (Yu *et al.*, 2012).

Auxin and GA during tuberization

Several hormones have been studied for their effect on tuber initiation and development. Until recently, the hormone with the most prominent role in tuber initiation was attributed to GA. Genes involved in GA degradation do exhibit an upregulated profile during early stages of tuber development, leading to a rapid decrease of active GA content, thereby facilitating stolon-tip swelling. While GA is known to be involved in shoot and stolon elongation, the development of the new tuberorgan requires changes in meristem identity and the reorientation ofthe plane of cell division. In other developmental processes, such as embryo patterning, flower development and lateral root initiation auxin plays a key role. Recent evidence on the involvement of auxin in tuber formation was providedby the measurement of auxin content in swelling stolons. Auxin content in the stolon tips increased several fold prior to tuber swelling. In vitro tuberisation experiments with auxin applications support the role of auxin during tuber initiation. Taken

together, it is becoming clear that the initiation and induction of tubers in potato is a developmental process that appears to be regulated by a crosstalk between GA and auxin.

Expressed mRNA with homology to steroid dehydrogenases affects gibberellin levels and tuberization

Using cDNA-AFLP RNA fingerprinting throughout potato tuber development, a transcript-derived fragment (TDF511) with strong homology to plant steroid dehydrogenases was isolated. During in vitro tuberization, the abundance profile of the TDF shows close correlation to the process of tuber formation. However, when tuberization is inhibited by the addition of gibberellins (GAs) to the growth medium, the appearance of TDF511 in the fingerprint is delayed, then steadily increases in intensity during later stages of development. TDF511 was used to isolate the corresponding cDNA (CB12). The DNA and deduced amino-acid sequences of the cDNA show high homology to a fruit-ripening gene from tomato, a series of steroid dehydrogenases, and the maize Ts2 gene. A section of the cDNA was cloned in antisense orientation behind a 35S CaMV promoter and transformed into potato. Transgenic plants expressing the antisense gene showed significantly earlier emergence, an increase in height, and longer tuber shape. In vitro tuberization experiments reveal extended stolon lengths in comparison to the controls. The analysis of endogenous GA levels showed that the transgenic antisense plants have elevated levels of biologically active GAs and their respective precursors. We suppose that this gene plays a role in the metabolism of plant-growth substances important for tuber life cycle and plant development (Bachem *et al.*, 2001).

Acid phosphatase activity may affect the tuberization

Acid phosphatase activity is involved in regulating many physiological and developmental events by affecting the resorption process. APase activities were mainly localized in cytoplasm, gaps among cells and stroma of amyloplasts of parenchyma cells at the stage of tuber swelling. Acid phosphatase 1, encoding a putative Acid phosphatase, was also highly expressed in swelling tubers and a low level of expression was observed in elongated stolons and matured tubers. Inhibition of Acid phosphatase activity by applying Brefeldin A, an inhibitor of Acid phosphatase production and secretion, significantly suppressed the tuber swelling and moderately affected the stolon elongation and the tuberization frequency. During tuber development, sucrose serves as the main soluble sugar for long-distance transportation and resorption. In addition, inhibition of Acid phosphatase activity by Brefeldin A markedly reduced the sucrose content in tubers and further decreased the starch accumulation, suggesting that the function of Acid phosphatase in regulating the tuber swelling might be at least partially mediated by the sugar resorption. Exogenous sucrose treatments further indicate the important role of sucrose-mediated sugar resorption in tuber swelling. These results suggest that the Acid phosphatase activity might affect the tuber swelling by partially regulating the sucrose-mediated sugar resorption.

Auxin acts independently of DELLA proteins in regulating gibberellin levels during tuberization

Auxin and bioactive gibberellins (GAs), such as GA1, play critical roles in the control of elongation, along with environmental and endogenous factors, including other hormones such as the brassinosteroids. The effect of auxins, such as indole-3-acetic acid (IAA), is at least in part mediated by its effect on GA metabolism, since auxin up-regulates biosynthesis genes such as GA 3-oxidase and GA 20-oxidase and down regulates GA catabolism genes such as GA 2-oxidases, leading to elevated levels of bioactive GA 1. Evidence that this action of IAA is largely independent of DELLA proteins, the negative regulators of GA action, since the auxin effects are still present in the DELLA-deficient la cry-s genotype of pea. This was a crucial issue to resolve, since like auxin, the DELLAs also promote GA 1 synthesis and inhibit its deactivation. DELLAs are deactivated by GA, and thereby mediate a feedback system by which bioactive GA regulates its own level. However, these results, do not show the generality of the auxin-GA relationship across species and phylogenetic groups or across different tissue types and responses. Furthermore, they do not touch on the ecological benefits of the auxin-GA interaction (Reid *et al.*, 2011).

StPPI1, a proton pump interactor up-regulated during tuberization

Plasma membrane proton pumps (PM H(+)-ATPases) are involved in several physiological processes, such as growth and development, and abiotic stress responses. The major regulators of the PM H(+)-

ATPases are proteins of the 14-3-3 family, which stimulate its activity. In addition, a novel interaction partner of the AHA1 PM H(+)-ATPase, named PPI1 (proton pump interactor, isoform 1), was identified in Arabidopsis thaliana. This protein stimulates the activity of the proton pump in vitro. thaliana PPI1 homolog in potato (*Solanum tuberosum* L.) named StPPI1. The full-length coding sequence of StPPI1 was obtained. The open reading frame (ORF) encodes a protein of 629 amino acids showing 50% identity with A. thaliana PPI1 protein. The StPPI1 ORF is divided into seven exons split by six introns. Southern blot analysis suggests that StPPI1 belongs to a family of related genes. Recombinant StPPI1 stimulates H(+)-ATPase activity in vitro. Basal levels of StPPI1 transcripts are observed in all tissues, however, StPPI1 expression is higher in proliferative regions (shoot apex and flower buds), flowers and leaves than in shoots and roots. StPPI1 mRNA levels significantly increase during tuberization (Muñiz García *et al.,* 2011).

Changes in proteases and protease inhibitors during tuberization

The soluble protein fraction of fully developed potato (*Solanum tuberosum* L.) tubers is dominated by patatin, a 40 kD storage glycoprotein, and protease inhibitors. Potato multicystatin (PMC) is a multidomain Cys-type protease inhibitor. PMC effectively inhibits degradation of patatin by tuber proteases in vitro. It has been established that changes in PMC, patatin concentration, activities of various proteases, and their gene expression are temporally linked during tuberization, providing evidence that PMC has a role in regulating tuber protein content in vivo. PMC was barely detectable in non-tuberized stolons. PMC transcript levels increased progressively during tuberization, concomitant with a 40-fold increase in PMC concentration (protein basis) as tubers developed to 10 g fresh wt. Further increases in PMC were comparatively modest (3.7-fold) as tubers developed to full maturity (250 g). Protease activity declined precipitously as PMC levels increased during tuberization. Proteolytic activity was highest in non-tuberized stolons and fell substantially through the 10-g fresh wt stage. Cys-type proteases dominated the pre-tuberization and earliest stages of tuber development. Increases in patatin transcript levels during tuberization were accompanied by a notable lag in patatin accumulation. Patatin did not begin to accumulate substantially on a protein basis until tubers had reached the 10-g stage, wherein protease activity had been inhibited by approximately 60%. These results indicate that a threshold level of PMC (ca. 3 microg tuber(-1), 144 ng mg(-1) protein) is needed to favor patatin accumulation. Taken together, these results are consistent with a role for PMC in facilitating the accumulation of proteins in developing tubers by inhibiting Cys-type proteases (Weeda *et al.,* 2009).

Tuberization and and induction of α-amylases and β-amylases

Gibberellins (GA) are involved in bud dormancy release in potato. It has been established that GA-treatment released bud dormancy, initiated bud sprouting and promoted sprout growth of excised potato tuber bud discs ('eyes'). Monoterpenes from peppermint oil (PMO) and S-(+)-carvone (CAR) interact with the GA-mediated bud dormancy release in a hormesis-type response: low monoterpene concentrations enhance dormancy release and the initiation of bud sprouting, whereas high concentrations inhibit it. Otherwise, PMO and CAR did, not affect sprout growth rate after its onset. Furthermore GA-induced dormancy release is associated with tissue-specific regulation of α- and β-amylases. Molecular phylogenetic analysis shows that potato α-amylases cluster into two distinct groups: α-AMY1 and α-AMY2. GA-treatment induced transcript accumulation of members of both α-amylase groups, as well as α- and β-amylase enzyme activity in sprout and 'sub-eye' tissues. In potato sprouts, CAR interacts with the GA-mediated accumulation of α-amylase transcripts in an α-AMY2-specific and dose-dependent manner. Low CAR concentrations enhance the accumulation of α-AMY2-type α-amylase transcripts, but do not affect the α-AMY1-type transcripts. Low CAR concentrations also enhance the accumulation of α- and β-amylase enzyme activity in sprouts, but not in 'sub-eye' tissues. In contrast, high CAR concentrations have no appreciable effect in sprouts on the enzyme activities and the α-amylase transcript abundances of either group. The dose-dependent effects on the enzyme activities and the α-AMY2-type α-amylase transcripts in sprouts are specific for CAR but not for PMO. Different monoterpenes therefore may have specific targets for their interaction with hormone signalling pathways (Rentzsch *et al.*, 2012).

The BEL1 transcription factor St BEL5 and its protein partner POTH1 regulate tuberization

BEL1-like transcription factors interact with Knotted1 types to regulate numerous developmental processes. In potato (*Solanum tuberosum*), the BEL1 transcription factor St BEL5 and its protein partner POTH1 regulate tuber formation by mediating hormone levels in the stolon tip. The accumulation of St BEL5 RNA increases in response to short-day photoperiods, inductive for tuber formation. Therefore, RNA detection methods and heterografting experiments demonstrate that BEL5 transcripts are present in phloem cells and move across a graft union to localize in stolon tips, the site of tuber induction. This movement of RNA to stolon tips is correlated with enhanced tuber production (Ducreux *et al.*,2008) . Overexpression of BEL5 transcripts that include the untranslated sequences of the BEL5 transcript endows transgenic lines with the capacity to overcome the inhibitory effects of long days on tuber formation. Addition of the untranslated regions leads to preferential accumulation of the BEL5 RNA in stolon tips under short-day conditions. Using a leaf-specific promoter, the movement of BEL5 RNA to stolon tips was facilitated by a short-day photoperiod, and this movement was correlated with enhanced tuber production. These findings implicate the transcripts of St BEL5 in a long-distance signaling pathway that are delivered to the target organ via the phloem stream (Banerjee *et al.*, 2006).

StCDPK1 is a calcium dependent protein kinase expressed in tuberizing potato stolons

StCDPK1 is a calcium dependent protein kinase expressed in tuberizing potato stolons and in sprouting tubers. StCDPK1 genomic sequence contains eight exons and seven introns, the gene structure is similar to Arabidopsis, rice and wheat CDPKs belonging to subgroup IIa. There is one copy of the gene per genome and it is located in the distal portion of chromosome 12. In addition, Western blot and immunolocalization assays (using confocal and transmission electron microscopy) performed with a specific antibody against StCDPK1 indicate that this kinase is mainly located in the plasma membrane of swelling stolons and sprouting tubers. Sucrose (4-8%) increased StCDPK1 protein content in non-induced stolons, however the amount detected in swelling stolons was higher.Otherwise, transgenic lines with reduced expression of StCDPK1 (beta 7) did not differ from controls when cultured under multiplication conditions, but when grown under tuber inducing conditions some significant differences were observed: the beta 7 line tuberized earlier than controls without the addition of CCC (GA inhibitor), developed more tubers than wild type plants in the presence of hormones that promote tuberization in potato (ABA and BAP) and was more insensitive to GA action (stolons were significantly shorter than those of control plants). StCDPK1 expression was induced by GA, ABA and BAP. Taken together these results suggest that StCDPK1 plays a role in GA-signalling and that this kinase could be a converging point for the inhibitory and promoting signals that influence the onset of potato tuberization (Gargantini *et al.*, 2009).

KNOX and BEL1-like transcription factors of potato that may regulate tuberization

Using the yeast (Saccharomyces cerevisiae) two-hybrid system and a potato (Solanum tuberosum) KNOX protein, designated POTH1, as bait, we have identified seven distinct interacting proteins from a stolon library of potato. All seven cDNAs are members of the BEL1-like family of transcription factors. Among these proteins, there are at least four regions of high sequence conservation including the homeodomain, the proline-tyrosine-proline three-amino acid loop extension, the SKY box, and a 120-amino acid region upstream from the homeodomain. Through deletion analysis, a protein-binding domain present in the carboxy end of the KNOX domain of POTH1 was identified. The protein-binding domain in the BEL1 protein is located in the amino-terminal one-half of the 120-residue conserved region of the BELs. RNA-blot analysis showed differential patterns of RNA accumulation for the BELs in various potato organs. The level of StBEL5 mRNA increased in response to a short-day photoperiod in both leaves and stolons. Similar to sense mutants of POTH1, transgenic lines that overexpressed StBEL5 exhibited enhanced tuber formation even under noninductive conditions. Unlike POTH1 sense lines, however, these BEL lines did not exhibit the extreme leaf and stem morphology characteristic of KNOX overexpressers and displayed a more rapid rate of growth than control plants. Both StBEL5 and POTH1 sense lines exhibited an increase in cytokinin levels in shoot tips. StBEL5 lines also exhibited a decrease in the levels of GA 20-oxidase1 mRNA in stolon tips from long-day plants. Our results demonstrate an interaction between KNOX and

BEL1-like transcription factors of potato that may potentially regulate processes of tuberization (Chen *et al.*, 2003).

Regulation of tuberization by Superoxide anion

A higher concentration of H_2O_2 was detected in the sense transgenic potato plant (SS4) with the lily chCu, ZnSOD sequence, whereas higher levels of $O_2(-)$ was detected in the antisense transgenic plant (SA1) than the WT plant. In these plants, the elongation growth in SA1 was significantly inhibited by treatment with diphenyleneiodonium, an inhibitor of $O_2(-)$ generation, and promoted in the SS4 on treatment with herbicide methyl viologen, a generator of apoplastic $O_2(-)$. Higher concentrations of GAs were also detected during plant growth and the early stage of tuberization in SA1. Complete recovery of the above elongation growth and microtuberization pattern in transgenic plants following treatment of GA(3) or an inhibitor of gibberellin synthesis, paclobutrazol, shows that these changes were mainly caused by active GA levels. In conclusion, a specific ROS ($O_2(-)$) acts as a signal transducer via GA biosynthetic pathways for the regulation of plant growth and tuber development of potato (Kim *et al.*, 2007).

The effects of auxin and strigolactones on tuberization

Many transcriptional networks and plant hormones have been implicated in controlling different aspects of potato tuberization. Auxin levels increase dramatically in the stolon prior to tuberization and remain relatively high during subsequent tuber growth, suggesting a promoting role for auxin in tuber formation. In addition, in vitro tuberization experiments showed higher levels of tuberization from axillary buds of explants where the auxin source (stolon tip) had been removed. This phenotype could be rescued by application of auxin on the ablated stolon tips. In addition, a synthetic strigolactone analogue applied on the basal part of the stolon resulted in fewer tubers. The experiments indicate that a system for the production and directional transport of auxin exists in stolons and acts synergistically with strigolactones to control the outgrowth of the axillary stolon buds, similar to the control of above-ground shoot branching (Roumeliotis *et al.*, 2012).

The signal transduction pathways controlling in potato tuberization

Tuberization is one of the multiple outputs of a single-input phytochrome B sensory system, involving several regulatory genes. Phytochrome B- and GA-mediated photoperiodic perception occurs in the potato leaf, and then the RNA acts as a systemic signal in the long-distance signaling pathway to initiate tuberization in the subapical region of an underground stolon. It has been demonstrated that flowering and tuberizing signals might be similar. The hypthesis that the lipoxygenase cascade involved in the formation of the perimedullary tissue in a growing tuber and that the aquaporins regulate cell division, expansion and elongation during stolon growth and tuber induction in potato is suggested. We suppose that the adaptive diversity for tuberization is under varying photoperiods a micro-evolutionary indicator of differential transduction of cell-to-cell signal molecules under spatial and temporal expression of regulatory genes encoding transcriptional activators (Sarkar, 2008).

The role of a BEL1-like messenger RNA on potato tuberization

BEL1- and KNOTTED1-type proteins are transcription factors from the three-amino-loop-extension superclass that interact in a tandem complex to regulate the expression of target genes. In potato (Solanum tuberosum), StBEL5 and its Knox protein partner regulate tuberization by targeting genes that control growth and development. RNA movement assays demonstrated that StBEL5 transcripts move through the phloem to stolon tips, the site of tuber induction. StBEL5 messenger RNA originates in the leaf, and its movement to stolons is induced by a short-day photoperiod. The movement of StBEL5 RNA to roots correlated with increased growth, changes in morphology, and accumulation of GA2-oxidase1, YUCCA1a, and ISOPENTENYL TRANSFERASE transcripts. Transcription of StBEL5 in leaves is induced by light but insensitive to photoperiod, whereas in stolon tips growing in the dark, promoter activity is enhanced by short days. The heterodimer of StBEL5 and POTH1, a KNOTTED1-type transcription factor, binds to a tandem TTGAC-TTGAC motif that is essential for regulating transcription. The discovery of an inverted tandem motif in the StBEL5 promoter with TTGAC motifs on opposite

strands may explain the induction of StBEL5 promoter activity in stolon tips under short days. Using transgenic potato lines, deletion of one of the TTGAC motifs from the StBEL5 promoter results in the reduction of GUS activity in new tubers and roots. Gel-shift assays demonstrate BEL5/POTH1 binding specificity to the motifs present in the StBEL5 promoter and a double tandem motif present in the StGA2-oxidase1 promoter. These findings suggest that, in addition to tuberization, the movement of StBEL5 messenger RNA regulates other aspects of vegetative development.

Starch metabolism during tuberization

Sucrose is a substrate for starch biosynthesis, unloaded symplastically into the developing potato tubers. Sucrose is converted to glucose-6-phosphate in the cytosol and exported into the amyloplasts. Starch may be degraded either hydrolytically or phosphorolytically. Glucose and maltose are products of hydrolytic starch breakdown in the sprouting tubers. Glucose phosphates are products of the phosphorolytic activity, metabolized to glucose and fructose in cold-stored tubers. Development of molecular tools for assaying potato gene function provide opportunities to receive genetic progress in the sugar-starch potato breeding (Czyzewska and Marczewski ,2009).

4. Conclusion:

Genetic diversity in potato is usefull to select parents wich produce new lines tolerant to abiotic stress and with higher yield. Identification of genes of interest and proteins involved in tuber induction or repression during tuberization is very important for potato breeders to create new recombined genotypes. Establishing the genomic and proteomic profile of potato during tuberization provide new signalling proteins controlling this process with the hormonal balance and the antioxydant system.

Acknowledgements

I once again thank the Journal of New Sciences for inviting me to give this lecture and all of you for your patience in attending it.

5. References

Abelenda J., Navarro C., Prat S. (2014). Flowering and tuberization: a tale of two nightshades. .Trends Plant Sci. 19(2):115-22.

Agahi K., Fotokian M.H. and Younesi Z., (2011). Study of genetic diversity and important correlations of agronomic traits in rice genotypes (*Oryza sativa* L.). *Iranian Journal of Biology*. 25(1) 97-110.

Agrawal L., Chakraborty S., Jaiswal D., Gupta S. , Datta A. and Chakraborty N., (2008). Comparative Proteomics of Tuber Induction, Development and Maturation Reveal the Complexity of Tuberization Process in Potato (Solanum tuberosum L.) J. Proteome Res., 2008, 7 (9), pp 3803–3817.

Aremu C.O., (2012). Exploring statistical tools in measuring genetic diversity for crop improvement. In: *Genetic Diversity in Plants* edited by M. Caliskan (InTech) 340-348.

Bachem C.W., Horvath B., Trindade L., Claassens M., Davelaar E., Jordi W., Visser R.G., (2001). A potato tuber-expressed mRNA with homology to steroid dehydrogenases affects gibberellin levels and plant development. Plant J. 25(6):595-604.

Banerjee A.K., Chatterjee M., Yu Y., Suh S.G., Miller W.A., Hannapel D.J., (2006). Dynamics of a mobile RNA of potato involved in a long-distance signaling pathway. Plant Cell. 18(12):3443-57.

Bhaskar P.B., Wu L., Busse J.S., Whitty B.R., Hameruk A.J., Jansky S.H., Buell C.R., Bethke P.C. and Jiang J. (2010). Suppression of the vacuolar invertase gene prevent cold-induced sweetening in potato. *Plant Physiol* 154: 938-48.

Bou-Torrent J., Martínez-García J., García-Martínez J., Prat S., (2011). Gibberellin A1 metabolism contributes to the control of photoperiod-mediated tuberization in potato. PLoS One 22;6(9):e24458.

Bradshaw J.E. and Bonierbale M., (2010). Potatoes. Root and Tuber Crops. Springer, Berlin: 295p

Brummell D.A., CHEN R.K.Y., Harris J.C., Zhang H., Hamiaux C., Kralicek A. Y., and Mckenzie M.J., (2011) Induction of vacuolar invertase inhibitor mRNA in potato tubers contributes to cold-induced sweetening resistanceand includes spliced hybrid mRNA variants. *J Experiment Bot* 62: 3519–34.

Chen H., Rosin F.M., Prat S., Hannapel D.J., (2003). Interacting transcription factors from the three-amino acid loop extension superclass regulate tuber formation. Plant Physiol. 132(3):1391-404.

Czyzewska D., Marczewski W., (2009). Starch metabolism in potato tubers. Postepy Biochem. 55(4):441-6.

Davis P., (2009) . Potato Tuberization Signals Why do potatoes tuberize? Published in the May 2009 Issue Published online: DR., CORNELL UNIVERSITY.

Debast S, **Nunes-Nesi A**, **Hajirezaei MR**, **Hofmann J**, **Sonnewald U**, **Fernie AR**, **Börnke F.,(2011).** Altering trehalose-6-phosphate content in transgenic potato tubers affects tuber growth and alters responsiveness to hormones during sprouting. Plant Physiol.156(4):1754-71.

Destefano-Beltrán L., Knauber D., Huckle L., Suttle J.,(2006). Chemically forced dormancy termination mimics natural dormancy progression in potato tuber meristems by reducing ABA content and modifying expression of genes involved in regulating ABA synthesis and metabolism. J Exp Bot. 57(11):2879-86.

Ducreux L.J., Morris W.L., Prosser I.M., Morris J.A., Beale M.H., Wright F., Shepherd T., Bryan G.J., Hedley PE, Taylor MA.,(2008). Expression profiling of potato germplasm differentiated in quality traits leads to the identification of candidate flavour and texture genes. J Exp Bot. 59(15):4219-31.

FAO (2011). Potato. Available: www.fao.org

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

Fu Y. and Somers D., (2009). Genomo-wide reduction of genetic diversity in wheat breeding. *Crop Science* 49 161-168.

Gargantini P.R., Giammaria V., Grandellis C., Feingold S.E., Maldonado S., Ulloa R.M., (2009). Genomic and functional characterization of StCDPK1. Plant Mol Biol. 70(1-2):153-72.

González-Schain N., Díaz-Mendoza M., Zurczak M., Suárez-López P.., (2012). Potato CONSTANS is involved in photoperiodic tuberization in a graft-transmissible manner. Plant J.;70(4):678-90.

Gururani M., Upadhyaya C., Strasser R., Y., Park S., (2012). Physiological and biochemical responses of transgenic potato plants with altered expression of PSII manganese stabilizing protein. Plant Physiol Biochem;58:182-94.

Hamernik A.J., Hanneman R.E., and Jansky S.H ,(2009). Introgression of wild species germplasm with extreme resistance to cold-sweetening into the cultivated potato. *Crop Sci* **49**: 529-42.

Hannapel D .,(2013).A perspective on photoperiodic phloem-mobile signals that control development.Front Plant Sci, 2;4:295.

Jackson S.D. (1999). Multiple signalling pathways control tuber induction in potato. *Plant Physiology* 119 1-8.Kim M.S., Kim H.S., Kim Y.S., Baek K.H., Oh H.W., Hahn K.W., Bae R.N., Lee I.J, Joung H., Jeon JH.(2007). Superoxide anion regulates plant growth and tuber development of potato. Plant Cell Rep. 26(10):1717-25.

Kolachevskaya O., Alekseeva V., Sergeeva L., Rukavtsova E., Getman I., (2014). Expression of auxin synthesis gene tms1 under control of tuber-specific promoter enhances potato tuberization in vitro. J Integr Plant Biol, 24 .

Licausi F., Giorgi F.M., Schmälzlin E., Usadel B., Perata P., van Dongen J.T., Geigenberger P.,(2011). HRE-type genes are regulated by growth-related changes in internal oxygen concentrations during the normal development of potato (Solanum tuberosum) tubers. Plant Cell Physiol. 52(11):1957-72.

Lin T., Sharma P., Gonzalez D.H., Viola I.L., Hannapel D.J.,(2013). The impact of the long-distance transport of a BEL1-like messenger RNA on development. Plant Physiol. 161(2):760-72.

Morris W., Hancock R., Ducreux L., Morris J., (2014). Day length dependent restructuring of the leaf transcriptome and metabolome in potato genotypes with contrasting tuberization phenotypes. Plant Cell Environ.;37(6):1351-63.

Morris W.L., Ducreux L.J., Hedden P., Millam S., Taylor M.A..,(2006). Overexpression of a bacterial 1-deoxy-D-xylulose 5-phosphate synthase gene in potato tubers perturbs the isoprenoid metabolic network: implications for the control of the tuber life cycle. J Exp Bot. 57(12):3007-18.

Muñiz García M.N., País S.M., Téllez-Iñón M.T., Capiati DA. ,(2011).Characterization of StPPI1, a proton pump interactor from Solanum tuberosum L. that is up-regulated during tuber development and by abiotic stress. Planta. 233(4):661-74.

Nayar N., (2014). Tuber crop and food security with special reference to the potato. Potato J 41 (1): 1-15.

Noelia M., García M., Stritzler M., Capiati D., (2014). Heterologous expression of ArabidopsisABF4 gene in potato enhances tuberization through ABA-GA crosstalk regulation. Planta , 239, (3), pp 615-631.

Pasare S.A, **Ducreux L.J**, **Morris W.L**, **Campbell R**, **Sharma S.K**, **Roumeliotis E**, **Kohlen W**, **van der Krol S**, **Bramley PM**, **Roberts AG**, **Fraser PD**, **Taylor MA.,(2013).** The role of the potato (Solanum tuberosum) CCD8 gene in stolon and tuber development. New Phytol**.** 198(4):1108-20.

Platonova T.A., Evsiunina A.S., Korableva N.P., (2010). Changes in the plastid apparatus of apical meristem cells of potato tubers upon growth regulation with jasmonic acid. Prikl Biokhim Mikrobiol. 46(3):385-92.

Reid J.B., Davidson S.E., Ross J.J., (2011). Auxin acts independently of DELLA proteins in regulating gibberellin levels. Plant Signal Behav. ; 6(3):406-8.

Rentzsch S, **Podzimska D**, **Voegele A**, **Imbeck M**, **Müller K**, **Linkies A**, **Leubner-Metzger G**., **(2012).** Dose- and tissue-specific interaction of monoterpenes with the gibberellin-mediated release of potato tuber bud dormancy, sprout growth and induction of α-amylases and β-amylases.Planta**.** 235(1):137-51.

Rommens C.M., Ye J., Richael C., and Swords K., (2006). Improving potato storage and processing cha r a c t e r i s t i c s through a l l -na t ive DNA transformation. *J Agric Food Chem* **54**: 9882–87.

Roumeliotis E., Kloosterman B., Oortwijn M., Kohlen W., Bouwmeester H.J., Visser R.G., Bachem C.W.,(2012). The effects of auxin and strigolactones on tuber initiation and stolon architecture in potato. J Exp Bot. 63(12):4539-47.

Roumeliotis E., Visser R.G., Bachem C.W., (2012). A crosstalk of auxin and GA during tuber development. Plant Signal Behav. ; 7(10):1360-3.

Sarkar D., (2008). The signal transduction pathways controlling in planta tuberization in potato: an emerging synthesis. Plant Cell Rep.27(1):1-8.

Sharma P., Lin T., Grandellis C., Yu M., Hannapel D., (2014). The BEL1-like family of transcription factors in potato. J. Exp. Bot.65(2):709-23.

Stushnoff C., Ducreux L.J., Hancock R.D., Hedley P.E., Holm D.G., McDougall G.J., McNicol J.W.., Morris J, Morris W.L., Sungurtas J.A., Verrall S.R., Zuber T., Taylor M.A.,(2010). Flavonoid profiling and transcriptome analysis reveals new gene-metabolite correlations in tubers of Solanum tuberosum L. J Exp Bot. 61(4):1225-38.

Suárez-López P.,(2013). A critical appraisal of phloem-mobile signals involved in tuber induction. Front Plant Sci, 16;4:253.

Suttle J.C., Abrams S.R., De Stefano-Beltrán L., Huckle L.L., (2012).Chemical inhibition of potato ABA-8'-hydroxylase activity alters in vitro and in vivo ABA metabolism and endogenous ABA levels but does not affect potato microtuber dormancy duration. J Exp Bot. 63(15):5717-25.

Valkov V.T., Scotti N., Kahlau S., Maclean D., Grillo S., Gray JC., Bock R., Cardi T.,(2009). Genome-wide analysis of plastid gene expression in potato leaf chloroplasts and tuber amyloplasts: transcriptional and posttranscriptional control. Plant Physiol.150(4):2030-44.

Wang D.Y., Lian Y., Zhu D.W.,(2008). Acid phosphatase activity may affect the tuber swelling by partially regulating sucrose-mediated sugar resorption in potato. J Integr Plant Biol. 50(6):733-41.

Weeda S .M., Mohan Kumar G.N., Knowles N.(2009). Developmentally linked changes in proteases and protease inhibitors suggest a role for potato multicystatin in regulating protein content of potato tubers. Planta. ; 230(1):73-84.

Yu J.W., Choi J.S., Upadhyaya C., (2012). Dynamic proteomic profile of potato tuber during its in vitro development. Plant Sci ; 195:1-9.

Yu J.W., Choi J.S., Upadhyaya C.P., Kwon S.O., Gururani MA, Nookaraju A, .(2012). Dynamic proteomic profile of potato tuber during its in vitro development. Plant Sci. 195:1-9.

Zhou J., Fang H., Shan J., Gao X., Chen L., Xie C., Xie T., Liu J., (2014). A major QTL located on chromosome V associates with in vitro tuberization in a tetraploid potato population. Mol. Genet. Genomics.12; 289(4):575-87.