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Gene Expression during Transition from Dormancy to Sprouting in Potato Tubers

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ABSTRACT

This article reviews the knowledge on the genetic regulation during transition from dormancy to sprouting in potato tuber, to elucidate the molecular mechanisms underlying the physiological and cellular changes driving this complex developmental process. Extensive studies on gene expression were carried out with different tools: differential display, cDNA-AFLP, subtractive libraries, microarrays, quantitative Real Time PCR and proteomic analysis. A high proportion of sequences up- or down-regulated at the end of dormancy are still unknown or match with proteins in databases that have not yet been characterised. Other sequences encode genes involved in hormone metabolism and response, or are transcriptional factors, ribosomal and carrier proteins, or putative regulators of cell growth and division. At the end of dormancy, when the tuber enters in a source condition, a general decrease of synthesis of storage proteins and enzymes involved in biosynthetic metabolism has been observed, as well as modifications in the pattern of proteins associated to membranes. Studies conducted on transgenic plants suggest that phosphate and sucrose levels may play a crucial role in the sink-to-source transition during sprout growth, as well as the reactive oxygen species.

Keywords: EST isolation, meristem activation, sink-source transition, sprouting

Abbreviations: **ABA**, abscisic acid; **ADH**, alcohol dehydrogenase; **ARF**, auxin response factor; **BE**, bromo-ethane; **DDRT-PCR**, differential display reverse transcriptase-polymerase chain reaction; **EST**, expressed sequence tag; **FLD**, fluridone; **GAs**, gibberellins; **IAA**, indole acetic acid; **LUC**, luciferase; **NCED**, 9-*cis*-epoxycarotenoid dioxygenase; **qRT-PCR**, quantitative real-time PCR; **TDFs**, transcripts derived fragments; **ZEP**, zeaxanthin epoxidase

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INTRODUCTION

A potato tuber is hypogeal stem (stolon) which, under appropriate conditions, stops longitudinal growth and swells. Cells located in the pith and the cortex of the apical region of the stem first enlarge and then later divide longitudinally. In this way the subapical part of the stolon swells and subsequently, after reaching a diameter of approximately 2 to 4 mm, the longitudinal divisions stop and are replaced by randomly oriented divisions and cell enlargement (Sanz *et al.* 1996). During its formation, the potato tuber accumulates starch, reserve proteins, in particular the glycoprotein patatin, and other metabolites, becoming the strongest sink organ of the plant. Eventually, the tuber decreases its metabolic activity and as such behaves as a typical storage sink organ.

At the beginning of the swelling, the meristematic activity of the apical region of the stolon and the lateral buds of the developing tuber is repressed, and in this way, dormancy is established (Burton 1989; Claassens and Vreugdenhil 2000). At maturity, the tuber represents a compressed stem and the eyes correspond to apical and lateral axillary buds, which for a period varying from 2 to 6 months, are unable to grow. Thus, tubers can be grouped with other vegetative over-wintering organs such as seeds, corms and buds that also exhibit varying degrees of dormancy, depending on internal or external factors (Okubo 2000). Although the definition of dormancy has been controversial, it is generally accepted that dormancy refers to the physiological state in which autonomous sprout growth does not occur, when the tuber is placed under optimal growing conditions: darkness, temperature 15-20°C, relative humidity about 90% (Reust 1986; Coleman 1987; Burton 1989). Several authors (Lang *et al.* 1985, 1987) classified dormancy into three

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classes: i) endodormancy: conditioned by internal factors of the bud; ii) paradormancy: related to plant tissues external to the bud and iii) ecodormancy: dependent on environmental conditions to which tubers are exposed. From a few days to some months after their maturity, tubers switch from a sink to a source condition, mobilizing their reserves and supplying energy and substrates to the growing sprouts. Breaking dormancy involves the resumption of cellular divisions in the bud meristem and mobilization of reserve substances to support growth and responses to bio-molecular and environmental triggers.

The length of dormancy is a controversial matter: it has been largely discussed because the physiological stage in which tuber seeds are planted greatly affects the yield of growing plants. Burton (1963) proposed of measuring the duration of dormancy from the date of tuber initiation, when the stolon tip starts to swell. Dormancy is deemed to have ended when the buds on the tuber, stored under optimal conditions for sprouting, start to grow. The definition for the end of the dormancy of a tuber lot is the moment when 80% of tubers have formed sprouts (Reust 1986). The presence of at least one sprout per tuber 2 mm long is conventionally taken as the indication of end of dormancy (Reust 1986; van Ittersum *et al.* 1992). This criterion is integrated by the term of physiological age which relates to the conditions or state of tubers, and is usually used to define the stage of development of seed tubers at planting. The main factors influencing physiological age are cultivar, environmental conditions during crop growth, time of harvest and importantly storage temperature regime (O'Brien *et al.* 1983; Caldiz *et al.* 2001).

The length of dormancy is a quantitative, genotype-dependent character: van den Berg *et al*. (1996) detected quantitative trait loci on nine chromosomes, either alone or through epistatic interactions.

The dormancy period varies greatly not only among potato cultivars, but also in the same lot of harvest. The length of dormancy is highly influenced by temperature and humidity in which tubers are stored (van Ittersum and Scholte 1992), by the growth conditions of mother plant such as nitrogen supply, temperature and light (van Ittersum 1992a, 1992b, 1992c). Also the size of tubers can influence the length of dormancy (Reust 1982).

The transition from dormancy to sprout development involves several physiological and biochemical changes, which are bridged by complex genetic regulatory networks. Some physiological mechanisms underlying this transition have been reviewed by Wiltshire and Cobb (1996) and by Suttle (2004a). For example, it has been noted that during dormancy meristems are metabolically active, but the efficiency of respiration, transcription and translation is reduced (Macdonald and Osborne 1988). During progression towards sprouting, tuber accumulates sugars (Wiltshire and Cobb 1996) and undergoes peroxidative damages to membrane lipids (Fauconnier *et al.* 2002).

The onset of sprout growth is accompanied by an increase in cell metabolism, which is strictly dependent on changes in gene expression (Bachem *et al.* 2000; Ronning *et al.* 2003). Other authors associated the resumption of sprout growth with changes in the methylation of meristem DNA (Law and Suttle 2003) and in histone acetylation (Law and Suttle 2004), which usually precede transcriptional activation of genes, leading to cell division and meristem growth in potato tubers.

Other dormancy-related mechanisms involve hormone changes (i.e. abscisic acid (ABA), auxin, cytokinins, gibberellic acid and ethylene) and the influence of other compounds as phenolics, jasmonates and brassinosteroids (Suttle 2004a, 2004b).

Advances in plant genetics and genomics have provided tools to investigate the molecular mechanisms that control dormancy in potato tubers. Analysis of transgenic plants (Carrera *et al.* 2000; Marmiroli *et al*. 2000; Farré *et al.* 2001; Bajji *et al.* 2007), differential display (Liang and Pardee 1992; Agrimonti *et al*. 2000), cDNA-AFLP (Bachem *et al*. 1996, 2000), subtractive libraries (Faivre-Rampant *et al.* 2004a), microarrays (Campbell *et al*. 2008; Kloosterman *et al.* 2008), qRT-PCR (Campbell *et al*. 2008) and proteomic analysis (Lehesranta *et al.* 2006) have been used to investigate genes associated with dormancy in potato tubers.

The goal here is to review the current status of knowledge on the genetic regulation of tuber dormancy and its bearing with physiological and metabolic triggers.

GENE EXPRESSION AND PHYSIOLOGICAL REGULATION OF TUBER DORMANCY

The role of hormones, ethanol and phenolic compounds, in regulation of tuber dormancy has been extensively reviewed by Suttle (2004a). The levels of five hormones (auxins, gibberellins, cytokinins, ABA and ethylene) change during storage and during the transition from dormancy to sprouting, suggesting that they may all be involved. Unfortunately, the genes involved in the metabolism of hormones, as well as their target genes are poorly characterised in potato tuber and until now little information is available on the regulatory network during dormancy and bud growth resumption. In this review, only those compounds for which some genetic determinants have been described will be discussed: a summary is given in **Table 1**.

Auxins

The first studies, started in the 1940s, were conducted to elucidate the role of free and esterified auxins in regulation of dormancy. Pioneering studies indicated that the endogenous levels of auxins were low in tissues of dormant tubers and increased during early sprout growth. At relatively high doses, indole acetic acid (IAA) and the more stable 1-naphthalene acetic acid (NAA) were found to be potent inhibitors of sprout growth when supplied exogenously (Denny 1945). Extremely low concentrations of auxin stimulated the growth of non-dormant sprouts, but had no discernable effects on dormant eyes (Hemberg 1949). Studies using high performance liquid chromatography (HPLC) coupled with fluorometric detection report no increase in free IAA content until the end of dormancy (Sukhova *et al*. 1993). More recent data describe the progressive increase in both free and conjugated IAA levels in tuber apical meristems during dormancy, which rapidly fall on sprouting (Sorce *et al.* 2000). A continuous increase of IAA has been observed also in tuber slices stored at 4°C during progression toward sprouting (Reverberi *et al*. 2001)

The role of auxins in the regulation of gene expression has been poorly characterised. In potato, there are two families of proteins which have been found to be key regulators of auxin-responsive gene expression in other plants: the auxin/indole-3-acetic acid (Aux/IAA) proteins and the auxin response factors (ARFs), (Hagen and Guilfoyle 2002; Liscum and Reed 2002; Wei *et al.* 2006). ARFs are regulatory proteins that bind with the amino terminal of a specific sequence in the promoters of auxin-responsive genes (Ulmasov *et al*. 1999a). These factors can act as repressors or activators of transcription depending on their structure: glutamine-rich central domains activate transcription whereas proline/serine/threonine-rich central domains confer repressor activity (Ulmasov *et al*. 1999b). Aux/IAA proteins do not bind DNA, but interact with ARFs, modulating their activity (Guilfoyle *et al.* 1998; Wei *et al.* 2006). cDNA with strong sequence similarity to *Arabidopsis thaliana ARF6* was isolated by Faivre-Rampant *et al.* (2004a) in a subtractive hybridization library enriched in clones representing genes up-regulated in the potato tuber apical buds on dormancy release (**Table 1**). Expression of *ARF6* is particularly high in the group of cells associated with the development of vascular tissues which lead to the meristem. The transcript was also found in the tunica and corpus. The authors provided evidence that changes in *ARF6* expression occur early in the process of meristem activation and may be of

Table 1 Genes involved in hormonal regulation of tuber dormancy.

Gene/ Sequence	Protein	Function (established or presumed)	Regulation/expression	Transgenic potato lines	Reference
ARF6	Homologous to auxin responsive factor	Transcriptional factor	Up regulated on release from dormancy		Faivre-Rampant *et al*. 2004
StZEP	Zeaxanthin epoxidase	ABA biosynthesis	See **Fig. 2**		Destefano-Beltrán *et al*. 2006a, 2006b
StNCED1	9-*cis*-epoxycarotenoid dioxigenase	ABA biosynthesis	See **Fig. 2**		Destefano-Beltrán *et al*. 2006a, 2006b
StNCED2	9-*cis*-epoxycarotenoid dioxigenase	ABA biosynthesis	See **Fig. 2**		Destefano-Beltrán *et al*. 2006a, 2006b
StCYP707A1	ABA-8-hydroxylase	ABA catabolism	See **Fig. 2**		Destefano-Beltrán *et al*. 2006a, 2006b
StCYP707A2	ABA-8-hydroxylase	ABA catabolism	See **Fig. 2**		Destefano-Beltrán *et al*. 2006a, 2006b
StCYP707A3	ABA-8-hydroxylase	ABA catabolism	See **Fig. 2**		Destefano-Beltrán *et al*. 2006a, 2006b
Stgan	Short-chain alcohol dehydrogenase	ABA biosynthesis		Antisense: premature sprouting	Bachem *et al*. 2001
RD22	Homologous to RD22 of *Arabidopsis thaliana*	Seed development	ABA responsive gene, down-regulated at the end of dormancy		Campbell *et al.* 2008
TUBBY	Homologous to TUBBY protein of *Arabidopsis thaliana*	ABA signalling	Down-regulated at the end of dormancy		Campbell *et al.* 2008
Cysteine protease inhibitors	Cysteine protease inhibitors	Inhibitors of proteases	ABA responsive gene, down-regulated at the end of dormancy		Campbell *et al.* 2008
StGA20ox1	GA 20-oxidase	Gibberellin biosynthesis		Constitutive over expression: shorter dormancy Leaf-specific over expression: shorter dormancy Antisense: no effect on dormancy	Carrera *et al.* 2000
IPT	Isopentenyltransferase	Cytokinin biosynthesis		Constitutive over expression: premature sprouting	Galis *et al.* 1995
Sho	Isopentenyltransferase of *Petunia hybrida*	Cytokinin biosynthesis		Constitutive over expression: premature sprouting	Zubko *et al*. 2005
dxs	Bacterial 1-deoxy-D-xylulose 5-phosphate synthase	Isoprenoid biosynthesis		Constitutive over expression: premature sprouting	Morris *et al*. 2006

primary importance. In particular, *ARF6* expression level decreases upon tuber initiation, when stolon growth changes from a longitudinal to a lateral expansion and there is a marked decline in the mitotic index in the shoot apical meristem. On the other hand, *ARF6* expression is strongly induced in buds upon endodormancy release, when meristematic activity recommences. Expression of *ARF6* increases strongly in axillary buds on release from the effects of apical dominance (paradormancy or correlative inhibition). A high level of *ARF6* transcript was found in leaf tips, supporting a correlation with high meristematic activity.

In *Arabidopsis*, ARF6 functions as an activator of gene expression (Tiwari *et al.* 2003), if the same is the case in potato, the ARF6-induced changes in gene expression may be a key process in the control of dormancy release.

Abscisic acid

Originally isolated and characterised from abscising lupin (*Lupinus albeus et luteus*), cotton (*Gossypium hirsutum*) fruit and dormant sycamore (*Platanus occidentalis*) buds and leaves, ABA has been considered to play a pivotal role in the regulation of plant dormancy (Addicott and Cairns 1983). The ABA synthesis in vascular plants is described in the **Fig. 1A** (Nambara and Marion-Poll 2005). In the potato tuber, the 8'-hydroxylase pathway leading to phaseic acid (PA) and its metabolite dihydrophaseic acid (DPA) is the predominant route of ABA degradation (**Fig. 1B**) (Suttle 1995; Nambara and Marion-Poll 2005).

In the potato tuber, ABA levels are generally highest after harvest, during endodormancy, and decline as sprouting commences (van den Berg *et al.* 1991), but its role in regulating tuber dormancy is rather controversial: endogenous ABA level rises at the end of dormancy when tubers are stored at low temperatures (Coleman and King 1984).

Suttle and Hulstrand (1994) have studied the role of ABA in maintaining dormancy in potato microtubers generated *in vitro*. Microtubers grown in the presence of the herbicide fluridone (FLD), an inhibitor of ABA synthesis, exhibited premature sprouting. When accompanied with the simultaneous application of exogenous racemic ABA, the result is a dose-dependent increase in endogenous ABA and a concomitant decrease in premature sprouting, suggesting a role of this hormone in dormancy maintenance. However, Sorce *et al.* (1996) reported that ABA decreases in the whole tuber after the loss of dormancy, but in bud eyes ABA concentration falls before the mother plant senescence, whereas a gradual rise accompanies overcoming of dormancy during storage.

To further elucidate the role of ABA in dormancy regulation, Destefano-Beltrán *et al.* (2006a) measured the endogenous ABA content in tuber meristems and their surrounding periderm and underlying cortical tissue during postharvest storage. The ABA content was found to be relatively low and constant in the periderm up to 100 days of storage and declined further. In contrast, the ABA content of meristems rose to about 43% during the first 27 days of storage, and then declined until 97 days, slightly increasing after 110 days, to decline definitively. A similar trend, although with lower values, was observed in the cortical tissue.

One gene sequence (*StZEP*) encoding the biosynthetic enzyme zeaxanthin epoxidase (ZEP) (**Fig. 1A**), two putative sequences (*StNCED1* and *StNCED2*) coding for 9-*cis*-epoxycarotenoid dioxygenase (NCED) (**Fig. 1A**) and three sequences coding for the catabolic enzyme ABA-8'-hydroxylase (*StCYP707A1*, *StCYP707A2*, *StCYP707A3*) (**Fig. 1B**) were monitored to quantify the relative abundance of their mRNAs in the three specific tuber tissues mentioned above:

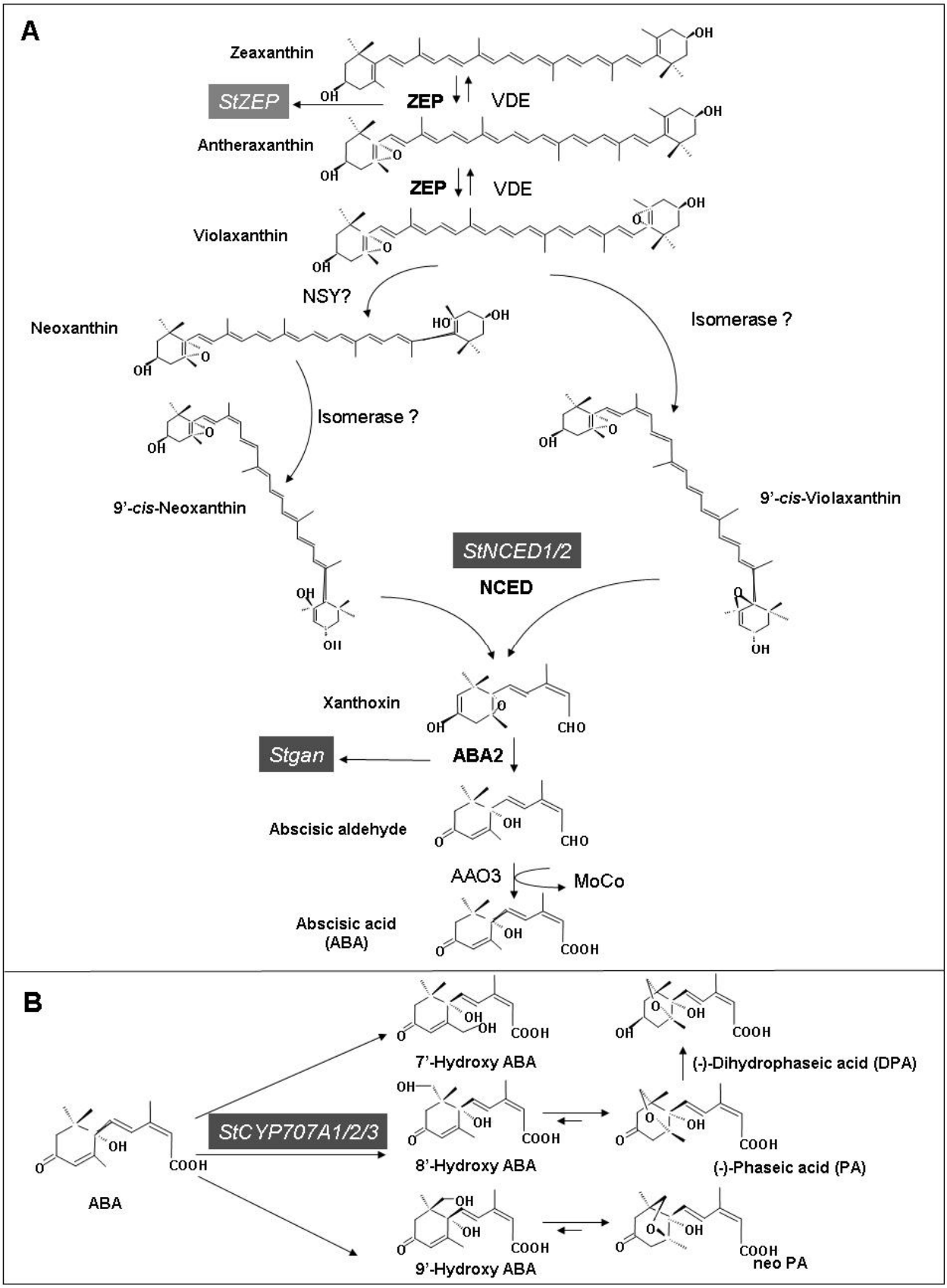

Fig. 1 ABA metabolism in vascular plants and metabolic genes cloned and studied in *Solanum tuberosum*. (**A**) Key steps and enzymes involved in ABA biosynthesis: zeaxanthin epoxidase (ZEP) catalyzes conversion of zeaxanthin to violaxanthin. A reverse reaction occurs in chloroplasts catalyzed by violaxanthin de-epoxidase, (VDE). In the box the gene *StZEP* coding for ZEP in *Solanum tuberosum* is indicated. The formation of *cis*-isomers of violaxanthin and neoxantin requires an isomerase and the enzyme neoxanthin syntase (NSY). Violaxanthin and neoxantin are cleaved by a family of 9-*cis*-epoxycarotenoid dioxygenases (NCED). *StNCED1/2*, indicated in the box are two cDNAs cloned in *Solanum tuberosum* coding for NCED. Xanthoxin is then converted by a short-chain alcohol dehydrogenase (ABA2) into abscisic aldehyde, *Stgan* is a sequences coding for a short-chain alcohol dehydrogenase homologous to ABA2. Abscisic aldehyde is finally oxidized into ABA by an abscisic aldehyde oxidase (AAO3). (**B**) Key steps and enzymes involved in ABA catabolism: ABA is hydroxylated in three different pathways, but the 8'-hydroxylation, catalyzed by ABA-8-hydroxylase is thought to be the predominant pathway. S*tCYP707A1/2/3,* are three cDNAs coding for ABA-8-hydroxylase in *Solanum tuberosum*. Reprinted from: **Nambara E, Marion-Poll A** (2005) Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology* **56**, 165-185, with kind permission of the authors and Annual Reviews, ©2005.

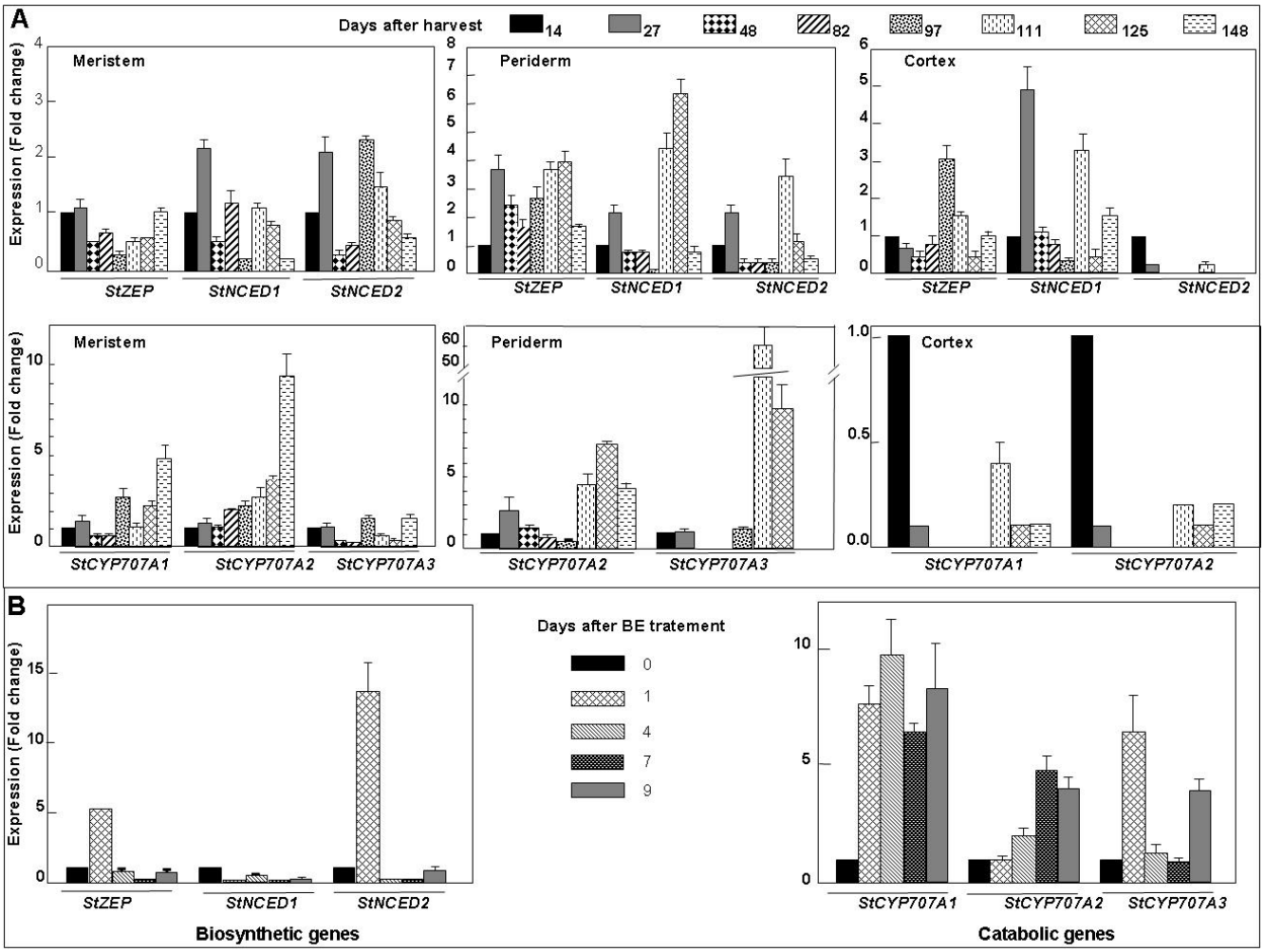

Fig. 2 Quantitative expression analysis of ABA metabolic genes. (**A**) Quantitative expression analysis of ABA biosynthetic (*StZEP, StNCED1/2*) and catabolic (*StCYP707A1/2/3*) genes. Expression analysis was conducted by qRT-PCR in meristem, periderm and cortex of tubers sampled at different times of storage: 14, 27, 48, 82, 97, 111, 125 and 148 days after harvest. Reprinted from: **Destefano-Beltrán L, Knauber D, Huckle L, Suttle J** (2006a) Effects of postharvest storage and dormancy status on ABA content, metabolism, and expression of genes involved in ABA biosynthesis and metabolism in potato tuber tissues. *Plant Molecular Biology* **61**, 687-697, with kind permission of Springer Science + Business Media, ©2006. (**B**) Quantitative expression analysis of biosynthetic (*StZEP*, *StNCED1/2*) and catabolic ABA genes (*StCYP707A1/2/3*) in meristems of potato tubers sampled 0, 1, 4, 7 and 9 days after BE treatment. Reprinted from: **Destefano-Beltrán L, Knauber D, Huckle L, Suttle J** (2006b) 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. *Journal of Experimental Botany* **57**, 2879-2886, with kind permission of the authors and Oxford University Press, ©2006.

bud meristem, periderm and cortical tissues at different times after harvest (Destefano-Beltrán *et al*. 2006a).

Expression of *StZEP* does not mirror changes in ABA content in any of the tissues examined: it is relatively constant in meristems, exhibits a transient increase during early and mid-to-late storage in periderm and peaks in the cortex during mid-storage (**Fig. 2A**). A lack of correlation between ZEP activity and ABA content was also observed in other experiments. Transgenic tubers with both a sense and antisense construct of ZEP, under the control of a tuber-specific promoter, accumulated high levels of ZEP activity, but the amount of ABA was the same as in the controls (Römer *et al*. 2002).

The expression of *StNCED1* and *StNCED2* increases transiently in the early storage in the meristem, mirroring the ABA content (Destefano-Beltrán *et al*. 2006a). This effect is more pronounced for *StNCED2* suggesting that this gene has a key role in the ABA biosynthesis in this tissue (**Fig. 2A**). The situation in the periderm is less clear: despite constant or decreasing levels of ABA, expression of both genes is biphasic, with transient increases at early and late storage (**Fig. 2A**). A different pattern was observed in the cortex, where expression of *stNCED1* correlates with ABA content, with transient increases during early and late storage, while *StNCED2* transcript abundance declined significantly during storage (**Fig. 2A**).

All three catabolic genes *StCYP707A* are expressed in meristems during storage: the expression of *StCYP707A1* increases at the end of storage, *StCYP707A2* rises steadily, while *StCYP707A3* does not change significantly (**Fig. 2A**). An increase in ABA catabolism, therefore, is correlated with the expression of these genes. In the periderm, expression of *StCYP707A1* is not detectable, while *StCYP707A2* and *StCYP707A3* are up-regulated in late storage, along with a decline in ABA content (**Fig. 2A**). In the cortex, expression of *StCYP707A3* is not detectable, whereas *StCYP707A1* and *StCYP707A2* are down-regulated during storage (Destefano-Beltrán *et al*. 2006a) (**Fig. 2A**). In general, a relatively high expression of ABA metabolic genes in the meristem suggests that the tissue is a basic site of ABA metabolism. The ability of tuber tissues to accumulate ABA declines during storage as a result of down-regulation of key ABA biosynthetic genes and the up-regulation of catabolic genes.

Since the interpretation of these data is complicated due to the natural asynchrony in sprouting, to minimise these differences, the experiments were repeated on meristems treated with the synthetic dormancy-terminating agent bromoethane (BE) that induces a rapid and highly synchronous sprouting of dormant tubers. The endogenous ABA content in tuber meristems increases 2-fold 24 hours after BE treatment and then declines dramatically. Seven days

after treatment, ABA content in the meristem declined by > 80%, and exogenous ABA was readily metabolized by isolated meristems to PA and DPA, showing how the BE treatment can almost double the rate of ABA metabolism (Destefano-Beltrán *et al*. 2006b). In these meristems, a correlation was found between the increase in ABA content and expression of *StNCED2*, while a decrease in ABA content was correlated with an increased expression of the three *StCYP707A* genes (**Fig. 2B**). These results confirm that NCED and the catabolic ABA-8'-hydroxylase are key enzymes in ABA metabolism in tuber meristems. Therefore, the ABA content in the tuber balances between synthesis and metabolism that increasingly favours catabolism as dormancy ends.

The biosynthetic enzyme ABA2 which catalyzes the conversion of xanthoxin to abscisic aldehyde (**Fig. 1A**) is a short-chain alcohol dehydrogenase (Gonzales-Guzman *et al*. 2002) belonging to a family of short proteins involved in many developmental processes (Wu *et al.* 2007). cDNA, referred to as *Stgan* that shared a high homology with a short-chain alcohol dehydrogenase, was isolated in potato by Bachem *et al*. (2001). Transgenic tubers expressing the antisense *Stgan* gene showed a premature sprouting and elevated levels of biologically active gibberellins and their relative precursors (Bachem *et al*. 2001) (**Table 1**). The levels of ABA in these transgenic tubers have not yet been determined, but these data suggest once more that biosynthesis of this hormone is crucial in maintaining the dormancy.

Termination of dormancy is associated with decreased expression of some ABA-responsive genes, isolated by high throughput screening and reviewed by Campbell *et al*. (2008) (**Table 1**). Of interesting is the down-regulation of cDNAs coding RD22, a protein first characterised in *Arabidopsis thaliana*, expressed during the early and middle stages of seed development (Yamaguchi-Shinozaki *et al*. 1993). The termination of dormancy in meristems also resulted in the down regulation of a putative TUBBY protein. In *A. thaliana* a TUBBY-like protein has been shown to be involved in ABA signalling (Lai *et al.* 2004). Also cysteine protease inhibitors, whose expression is down regulated at the end of dormancy, are induced by ABA (Seki *et al*. 2002). These observations are consistent with the fact that dormancy is regulated, at least partially, by ABA (Suttle and Hulstrand 1994) and support the existence of a connection between the dormant state and the level of ABA-related transcripts in the potato tuber.

Gibberellins

Typically, treatment with GA_3 induces rapid sprouting in seed tubers when required. Bioassays have demonstrated the presence of GA-like activities in tuber extracts and indicated that endogenous levels of certain GAs increased as sprout growth commences (Suttle 2004a). At present, over 100 GAs have been identified in seed plants. The biosynthetic pathway of GAs, reviewed by Hedden and Phillips (2000) is shown in its principal components in **Fig. 3**; it involves three cellular compartments: plastids, endomembranes and cytoplasm.

In all species of *Solanaceae* examined, the predominant GAs are members of the early 13-hydroxylation pathway leading to GA_1 (**Fig. 3**); in addition, the occurrence of GA_4 in certain potato tissues has also been reported (Jones *et al*. 1988; van den Berg *et al.* 1995; Carrera *et al*. 2000). Although exogenous GA_3 is able to terminate dormancy, the role of GAs in regulating dormancy is not well clarified. Quantification of endogenous GAs by gas chromatography-mass spectrometry (GC-MS) revealed a dynamic pattern of GAs content during progression of tuber dormancy (Suttle 2004b). The concentration of GAs is initially relatively high, declines during postharvest storage and rises again as sprout growth commences. Surprisingly, the content of GAs is higher in deeply dormant tubers than in tubers beginning to exit dormancy. The content of GA_1, GA_{19}, and GA_{20} is greatest in tubers exhibiting robust sprout growth. These observations suggest that endogenous GAs play a role in the regulation of sprout growth after the termination of dormancy, but not in the progression of tuber dormancy.

Suttle (2004b) explored the ability of exogenous gibberellin precursors or members of the early 13-hydroxylation pathway to interrupt dormancy. These compounds exhibited a time and dose-dependent ability to prematurely terminate tuber dormancy. As expected, GA_1 which is the active hormone is the most efficient, followed by GA_{20} and GA_{19}. Earlier members in this pathway (kaurene and GA_{12}) are ineffective. Injection of GA_{19} had no effect on tuber dormancy, but enhances sprout growth of non-dormant tubers. Although levels of GA_1 and GA_{20} rose modestly as sprout growth commences, GA_{19} levels increased significantly at the late storage. This suggests that conversion of GA_{19} to GA_{20} is the rate-limiting step in the GA_1 biosynthetic pathway in tubers. This metabolic step is catalyzed by GA 20-oxidase, a multifunctional enzyme, whose levels and activities are tightly regulated in many plant tissues. Three cDNAs encoding potato GA 20-oxidase (*StGA20ox1/2/3*) were isolated (Carrera *et al.* 1999) (**Table 1**). To investigate the role of this enzyme in tuber dormancy and other developmental processes, transgenic potato lines that over- and down-regulate this gene were produced. Two lines, with constitutive (expressing the transgene in the whole plant) and leaf-specific over-expression of the gene were selected. Tubers harvested from both types of transgenic plants exhibited a shorter dormancy period and formed longer and thinner sprouts than the control tubers. Shortening of dormancy was greater in the constitutive over-expressing lines than in the leaf-specific over-expressors, indicating that dormancy was probably regulated by GAs synthesized directly in the tuber rather than by GAs imported from the leaves. Transgenic lines with the antisense *StGA20x1* showed a reduction in the level of GA_1. Tubers harvested from these lines developed shorter sprouts than those of untransformed lines, but no effect on of dormancy duration was observed (Carrera *et al.* 2000).

The application of growth retardants that inhibit GA biosynthesis on *in vitro* tubers was used to study the role of GAs on tuber dormancy. The application of growth retardants resulted in a premature dormancy release different to the expected result if GA biosynthesis was required for breakage of dormancy (Suttle 2004b). Studies conducted with tubers of dwarf mutants, *Solanum tuberosum*, spp. *andigena*, showed that endogenous levels of GA_1 were below the limit of detection during the entire period of postharvest dormancy. In the same tubers, sprout growth was severely reduced as compared to the growth of the wild-type potato, but the duration of dormancy remained unchanged (Suttle 2004b). These results are consistent with observations in transgenic lines by Carrera *et al*. 2000 (see above) and illustrate how the reduction of GA_1 affects sprout growth, but not the duration of dormancy.

Although the role of GAs in dormancy regulation remains controversial, it has been observed that their artificial increase, either by exogenous injection or by ectopic expression of biosynthetic genes, typically results in premature sprouting of tubers. The endogenous level increases only when sprout growth commences; the GAs, therefore, seem to act preferentially in promoting the growth of non dormant sprouts rather than in interrupting dormancy.

The studies of Bachem *et al.* (2001), discussed earlier, suggest that the levels of GAs are affected by the metabolism of ABA.

Cytokinins

Natural cytokinins are adenine derivatives and can be classified by the configuration of their N^6-side chain as isoprenoid or aromatic cytokinins (Mok and Mok 2001).

It is thought that cytokinins stimulate cell division in plant tissues by releasing the G1 cell cycle block (Francis and Sorrell 2001). All cells in the dormant tuber meristem are arrested in the G1 phase of the cell cycle, and thus a role

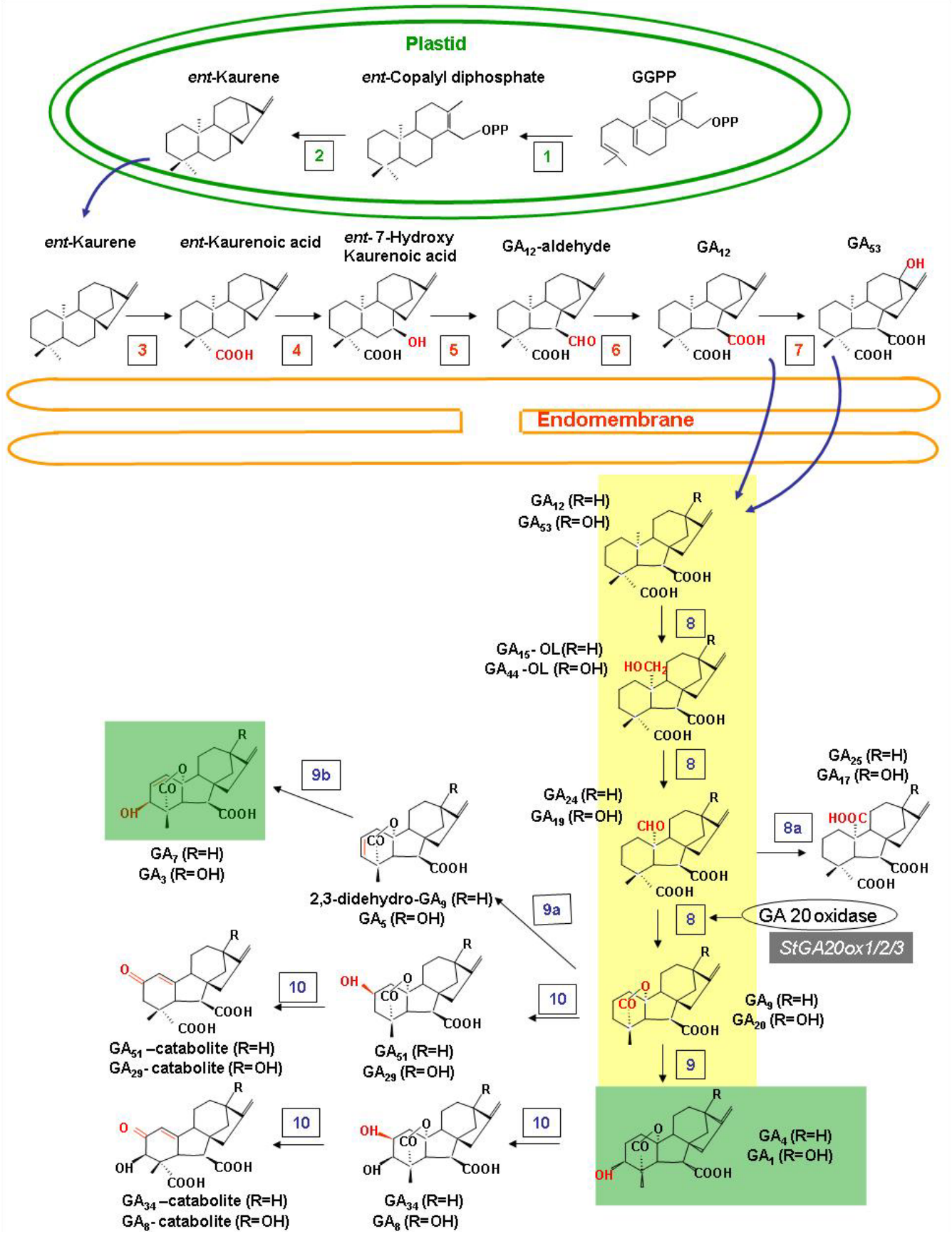

Fig. 3 The pathways of gibberellin (GA) metabolism in higher plants. The synthesis of GAs occurs in three compartments: plastids, endomembranes and cytoplasm. The functional groups that are introduced or modified at each step are indicated in red and the biologically active GAs are highlighted in green. In yellow box the early 13-hydroxylation pathway, predominant in potato, is evidenced with the enzyme GA 20-oxydase and the respective genes *StGA20ox1/2/3*, discussed by Carrera *et al*. (2000). Reprinted from: **Hedden P, Phillips A** (2007) Gibberellin metabolism: new insights revealed by the genes. *Trends in Plant Science* **5**, 523-530, with kind permission of Elsevier, Ltd, ©2007.

of cytokinins in the termination of dormancy has been suggested. Studies have demonstrated that the application of cytokinins to dormant tubers may reduce the dormant period and elicit early sprouting. Endogenous levels of isopentenyladenine (IP) and *trans*-zeatin-type cytokinins increase in tubers prior to the onset of sprout growth (Turnbull and

Hanke 1985b; Suttle 1998). Moreover, injection of IP or *trans*-zeatin in dormant tubers accelerates the termination of dormancy and the start of sprout growth (Suttle 1998). Indeed the efficiency of these supplements depends on their concentration and on the physiological stage of the test tubers. When added immediately after harvest or during the initial period of storage, exogenous cytokinins are unable to stimulate sprouting. The effect is better documented when they are supplied during late storage; in this case the still-dormant tubers exhibit a dose-dependent premature sprouting (Turnbull and Hanke 1985a; Suttle 1998).

The role of these hormones in the regulation of dormancy is also supported by the observation that cytokinin overproducing plants expressing the gene for isopentenyltransferase (IPT), an enzyme involved in cytokinin biosynthesis, exhibit very early sprouting (Galis *et al.* 1995). Zubko *et al*. (2005) obtained similar results with potato transformed with the gene (*Sho*) coding for IPT that was isolated in *Petunia hybrida*. Overexpressing *Sho* resulted in an increase in the level of total cytokinins and in particular of N^6-(^{2}-isopentenyl) adenine (2iP). Transgenic lines with the most pronounced cytokinin biosynthesis produced tubers with a reduced dormancy period and in some cases, with no dormancy at all (**Table 1**).

Morris *et al.* (2006) examined the effect of the expression of the bacterial gene *dxs*, encoding 1-deoxy-D-xylulose 5-phosphate synthase (DXS) in potato. This is a key enzyme in the isoprenoid biosynthetic pathway which produces a wide range of metabolites, including cytokinins. Transgenic tubers expressing the *dxs* gene showed a significant increase in the levels of *trans*-zeatin, associated with a reduction in the length of dormancy (**Table 1**).

Ethanol

The first evidence that ethanol can break dormancy was demonstrated with Jerusalem artichoke (*Helianthus tuberosus*) (Petel *et al.* 1993). Claassens *et al*. (2005) conducted a detailed analysis of the action of ethanol on dormancy and sprouting in potato tubers, studying the effects at the level of visible sprouting and gene activity. The experiments were conducted on *in vitro* tubers grown in a medium containing 0.5% ethanol and with 1% or 8% of sucrose. Ethanol treatment in a medium with 1% of sucrose induced the growth of sprouts. Ethanol in combination with 8% of sucrose induced the growth of secondary tubers. In both cases, ethanol terminated dormancy, whereas the nature of the growth pattern was determined by the level of sucrose.

The effect of ethanol on sprout growth persisted even after the tubers were transferred to an ethanol-free medium. Ethanol vapours were able to induce sprout growth as well, and tubers stored under anaerobic conditions accumulated ethanol and showed premature sprouting. The effect of ethanol on *in vivo* gene expression during the termination of dormancy was studied using potato luciferase (LUC) reporter lines. Particular attention was paid to genes involved in the regulation of the cell cycle and to storage-related genes. Reporter plants containing the promoter of the cell-cycle genes *cycB1;1* and *CDC2a* and the storage-related genes *AGPaseS* and *λPat21*, involved in the synthesis of the storage protein patatin, fused to the coding sequence of the LUC reporter gene, were used.

In microtubers the expression of these genes, rapidly declined after the addition of ethanol with both 1% and 8% of sucrose in the medium (**Fig. 4**). This means that processes like cell division and synthesis of reserves, which are normally associated with tuber development, are blocked by ethanol. Ethanol affects the expression of the cell cycle and

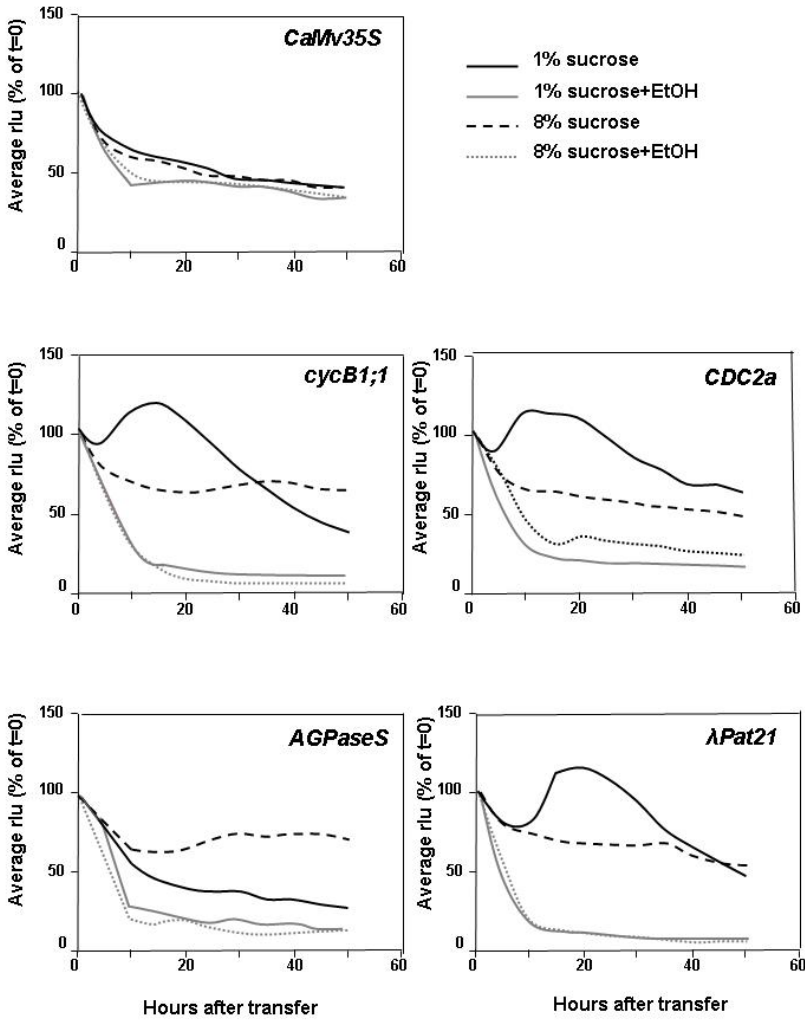

Fig. 4 Effects of ethanol on potato promoters. The effects of ethanol were determined in microtubers expressing luciferase (LUC) under the control of the promoters: *CaMV 35S*, *cycB1;1*, *CDC2A*, *AGPaseS*, *λpat21*. Luciferase activity was measured in microtubers in presence of 1% and 8% of sucrose, with or without ethanol addition. The LUC activities, are expressed as light intensity (rlu) as a percentage of the initial LUC activity at t=0. LUC. Reprinted from: **Claassens MMJ, Verhees J, van der Plas LHW, van der Krol AR, Vreugdenhil D** (2005) Ethanol breaks dormancy of the potato tuber apical bud. *Journal of Experimental Botany* **56**, 533-541, with kind permission of the authors and Oxford University Press, ©2005.

storage-related genes early, when there is no detectable effect on sprout growth. Down-regulation of gene expression is visible within 10 h after ethanol addition, whereas the growth of sprouts starts only 2-3 days after treatment.

In the apical bud, an up-regulation of cell cycle-related genes is observed when tubers are placed in the presence of ethanol with 8% of sucrose in the medium, which promotes the growth of secondary tubers, but not in the presence of ethanol and with 1% of sucrose which promotes sprout growth. Thus, a high activity of the cell cycle genes appears related to tuber development with no effect on resumption of sprout growth. In the presence of ABA, treatment with ethanol is not able to promote sprouting, and the activity of the cell cycle genes is not affected.

The shortening of dormancy in tubers stored in anaerobic conditions, with consequent accumulation of ethanol, suggests that alcohol dehydrogenase (ADH) may play a role during dormancy. Treatment of tubers with 4-methylpyrazole (4-MP), an inhibitor of ADH, inhibited sprouting and prevented ethanol-induced down-regulation of storage and cell cycle genes. These data suggest that ADH activity is important for breaking dormancy. However, the products of ADH have no effect: treatments with acetaldehyde or acetic acid do not induce sprouting or affect gene expression in the tubers. However, the conversion of ethanol in aldehydes by ADH increases the NADH level, which, as observed in other species (Gallais *et al.* 1998), promotes sprouting.

Reactive oxygen species

Generation of reactive oxygen species (ROS), including superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxylradicals (OH^-) is ubiquitous in biological systems, and occurs either as unavoidable by-products of metabolic reactions or through signal-regulated processes under normal and stress conditions (Bolwell 1996, Reverberi *et al*. 2001). In plants, ROS levels are determined by some enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT); this last, in particular is considered responsible for removal of excess of H_2O_2 during stress (Mittler 2002).

In potato tubers dormancy release is accompanied by a transient but remarkable increase in H_2O_2 content, moreover the application of inhibitors of CAT or of exogenous H_2O_2 results in a reduction of the dormancy period and in rapid and synchronised sprouting (Bajji *et al.* 2007). The effects of CAT inhibition on potato tuber dormancy and sprouting were evaluated using the transgenic technology. Plants partially repressed in their CAT activity by antisense mRNA showed acceleration in sprouting kinetics in transgenic tubers compared to those from the wild type, confirming that accumulation of H_2O_2 plays a role in breaking dormancy (Bajji *et al.* 2007). It has been suggested that H_2O_2 may favour the oxidative pentose phosphate pathway (Fontaine *et al.* 1994) or would yield oxygen for respiration and other oxidation processes (Roberts 1969) and for monooxygenases implicated in gibberellin biosynthesis (Fontaine-Roux *et al.* 1997). Other effects of H_2O_2 in cellular mechanisms involved in germination cannot however be excluded, in fact the endogenous level of H_2O_2 modulates the expression of many genes and is involved in the control of growth and differentiation (Penel 1997). In a recent review, Bailly (2004) has reported that the control of dormancy by hormones such as ABA and ethylene could be connected to H_2O_2 signalling and such kind of interplay constitutes a challenge for future research in this area.

METABOLIC EVENTS INVOLVED IN DORMANCY AND SPROUTING

As mentioned in the introduction, dormancy is strictly associated with tuber initiation and enlargement. One of the assumptions is that bud rest starts with the first phases of tuberisation when the tuber exhibits a high capacity to accumulate metabolites. As reported by Viola *et al*. (2001), tuberisation is accompanied with a switch from apoplastic to symplastic phloem unloading of sucrose. Symplastic unloading dramatically increases the intracellular concentration of sucrose with a strong impact on the metabolic and developmental processes. A high concentration of sucrose induces the transcription of several genes involved in tuber storage metabolism (Muller-Röber *et al*. 1990; Visser *et al.* 1994), which allows for the conversion of soluble assimilates (sugars and amino acids) into polymeric reserves (starch and proteins). On the other hand, the apical bud of the stolon, symplastically isolated from the rest of the tuber, results in a reduction in cell division (Viola *et al.* 2001). These functional differences between apical bud and swelling tuber are consistent with differences in sugar distribution and acid invertase activity along the axis of tuberisation. A cell-wall acid invertase was strictly localised in the apical bud region of the stolon, whereas the soluble acid invertase was more abundant in the sub-apical region. The same authors proposed that sucrose is unloaded by symplastic communication in the swelling tuber and is then hydrolysed in the cytoplasm, whereas in the apical bud, sucrose hydrolysis occurs in the intercellular space. This hypothesis is supported by the expression of the gene *invGE*, coding for an apoplastic invertase, in the stolon bud (Viola *et al.* 2001).

In a subsequent experiment, Viola *et al.* (2007) demonstrated that the availability of sugars is low in dormant buds, but increases greatly at the onset of sprouting. The availability of sugars during sprouting may be related with the establishment of symplastic connections between growing sprouts and the rest of tuber. Therefore, buds are symplastically isolated from the tubers during dormancy and this limits the sugar flux. At the end of dormancy, the symplastic connections between tuber parenchyma and buds are re-established, thus the nutrients can flow and growth starts. So far, changes in transcript profiles directly associated to sugar flux have not been found in growing sprouts.

Degradation of starch accumulated in the tuber parenchyma is the main source of energy for growing sprouts. However, not all the enzymes involved in starch degradation are equally involved in sprouting. Tubers of transgenic plants with a significant reduction of the R1 enzyme, involved in starch degradation, showed normal sprouting behaviour (Lorberth *et al*. 1998). On the other hand, transgenic plants in which the cytosolic isoform of a degrading starch phosphorylase was inhibited were not affected in their metabolism of carbohydrates, but displayed an increase in the number of sprouts and a shorter dormancy (Duwenig *et al.* 1997).

The sucrose formation and starch breakdown can be linked with the cellular level of inorganic pyrophosphate. Transgenic plants expressing an additional inorganic pyrophosphatase (Farré *et al*. 2001; Fernie and Willmitzer 2001) driven by a tuber-specific promoter, exhibited a shortened dormancy by 6 to 7 weeks. The inorganic pyrophosphatase would enhance the conversion of glucose-1-phosphate, resulting from starch breakdown, to UDP-glucose through UDP-glucose pyrophosphorylase by the removal of the inorganic pyrophosphate formed (Sonnewald 2001) (**Fig. 5**). This would increase the sucrose and cell wall biosynthesis required by the rapidly growing sprouts. However, it should be stressed that although these results were reproducible over a large number of tubers, premature sprouting was only observed over moderate enzymatic activity. Transgenic plants strongly expressing pyrophosphatase displayed the opposite phenotype, and in extreme cases they never sprouted (Hajirezaei and Sonnewald 1999). The authors suggest that in this case the lack of sprouting was due to a complete shut-down of glycolysis for the inhibition of the pyrophosphate-dependent phosphofructokinase. Sugar availability is, therefore, crucial as an energy supply which drives sprout growth but the studies concerning metabolic regulation of sprouting are very few. Hajirezaei *et al*. (2003) suggest that sucrose level may act as a signal triggering the mobilisation of reserve compounds in storage parenchyma according to the sink demand of growing sprouts. Due to increased suc-

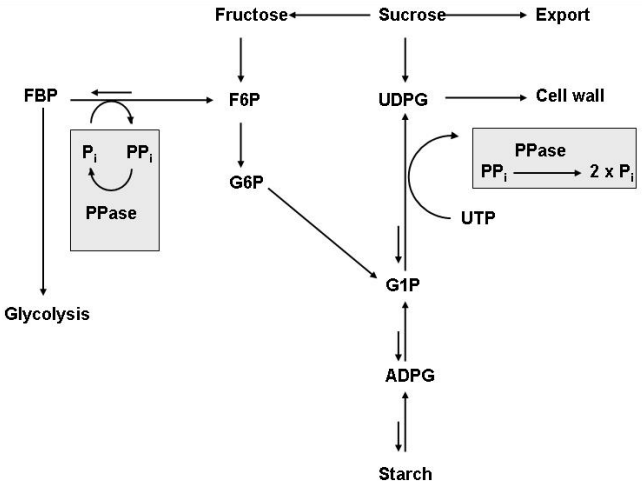

Fig. 5 The role of pyrophosphate in sugar catabolism in potato tubers. The removal of inorganic pyrophosphate (PPi), with formation of inorganic phosphate (Pi) catalyzed by an inorganic pyrophosphatase (PPase), is critical in two steps: in the conversion of glucose-1-phosphate (G1P) to UDP-glucose (UDPG) and in the conversion of fructose-6-phosphate (F6P) into fructose-1,6-bisphosphate (FBP). In presence of low level of PPi and high level of inorganic phosphate (Pi) FBP is converted to F6P and Pi to PPi and glycolisis is inhibited. Other abbreviations: ADPG: ADP-glucose; G6P: glucose-6-phosphate. Reprinted from: **Sonnewald U** (2001) Control of potato tuber sprouting. *Trends in Plant Science* **6**, 333-335, with kind permission of Elsevier, Ltd., ©2001.

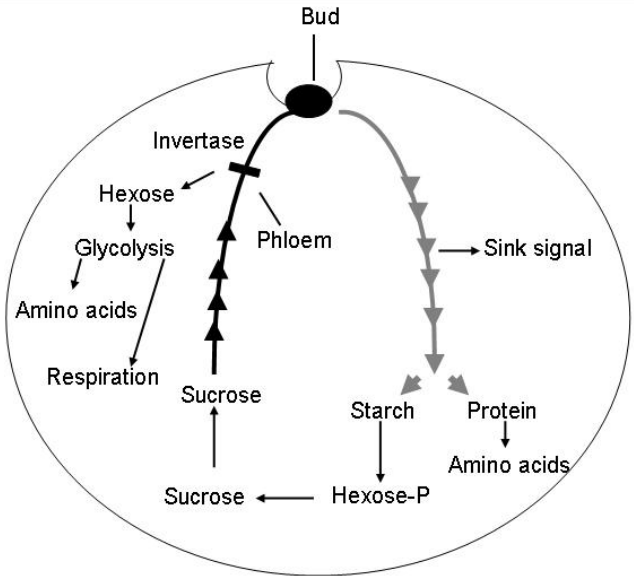

Fig. 6 Effect of phloematic sucrose invertase on reserve mobilisation in the tuber. As consequence of sink signals of the buds (grey arrows) the tuber converts proteins into aminoacids and starch into sucrose. The sucrose fluxes into phloem towards the bud (black arrows), but it is converted into hexoses by the invertase and it could not be utilized for sprouting. In this way, although a high catabolic activity is present in the tuber the sprouting does not occur. Reprinted from: **Hajirezaei M, Börnke F, Peisker M, Takahata Y, Lerchl J, Kirakosyan A, Sonnewald U** (2003) Decreased sucrose content triggers starch breakdown and respiration in stored potato tubers (*Solanum tuberosum*) *Journal of Experimental Botany* **54**, 477-488, with kind permission of the authors and Oxford University Press, ©2003.

rose demand in developing sprouts, soluble sugar level decreases in storage parenchyma cells and this may act as a signal to drive the catabolism of reserves. To study the possible regulatory role of sucrose in potato tuber metabolism, the phloem transport of sucrose was blocked by the expression of a yeast invertase in phloem cells (**Fig. 6**). As a consequence, reserve mobilization in the transgenic tubers was highly accelerated, but sprouting was strongly impaired. Based on these results, it was suggested that a low sucrose level may trigger starch mobilisation, but sucrose flux toward buds is necessary to promote sprout growth.

SEARCH FOR NEW GENES INVOLVED IN DORMANCY REGULATION

Transcriptome analysis has been used to mine genes involved in potato dormancy. A schematic representation of the different methodologies used and the results are shown in **Fig. 7**. Agrimonti *et al.* (2000) made use of Differential Display Reverse Transcriptase PCR (DDRT-PCR) as described by Liang and Pardee (1992) to isolate genes that are up- or down-regulated during dormancy and sprouting in potato tubers: two cDNA clones were isolated and named *G1-1* and *A2-1* (**Table 2**). These cDNAs hybridize with mRNAs expressed at a low level in the tuber; *G1-1* is turned on at the end of dormancy, while *A2-1* is turned off. Sequence analysis of *G1-1* shows some similarities with an expressed sequence tag (EST clones) of the Solanaceae family (Agrimonti *et al.* 2007). *In silico* analysis showed that *A2-1* encodes for a putative ATPase with the Walker box ATP domain that is characteristic of a superfamily of proteins displaying a remarkable diversity of functions, such as active transport of low-molecular-weight compounds and plasmid partitioning in bacteria (Koonin 1993). Transgenic tubers, in which *G1-1* and *A2-1* were inactivated by antisense technology, were produced and analyzed. Statistical analysis on the length of dormancy in transgenic lines for the antisense *G1-1* gene showed a significant increase. Conversely, *A2-1* antisense plants did not reveal any significant change of dormancy (Marmiroli *et al.* 2000). Analysis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) performed on *G1-1* antisense transgenic tubers revealed that two small proteins, of 4292 and 4371 Da, disappeared after inactivation (Careri *et al*. 2003). The *G1-1* expression increased in tuber buds during the first stages of sprouting, whereas almost no transcript was found in the parenchyma (Agrimonti *et al*. 2007). The highest levels of expression were detectable in sprouts and in developing sink leaves. *In situ* hybridization on potato tissues showed that the *G1-1* transcript was mainly localized in the tunica, corpus and pro-cambium tissues of growing sprouts (**Fig. 8**). To get more insight into the function of the *G1-1* gene, the authors chose a correlative approach by comparing the expression of its tomato homologues (*LeG1-1* and *LeG1-2*) during seed germination. The expression of these sequences was highest after the primordial roots emerged from the teguments. Indeed, *LeG1-1* and *LeG1-2* transcript levels were low in meristematic structures formed *ex novo* in the *in vitro* culture of tomato tissues. These results suggest that *G1-1* and its homologues may be responsible for activation and growth of pre-existing meristems, but not for their *de novo* formation (Agrimonti *et al.* 2007).

Faivre-Rampant *et al*. (2004a) used suppression subtractive hybridization to produce a library of genes enriched with those up-regulated on the release of dormancy. A total of 385 different sequences was isolated: approximately 12% of which were similar to ribosomal proteins, which may reflect a resumption of biosynthetic activity on release from dormancy. Heat shock proteins or those with similarity to chaperonines made up some 9% of the sequences. A relatively high percentage of carrier proteins (4%) and transcription factors (3%) were also identified within these sequences. The possible role of an auxin responsive factor, isolated in the subtractive library was discussed earlier. The remaining 36% of these sequences were unknown or matched with unknown proteins.

An EST containing TCP bHLH, a domain characteristic of a family of transcription factors, TCP (Cubas *et al.* 1999) was isolated within a BAC clone (Faivre-Rampant *et al.* 2004b). The steady state level of mRNA for this sequence, called *sttcp1,* was higher in dormant than in sprouting buds and therefore its expression pattern correlates with inactivity of both apical and axillary meristems in potato.

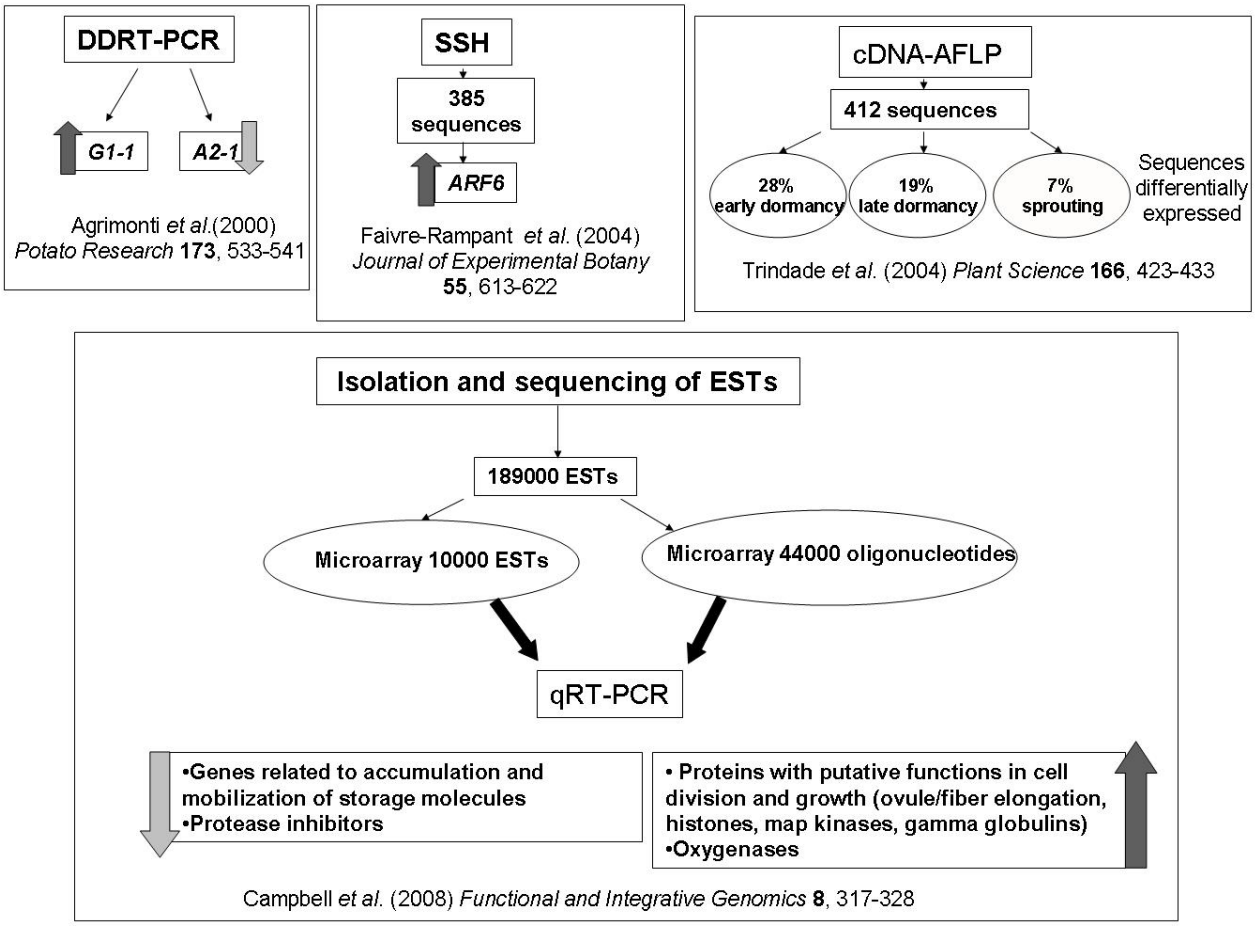

Fig. 7 Approaches of transcriptome analysis. Schematic representation of different tools used to isolate sequences differentially expressed during transition from dormancy to sprouting in potato tubers and the main results obtained. The ⬆ arrow indicates the sequences up-regulated at the end of dormancy, the ⬇ arrow indicates the sequences down-regulated at the end of dormancy.

Table 2 Summary of information on *G1-1* and *A2-1* sequences.

	G1-1	***A2-1***
Regulation	Up regulated in the apical and lateral buds at the end of dormancy.	Down regulated in the tuber at the end of dormancy.
Putative function	Homologous to two EST sequences of *Solanum lycopersicum.*	Homologous to a gene coding for ATPase with the Walker box ATP domain.
Transgenic lines	Tubers of antisense *G1-1* transgenic lines showed a delay in sprouting.	Tubers of antisense *A2-1* transgenic lines did not reveal any significant change in the length of dormancy.
Proteomic data	Analysis with MALDI-TOF-MS on antisense *G1-1* transgenic tubers revealed that two small proteins of 4292 and 4317 Da disappeared.	

Bachem *et al.* (2000) used cDNA-AFLP methodology (Bachem *et al.* 1996) to isolate genes differentially expressed during the life cycle of a tuber in a tuberisation and dormancy synchronised system. Three transcripts derived fragments (TDFs), related to the tuber life cycle showed homology to plant *nsLTP* genes, coding for a class of proteins capable of binding lipid compounds in plant tissues (Horvath *et al.* 2002). The function of these proteins remains substantially unknown; Sterk *et al*. (1991) suggests that they possibly have a role in transferring lipophylic compounds in the apoplast for epicuticular wax formation. The expression profile of the three *nsLTP* related potato TDFs shows a short induction in the stolon just prior to tuber formation and increased expression during sprout development. Histological analysis of transgenic tubers, carrying the GUS gene fused with the promoter of one of the three related *nsLTP* TDFs, showed that GUS activity was concentrated in the vascular bundles that lead to the eyes. The activity increased in the vascular system of growing sprouts after the end of dormancy. These genes may have a role in maintaining dormancy and in regulating sprouting, but in potato plants expression of their antisense RNA was not associated with phenotypic differences, as compared to the untransformed controls, and thus the real function of *nsLTP* is still undetermined.

Analysis of the expression profiles during the life cycle of the potato tuber, reported by Trindade *et al.* (2004), led to the isolation of 412 TDFs from dormant tubers. Eighty eight percent were expressed in the early stages of dormancy, 84% during the late dormancy and 78% during sprouting. The majority of TDFs was expressed during the first phase of dormancy (28%), followed by last period of dormancy (19%) and only 7% were differentially expressed during sprouting. The conclusion from these studies was that the number of expressed genes decreases as the life cycle progresses, with a slight increase during sprouting, but most of these genes showed a profile of constant expression. This agrees with the idea that a large number of genes are required for the metabolic processes occurring during the potato life cycle, and also with the notion that more genes are expressed during tuber induction and tuber growth than during dormancy and sprouting. Expression of several genes during tuber formation is altered by the addition of gibberellic acid, the effect resulted in a different expression profile and/or level, and in other cases a delayed expression of some sequences (Trindade *et al*. 2004).

More recently, a large number of genes that govern the developmental characteristics of potato have been isolated

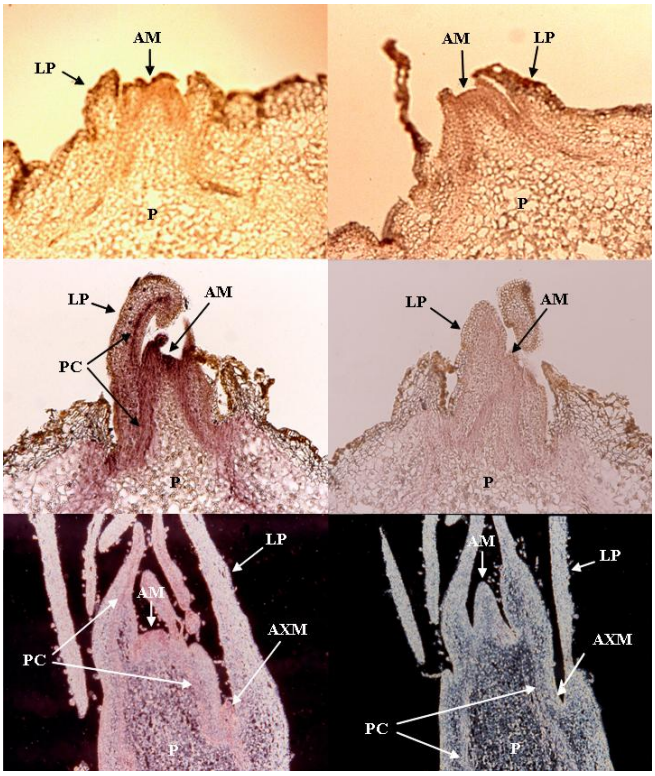

Fig. 8 *In situ* hybridization of G1-1 gene on potato tuber tissues during transition from dormancy to sprouting. A purple-brown stain indicates the presence of *G1-1* transcript. (A) Apical bud of dormant tuber (T0); B) Apical bud of tuber stored for 3 weeks at 20°C (T1); C) Apical bud 1 mm long; D) Apical bud 1 mm long hybridized with sense riboprobe; E) sprout apex; F) sprout apex hybridized with sense riboprobe. Samples E and F were visualized in dark- field microscopy. Abbreviations: AM: apical meristem; AXM: axillary meristem; LP: leaf primordia, PC: procambium; P: parenchyma. Reprinted from: **Agrimonti C, Visioli G, Bianchi R, Torelli A, Marmiroli N** (2007) *G1-1* and *LeG1-1*/*LeG1-2* genes are involved in meristem activation during breakage of dormancy and early germination in potato tubers and tomato seeds. *Plant Science* **173**, 533-541, with kind permission of Elsevier, Ltd., ©2007.

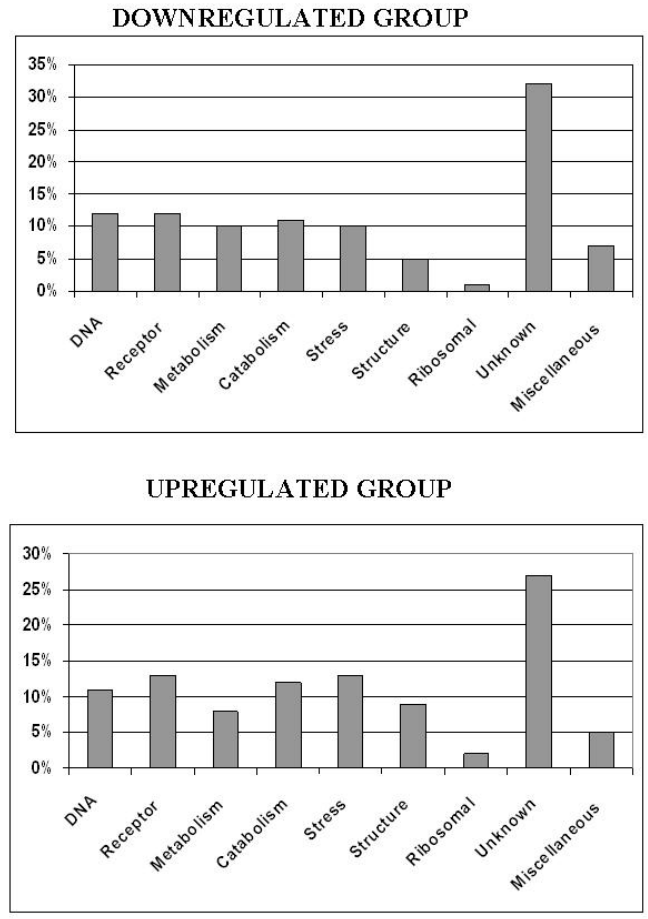

Fig. 9 Results of high throughput expression analysis of sequences isolated during transition from dormancy to sprouting. Functions encoded by cDNAs that exhibit twofold increase or decrease in expression during transition from dormancy to sprouting in tubers under storage conditions are indicated. Data obtained from: **Campbell M, Segear E, Beers L, Knauber D, Suttle J** (2008) Dormancy in potato tuber meristems: chemically induced cessation in dormancy matches the natural process based on transcript profiles. *Functional and Integrative Genomics* **8**, 317-328.

using a high throughput approach. Two big projects to isolate and sequence ESTs were conducted by the Institute for Genomic Research (TIGR) (Ronning *et al*. 2003) and by the Canadian Potato Genome Project (CPGP) (Flinn *et al*. 2005). Sequenced libraries represented the transcripts of tubers and flowers at different stages of development, leaves, cultured cells and roots. A total of 189,000 potato EST sequences were obtained by assembling the results of both projects. Particular attention was devoted to the tuber life cycle that consists of induction, initiation, enlargement, dormancy and sprouting stages, and for this purpose libraries were constructed from stolons, microtubers, dormant tubers and sprouting eyes. As reported by Faivre-Rampant *et al*. (2004a), most of the sequences were isolated at a higher frequency in sprouting than in dormant tubers, encoded ribosomal proteins, while other sequences belonged to a wide class of genes with no clear relationships with resumption of sprouting. A large number of sequences isolated did not match any known genes. Many sequences, were used in high throughput analysis of expression with microarrays. TIGR has developed a microarray containing 10,000 expressed sequences of potato while a consortium of laboratories has recently established a microarray with 44,000 oligonucleotides (60-mer) designed on the full set of EST sequences available in the databases (Kloosterman *et al.* 2008). Campbell *et al.* (2008) utilised TIGR microarray analysis and quantitative real-time PCR (qRT-PCR) to examine gene expression changes in potato tuber meristems both allowed for the termination of dormancy spontaneously after storage, or exposed to the synthetic inducer BE.

Natural progression of dormancy to sprouting resulted in a greater number of down-expressed than up-expressed cDNA. Even though the interpretation of this difference is problematic, it is substantially consistent with the observations of Trindade *et al.* (2004), discussed earlier. Characterisation of the cDNAs by putative function indicates that there are no large functional differences between down- or up-regulated transcripts during dormancy termination (**Fig. 9**). It appears conceivable that there is no global metabolic shift associated with the end of dormancy in tuber meristems, consistent with the observation of Bachem *et al.* (2000) who also failed to observe any gross changes in gene expression in whole microtubers during the progression of dormancy. However, changes observed in the expression of genes related to the accumulation and mobilization of the storage molecule e.g. patatin, are indicative of the shift of tuber metabolism from sink-to-source condition. In growing sprouts mobilisation of storage proteins is accompanied by an increase in expression of cDNAs encoding cysteine protease in growing sprouts (Ronning *et al*. 2003) along with a decrease in the expression of protease inhibitors of metallocarboxypeptidase, cysteine protease, aspartic protease, as well as in a number of other unspecified protease inhibitors. Thus, termination of dormancy in tuber meristems is associated with a decreased expression of inhibitors of all major classes of plant proteases (Callis 1995; Schaller 2004). Other genes induced by ABA are down regulated at the end of dormancy and they have been discussed in the paragraph

above.

In comparison to down-regulated cDNAs, there are fewer genes exhibiting an increase in expression after dormancy termination. During dormancy, tuber meristems are arrested in the G1 phase of the cell cycle, while a resumption of cell cycle activity and the resultant cell division accompanies the onset of sprout growth (Campbell *et al*. 1996). A number of cDNAs, including ovule/fiber elongation protein, histones B2, H3 and H4, map kinase, and γ tubulin encode proteins that have possible functions in cell division and growth, and all exhibited increased expression in sprouting meristems. These data support previous studies that have shown that acetylation patterns of histone proteins change as potato dormancy terminates (Law and Suttle 2004) and suggest that altered histone acetylation may be indicative of *de novo* synthesis of histone proteins.

A cDNA encoding for an oxoglutarate-dependent dioxygenase (ODO) exhibits significant increase in expression at termination of dormancy. ODO is a large group of proteins involved in the incorporation of molecular oxygen during catabolic and anabolic pathways (de Carolis and de Luca 1994). The ODO cDNA found in potato meristems has a high similarity to *Solanum chacoense* 2-oxoglutarate-dependent dioxygenase (SPP2) and *Lycopersicon esculentum* 2-oxoglutarate-dependent dioxygenase (GAD2). SPP2 encodes a transcript that is expressed during the pollen pistil developmental response (Lantin *et al.* 1999). GAD2 is regulated by GA and ABA interactions in tomato, and therefore, the expression of this homologue may be associated with a hormonal shift during dormancy termination (Jacobsen and Ne 1996).

To better understand the molecular aspects of transition from dormancy to sprouting, the analysis of specific protein synthesis during the process has been performed. Borgmann *et al*. (1994) analysed the protein pattern in potato tubers during the sink-to-source transition by two-dimensional electrophoresis (2-DE). Of 1072 polypeptide detected, 296 (28%) were specific to the sink tubers, 185 (17%) were uniquely associated with the source tubers and 591 (55%) were common to both developmental stages. Some of the sink specific proteins were identified as patatin isoforms and as sucrose synthase, a key enzyme involved in carbohydrate metabolism. Conversely, source specific proteins resulted associated with the membranes suggesting that the sink to source transition may be related to different transport characteristics of "sink" as compared with "source" tubers. These results were substantially confirmed by Espen *et al.* (1999) who analysed protein profiles in the soluble and microsomal fraction of parenchymatic tissues of tubers during the final period of growth and during storage. Many of changes in polypeptide profile were detected in the microsomal fraction, suggesting that the changes in cell membrane functionality during the different phases of the tuber life cycle could be linked also to the presence of specific proteins on cell membranes. As the sugar unloading in sink tubers seems to occur symplastically and the apoplastic loading in source involves active transport mediated by carrier proteins (Wright and Oparka 1989; Viola *et al.* 2001) changes in the proteins associated with membranes may be expected.

Some of the polypeptides that were no longer detected in mature tuber appeared again during storage, suggesting that the dormancy, characterised by the drop of some metabolic activities might be due to a lack of synthesis of specific proteins.

Significant changes in protein synthesis during the potato tuber life cycle, including development, dormancy, storage and sprouting, were also recently reported by Lehesranta *et al*. (2006). Proteomic profiling conducted by 2-DE combined with multivariate analysis showed that the different stages of tuber life are characterised by peculiar sets of proteins. Noticeably, sprouting tubers are characterised by a very peculiar profile. The identification of polypeptides, conducted by high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) confirmed the results mentioned above, highlighting the active synthesis of storage proteins and enzymes related to primary and secondary metabolism in developing tubers. The synthesis of these proteins decreases during storage and sprouting, according to a general drop out of biosynthetic metabolism.

CONCLUSIONS

The identification of genes that regulate the transition from dormancy to sprouting has increased our understanding of the physiological bases of the process. As expected, some genes are involved in hormone metabolism and response, and others are involved in mobilisation of reserve substances. Recently, the application of new tools has resulted in an overall picture of the transcription profile during tuberisation, dormancy and sprouting. Comparisons of the results obtained by different authors have identified some common features: 1) transcriptional activity is highest during tuberisation but decreases during dormancy and sprouting; 2) the end of dormancy is not accompanied by a significant increase in transcriptional activity; 3) many genes, up- and down-regulated at the end of dormancy, are unknown or code for unknown proteins; 4) no known genes related with the cell cycle appear regulated during the resumption of bud growth, even though modifications in methylation and acetylation of histones, events that usually precede the transcriptional activation of genes leading to cell division and meristem growth, have been observed.

Proteomic analysis of the potato tuber life cycle evidenced a general decrease of proteins related to active metabolism in the source tubers with respect to sink tubers, reflecting a general decrease in biosynthetic metabolism after tuber maturation. The sink-to-source transition is associated with changes of membrane proteins, probably related to the modification of metabolite flux in the different conditions.

In general all the gene sequences and proteins isolated provide a robust background for future research, but the comprehension of the overall process of dormancy and sprouting is still far because many of these sequences have unknown functions or encode for proteins apparently not related to the process. Silencing or over-expression of these sequences in transgenic plants does not always give clear results. Surely, integration of all the "omics" approaches is required: transcriptomics and metabolomics as well as proteomics together with the transgenic approach can help in understanding the role of these sequences. Additionally, genetic approaches based on identification of genes underpinning QTL should not be neglected to understand the global process of dormancy and sprouting regulation.

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