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Physiological and Biochemical Regulation of Dormancy and Sprouting in Potato Tubers (*Solanum tuberosum* L.)

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ABSTRACT

At harvest, potato (*Solanum tuberosum* L.) tubers are in a dormant state and do not have the ability to sprout. After harvesting, tuber dormancy is released progressively. This release is accompanied by numerous physiological and biochemical changes, such as carbohydrate hydrolysis and the accumulation of saccharides, which are detrimental to the nutritional and processing qualities of potatoes. Endogenous hormones are thought to play a significant role in the regulation of tuber dormancy and in the onset of sprouting. This review aims to describe the involvement of all major classes of endogenous hormones in tuber dormancy, as well as the physiological and biochemical changes during these steps. Based on current scientific evidence, both abscisic acid (ABA) and ethylene are required for the induction of dormancy, however, only ABA plays a role in maintaining bud dormancy. Moreover, an increase in cytokinin sensitivity and content appear to be the principal factor leading to the release of dormancy. Changes in endogenous indole-3-acetic acid (IAA) and gibberellin (GA) content appear to be more closely related to the regulation of subsequent sprout growth. Physiological and biochemical changes during tuber dormancy and the termination of dormancy centre on carbohydrate levels and the activity of enzymes involved in carbohydrate metabolism. In particular, starch breakdown, accompanied by an increase in the concentration of sucrose and reducing sugars, appears to play an important role in the onset of sprouting of tuber buds. Many of these changes are initially observed in tissues in the region of the buds, but subsequent growth of the sprout significantly affects the metabolism of tissues in other regions of the tuber. The application of dormancy-breaking chemicals, such as bromoethane, or phytohormones (GA_3), to potato tubers induces metabolic changes that lead to dormancy breakage. These changes differ between treatments and also from those occurring when dormancy is broken naturally.

Keywords: biochemistry, physiology
Abbreviations: **ABA**, abscisic acid; **BA**: benzyl adenine; **BS**, brassinosteroid; **CIP**, isopropyl N-phenyl carbamate; **CIPC**, chlorinated isopropyl N-phenyl carbamate; **GA**, gibberellin; **MH**, maleic hydrazide; **IAA**, indole-3-acetic acid; **NAA**, 1-naphthalene acetic acid; **PA**, polyamine; **TPS**, true potato seed

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Invited Review

INTRODUCTION

From antiquity, potato (*Solanum tuberosum* L.) is perhaps one of the oldest vegetables that is thought to have improved human health and nutrition, influenced wars, patterns of human migration, and the economy of many nations (Salaman *et al*. 1985). The potato is indigenous to various parts of South America; plants in a wild state have been found along the Peruvian coast, as well as on the mountainous lands of Central Chile and Argentina (**Fig. 1**). It is believed that the Spaniards first brought potatoes from Quito, Ecuador to Europe in the early part of the 16th century. European immigrants introduced potatoes to North America throughout the 1600s, but they were not widely grown in America until after 1719. According to the FAO, potatoes are the most important vegetable in the world. The release of different types of processed potatoes in the market over the past several decades has been accompanied by an increased consumption of frozen potato in developed countries and a decline in the consumption of fresh potato. During the last five years (2002-2006), worldwide production of potatoes fluctuated between 313 and 333 million metric tons (MT) (FAO 2006), and 70% of the potato crop is stored for medium-long-term according to the demands of consumers and processors. Unlike other major horticultural crops, the potato is stored as fresh produce in a perishable form. Annual estimated postharvest losses in the world average 10-15% of the harvested crop but can be as high as 30% in developing countries, where storage facilities are lacking and the best practices of harvesting and handling have not been fully adopted. Maintenance of postharvest quality attributes of marketed potatoes is of prime importance to producers, processors and consumers. Potato tuber deterioration during storage can result from either physiological disorders or disease-related processes (Salaman *et al*. 1985).

Sprouting is one of the most important physiological processes affecting postharvest tuber quality (**Fig. 2**). This process is accompanied by other physiological and biochemical changes, including increases in respiration, water loss, reducing sugar content and glycoalkaloid content (Burton 1989; Claassens and Vreugdenhil 2000) (**Fig. 3**). All these changes are detrimental to quality attributes of the fresh potato. For these reasons the majority of harvested potatoes are stored for medium-long-term periods and are often treated with synthetic sprout inhibitors - mainly with carbamate isopropyl N-Phenyl [CIP] or carbamate isopropyl N-Phenyl-chloride [CIPC] - prior to storage. However, the situation is reversed for seed-potato where it may be desirable to break tuber dormancy rapidly. Regardless of the intended end-use, improvements in sprout control technologies are hampered by our insufficient knowledge of the physiological basis of tuber dormancy.

Fig. 1 Colourful edible potatoes (*Solanum tuberosum* L.).

Fig. 2 Sprouting old potatoes.

Fig. 3 Potato brown fleck, a physiological disorder occurring during the postharvest life.

This review aims to present the role of endogenous hormonal factors in the regulation of potato tuber dormancy with regard to changes in tuber metabolism during this period and the effect of exogenously applied treatments to break or extend dormancy.

THE CHARACTERISTICS OF POTATO TUBER DORMANCY

From a botanical point of view, the potato tuber is a thickened stem, and the eyes correspond to apical and lateral axillary buds. Potato tubers are vegetative over-wintering organs and like many other organs of propagation and perennation, such as seeds, corms, buds, exhibit varying degrees of dormancy (Okubo 2000). However, dormancy in potato tubers, unlike that in seeds, is confined to the buds and is not a characteristic of the whole organ. Examples of dormancy can be found at all levels of biological complexity. Because dormancy is considered to be a survival mechanism, dormant organs are more resistant to biotic and abiotic stress resulting from their insensitivity to environmental factors during this period of "rest".

Potato (*Solanum tuberosum* L.) tuber dormancy, which is considered to be a condition of physiological rest, is reported to start at the beginning of tuber initiation or "tuberization" and continue until 6 to 12 weeks after harvest, depending on varietal characteristics (Emilsson 1949; Burton 1989), although in some cultivars, dormancy may be as long as 15-27 weeks. Thus, during tuber development on the mother plant, the buds differentiate and progressively enter dormancy, beginning first from the bud that is formed in the region of the stolon, and ending with the bud at the apex of the tuber (van Es and Hartmans 1987). For this rea-

son, the duration of dormancy of potato tubers depends both on factors that are present during tuber growth on the mother plant as well as on those that exist after harvest and during storage (Burton 1989).

From a genetic point of view, quantitative trait loci (QTL) analyses have indicated that tuber dormancy is controlled by no less than nine distinct loci (van den Berg *et al*. 1996), although the inheritance pattern of tuber dormancy is more complex. Given the complexity of dormancy control it is likely that the physiological and genetic processes regulating dormancy are equally complex whereas dormancy can be affected by both preharvest and postharvest environmental conditions. Burton (1989) reported that under a temperature regime varying between 3 and 25°C, the period of tuber dormancy is in inverse proportion to storage temperature. Moreover, tuber dormancy can also be released by a variety of chemicals, such as gibberellic acid (GA) and bromoethane, although their mechanisms of action are not clearly understood. These two chemical agents are mainly used to stimulate the sprout growth of seed potatoes (Coleman 1987; Allen *et al*. 1992). Physiologically, dormancy is defined as the absence of any visible growth. Although many cellular processes, such as respiration (Perez-Trejo *et al*. 1981) and protein biosynthesis (Kaur-Sawhney *et al*. 1982), are suppressed during dormancy, the meristematic regions remain metabolically active. Dormant organs are also characterized by their inability to sprout under conditions (e.g. water, light and temperature) that are favourable to sprouting (Macdonald and Osborne 1988; Lang 1996). According to the European Association for Potato Research (EAPR), potato tuber dormancy is defined as "the physiological stage of tubers during which no autonomous growth of the buds occurs even when the environmental conditions for sprouting are favourable, i.e. dark, 15-20°C temperature and RH about 90%" (Reust 1986). However, the concept of dormancy is more complex than it appears at first glance and Lang *et al*. (1987) defined three types of dormancy:

1) Endodormancy: The growth of the meristem is arrested due to factors within the tuber itself.

2) Ecodormancy: The meristem is arrested due to environmental factors, e.g., low temperatures prevent or delay the sprouting of buds.

3) Paradormancy: The meristem is arrested due to external physiological factors, e.g., a dominant bud inhibits the growth of other buds.

According to Vreugdenhil (2007), potato tubers (i.e. the 'eyes' or buds) may exhibit all three types of dormancy: immediately after harvest, the meristems (eyes) are in a state of endodormancy, and do not sprout even when temperature, moisture and other conditions are favourable. When the dormancy of one of the eyes has ended (usually the apical eye), the other eyes remain paradormant for a while, because of the inhibition of growth by the growing apical shoot. This phenomenon has clear links with physiological ageing. When temperatures are too low, tuber buds (eyes) remain dormant due to ecodormancy.

IMPORTANCE OF POTATO DORMANCY

The onset of sprout growth, that follows the termination dormancy, is accompanied by increases in cell metabolism and physiological activities. Recent investigations have demonstrated that major changes in gene expression occur during the progression of dormancy (Bachem *et al*. 2000; Ronning *et al*. 2003). Thus, understanding and controlling potato tuber dormancy and sprouting are essential for growers, processors and fresh markets. As stated above, the aims of understanding and controlling potato tuber dormancy and sprouting might be either to delay sprouting or accelerate sprouting, depending on the final use of the crop. Most potatoes for the processing and fresh markets are stored for long periods of up to 9 months, and in this case to control the quality during storage the avoidance of precocious sprouting of tubers is crucial. Although this is usually achieved by low storage temperatures (3-4°C), low temperatures also induce the hydrolysis of starch and its conversion to reducing sugars, which interfere with technological qualities such as good behaviour during processing and transformation (French fries, mashed potato, etc.) (Haase 2007). In contrast, the acceleration of sprouting and early termination of dormancy is often required, especially when seed potato is exported to other growing regions.

POTATO TUBER DORMANCY AND ITS HORMONAL REGULATION

Abscisic acid (ABA)

Abscisic acid (ABA) is a plant hormone that plays important roles during many phases of the plant life cycle, including seed development and dormancy, and in plant responses to various environmental stresses. Initially known as "*dormine*" or "*dormines*", Hemberg (1949) was the first to recognize the potential importance of these inhibitors in dormancy regulation. The length of potato tuber dormancy depends on both the genotype and the environmental conditions during growth and storage. 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 still unknown (Seo and Koshiba 2002).

Primarily, ABA was isolated and characterized from abscising lupin and cotton fruit and dormant sycamore buds and leaves. This phytohormone is known to play a fundamental role in the regulation of plant dormancy (Addicott and Cairns 1983). Studies with genetically defined and physiologically characterized ABA-synthesis and -response mutants provide unequivocal proof for a role for endogenous ABA in the process of seed dormancy. In *Arabidopsis*, seed dormancy is absent in both these types of mutants, whereas in tomato, dormancy is absent in the ABA-deficient *sit* mutant (Karssen *et al*. 1990). In potato tubers, the concentration of ABA is high during tuber development on the mother plant and for this reason ABA is considered to play a significant role in the establishment and maintenance of tuber dormancy (Claassens and Vreugdenhil 2000). Complementing these data, there are studies using inhibitors of carotenogenesis. ABA is now thought to be synthesized from a C40 carotenoid precursor via a series of cleavage and oxidation steps (Parry and Horgan 1991).

The results of a series of successive studies carried out on potato, demonstrated that (i) endogenous contents of the growth inhibitors are highest in dormant tuber periderm extracts, but decline during storage as dormancy is released, and (ii) treatments with phytohormones that prematurely terminate tuber dormancy also result in a rapid decline in endogenous inhibitor content. However, the obviously heterogeneous biochemical nature of the different fractions of inhibitors separated and isolated from plants complicated simple interpretation of these observations (Blumenthal-Goldschmidt and Rappaport 1965; Franklin and Hemberg 1980; Hemberg 1985). Milborrow (1967) identified ABA as a bioactive component of the crude inhibitor complex, and El-Antably *et al*. (1967) reported that exogenous ABA elicits a transient, dose-dependent inhibition of potato sprout growth. *In situ* analyses demonstrated that the endogenous level of ABA was also high in extracts obtained from both dormant tubers and periderm samples, but this level declined during postharvest storage (Coleman and King 1984; Suttle 1995). However, a basal ABA content for dormancy maintenance was not clearly established, although Sorce *et al*. (1996) reported that the ABA content in the extracts of tuber eyes showed an increase as dormancy is released and sprouting commences.

On the other hand, Suttle and Hultstrand (1994) investigated the involvement of endogenous ABA in tuber dormancy using an *in vitro* microtuber system and the effect of an inhibitor of ABA biosynthesis (fluridone). They observed that in fluridone-treated microtubers ABA accumulation was inhibited by over 90% and resulted in premature sprou-

ting, while exogenous application of ABA to fluridone-treated microtubers restored ABA levels to the control values and suppressed precocious sprouting. Moreover, the application of ABA-inhibitor to dormant microtubers also resulted in a decline in ABA content and a concomitant increase in premature sprouting. These results clearly suggested that sustained biosynthesis of ABA is required for both the induction and maintenance of potato tuber dormancy.

Recently, 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. However, the protracted length of the natural dormancy process complicated the interpretation of these data. Destefano-Beltrán *et al*. (2006) reported that the changes in ABA content observed during tuber dormancy progression are the result of a dynamic equilibrium of ABA biosynthesis and degradation that increasingly favours catabolism as dormancy progresses. According to these authors the application of bromoethane to potato tubers causes an increase in the concentration of ABA on the first day after treatment, but thereafter (4-9 days after treatment), the ABA concentration decreases to levels lower than those detected prior to bromoethane application. Destefano-Beltrán *et al*. (2006) suggested that the breakage of bud dormancy of potato tubers by bromoethane (9 days after treatment) related to this decline in ABA concentration.

Auxins

Auxins were the first the first class of endogenous plant hormones to be characterized chemically, and their roles in nearly all aspects of plant developmental regulation attracted early attention (Went and Thimann 1937; Woodward and Bartel 2005). The first investigations using bioassays were carried out by Hemberg in 1949 and indicated that the endogenous levels of indole-3-acetic acid (IAA) were low in dormant tuber tissues and increased during early sprout growth. Some exogenous types of auxins, such as IAA and 1-naphthalene acetic acid (NAA), were found to be potent inhibitors of sprout growth at relatively high doses (Denny 1945). Free and esterified auxins also inhibited sprout growth in non-dormant potatoes. However, extremely low concentrations of auxins stimulated the growth of non-dormant sprouts effectively, while no effects on dormant eyes were observed (Hemberg 1949). According to Suttle (2004), the exogenous application of auxin (IAA or NAA) to potato tubers after dormancy breakage, promotes sprout growth probably due to cell extension but high concentrations may be inhibitory. The immersion of tubers in aqueous solutions of IAA did not affect the duration of dormancy of tubers produced by plants grown from true seed potato (TPS) (Alexopoulos 2006).

Subsequent, studies using modern analytical techniques, such as HPLC, have confirmed that free IAA is only observed after the total release of dormancy (Sukhova *et al*. 1993). On the other hand, recent investigations using GC-MS found that the content of free IAA in the eyes of potato tubers increased prior to the onset of visible sprout growth (Sorce *et al*. 2000). These data prompted these authors to propose a role for IAA in the control of dormancy. However, although there are no reports to date that demonstrate an involvement of exogenous IAA (or any other auxin) in the premature termination of tuber dormancy; investigations are still in progress. Indeed, the data reported do not support an effective role for endogenous IAA in tuber dormancy, however, they suggest a role of IAA and other endogenous auxins in subsequent sprout growth. This role is in agreement with the proposal that auxins are essential cognate regulators of cell cycle progression in all plant tissues (Francis and Sorrell 2001).

Interactions of auxins with other hormones

The interaction of auxins with other phytohormones makes their roles more complex. Various studies have demonstrated that auxins modulate hormone levels, and physiological studies suggest many links between these phytohormones. Furthermore, several other hormones modulate or are modulated by auxins levels and responses. Indeed, one of the oldest known relationships in plant cell biology is the interaction between auxins and cytokinins, which is frequently employed *in vitro* to induce root and shoot development, respectively (Skoog and Miller 1957).

There is an inverse correlation between the levels of auxin and cytokinin *in vivo* (Eklöf *et al*. 2000) and auxin treatment can rapidly inhibit cytokinin biosynthesis (Nordström *et al*. 2004). Moreover, there is a strong correlation between the levels of auxins and the gaseous hormone ethylene. Exogenous application of auxins stimulates ethylene production (Morgan and Hall 1962) through the induction of a gene encoding the rate-limiting enzyme in ethylene biosynthesis (Abel *et al*. 1995). Conversely, ethylene inhibits lateral (Burg and Burg 1966) and basipetal (Suttle 1988) auxin transport. Likewise, auxin causes increased gibberellic acid (GA_3) production in peas (Ross *et al*. 2000), and basipetally transported auxins are necessary for the production of the active gibberellins, GA_1 and GA_3, in barley (Wolbang *et al*. 2004). GAs act, at least in part, by promoting the degradation of *DELLA* repressors (Silverstone *et al*. 2001); disrupted auxin transport precludes GA-mediated *DELLA* protein degradation (Fu and Harberd 2003). Auxin response is also connected to brassinosteroids (BRs), which act in conjunction with auxins to promote root gravitropic curvature in maize (Kim *et al*. 2000). BR and auxin treatments induce the accumulation of many of the same transcripts (Goda *et al*. 2004; Nemhauser *et al*. 2004).

Exposure to ABA decreases free IAA levels while increasing esterified IAA conjugates in muskmelon ovaries (Dunlap and Robacker 1990). As an antagonist of auxins, exogenous ABA inhibits lateral root formation (de Smet *et al*. 2003).

Cytokinins

Cytokinins are present in all parts of potato tubers and are equally distributed between the apical, lateral and internodal tissues during dormancy. They are defined by their ability to stimulate cell division in hormone-depleted plant tissues by releasing a G-1 cell cycle block (Francis and Sorrell 2001). Growth inhibition in dormant tuber meristems is a result of the arrest of bud meristem cells in the G-1 phase of the cell cycle (Campbell *et al*. 1996). Cytokinins are likely candidates for endogenous dormancy-terminating hormones. Hemberg (1970) demonstrated that both natural and synthetic cytokinins can break tuber dormancy.

Van Staden and Dimalla (1978) reported that the breakage of dormancy coincided with a rapid increase in the levels of free cytokinins in both apical buds and adjacent tissues. They also noted that these high levels of cytokinins in the apical tissue were maintained as long as apical dominance was displayed. Once apical dominance was overcome, the cytokinin level in the lateral buds and in the tissue adjacent to them were similar to its level in the apical regions. From their data, these authors concluded that cytokinin glucosides are transported to the meristematic regions of the tubers, where they are hydrolysed to their free bases. Free bases in excess of those required for growth are apparently converted to storage forms (particularly zeatin glucoside) in the meristematic regions of the tubers and in the sprouts again. However, van Staden and Brown (1979) found that buds and sprouts do not synthesize cytokinins, but initial bud growth may be dependent on the supply of cytokinins within the mother tubers, while the increase in the sprouts could be the result of root development at their basal ends. Additionally, it is possible that towards the end of tuber dormancy, the sensitivity of the bud tissues to endogenous cytokinins increases (Turnbull and Hanke 1985a).

The concentration of cytokinins is high during the early stages of tuber growth and development (Turnbull and Han-

ke 1985b), but subsequently declines. According to Turnbull and Hanke (1985a), the exogenous application of cytokinins during the first six weeks after the start of tuber formation leads to faster breakage of dormancy.

On the other hand, Koda (1982) investigated the levels of cytokinins in potato tubers during storage under different regimes. This author noted that the level of butanol-soluble cytokinin in potato tubers decreased greatly after harvest of the tubers, remained low during the rest-period, but began to increase prior to the end of the period. Under a high temperature storage regime (25°C), its level rose faster than under a cold temperature storage regime (4°C). He also noted that the level of water-soluble cytokinin, which was high during the rest-period, decreased after rest was broken. Interestingly, he noted that wounding the tubers caused an increase in butanol-soluble cytokinin but this increase in cytokinin, within the first 24 h after wounding, was lower in warm-stored tubers than in cold-stored ones and decreased with increasing storage time of the tubers. The level of water-soluble cytokinins decreased with wounding. Therefore, he concluded that water-soluble cytokinin is a storage form and that the level of butanol-soluble cytokinin is regulated by interconversion between the two forms.

Similarly, Suttle (1998a) reported that an increase in bioactive cytokinins preceded the onset of sprouting in tubers stored under growth-permissive conditions and in tubers held in a cold regime (3°C). This author concluded that the increase in bioactive cytokinins was not a result of bud growth, but was more likely due to the re-initiation of meristematic activity. Moreover, potato tubers do not contain cytokinins of the *trans*-zeatin family only, but also *cis*-zeatin derivatives, which exhibit biological activity in a number of bioassays (Mauk and Langille 1978). Suttle and Banowetz (2000) reported that the application of exogenous cytokinins (*cis*- and *trans*-zeatin) was not only effective in breaking tuber dormancy, but also triggered an increase of endogenous levels of free *cis*-zeatin, suggesting that *cis*-zeatin might be a potential regulator of potato tuber dormancy.

Furthermore, dormancy is characterized by a progressive increase in sensitivity to cytokinins, and endogenous cytokinins levels increase even though the potato tuber is not sensitive to external application of cytokinins immediately after harvest or during the early postharvest period (Turnbull and Hanke 1985a; Suttle 2002; Alexopoulos *et al.* 2007b), either because other substances are responsible for dormancy breakage (Suttle 2001) or because exogenously applied cytokinins are rapidly metabolized (Kaminec *et al*. 1997).

Thereafter, dormant tubers exhibited a time-dependent increase in cytokinin sensitivity. The increase in sensitivity to cytokinins was not accompanied by changes in cytokinin metabolism, which suggested that elements of the perception of cytokinins and/or the signal transduction pathway were affected by dormancy status (Suttle 2002). These results suggest that endogenous cytokinins are natural dormancy-terminating agents in potato tubers.

Gibberellins

Unlike other phytohormones (mainly auxins), which are classified on the basis of their functions), gibberellins are classified both on the basis of their structure and on the basis of their function. All gibberellins are derived from the *ent*-kaurene skeleton, and the derivatives of this skeleton along with the structure of a few of the active gibberellins are described below. The gibberellins are named GA_1....GA_n in order of their discovery, while gibberellic acid, which was the first gibberellin to be structurally characterized, is GA_3. At present, 136 GAs have been identified in plants, fungi and bacteria, and over 100 GAs come from seed plants (Heddon and Kamiya 1997). This structural diversity of GAs, together with the usual concerns over bioassay data, renders interpretation of data difficult. Naturally occurring gibberellins may be intimately involved in controlling the rest period in potato tubers (Boo 1961; Smith and Rappaport 1961). As consistently reported and confirmed in numerous investigations, potato dormancy can be broken with exogenous GAs (Brian *et al*. 1955; Rappaport *et al*. 1958; Hemberg 1985). Bioassays have demonstrated the presence of GA-like activities in tuber extracts and indicated that endogenous levels of certain GAs increase as sprout growth commences (Lippert *et al*. 1958; Smith and Rappaport 1961; Bialek and Bielinska-Czarnecka 1975).

Breakage of dormancy may be achieved by the application of gibberellins during tuber growth on the mother plant (van Ittersum and Scholte 1993). According to Alexopoulos *et al*. (2006a), the effect of exogenous GA_3 depends on the stage of development of the tuber and mother plant at the time of application. GA_3 affects tuber growth and when it is applied at an early stage of tuberisation, it inhibits the growth of tubers that have already formed on the plant and encourages the induction of new tubers (Alexopoulos *et al.* 2006b). On the other hand, when GA_3 is applied towards the end of the growth cycle (20-30 days before harvest), it leads to the production of tubers with a shorter duration of dormancy, a higher rate of respiration and higher rate of weight loss during storage (Alexopoulos *et al*. 2007a).

In their study, Rappaport *et al*. (1965) worked with excised plugs containing buds from potato tubers which were treated with a number of growth-regulating substances. Interestingly, they observed that GA_3 stimulated sprouting over a wide range of concentrations, while GA_4, GA_5, and GA_7 stimulated sprouting, as well. Surprisingly, they noted that GA_6, GA_8, and GA_9 either had either no effect or slightly inhibited sprouting.

Suttle (2004) assessed the different chemical types of GA in detail, and found that the endogenous concentrations of these GAs in tubers releasing dormancy were almost identical to those of deeply dormant tubers, and the increases in endogenous GA content became evident only after the onset of sprouting. These data strongly suggest that endogenous GAs are not effectively involved in the direct control of dormancy breakage, but they seem to play a critical role in subsequent sprout elongation and development. According to Suttle (2004), the precise determination of the concentrations of GA and possible changes before or after dormancy breakage presents significant difficulties due to the large number of GA forms and their low concentrations within plant tissues (1-10 ppb). One form may change into another, and several forms constitute precursors of other GAs (Taiz and Zeiger 2002). In addition, the binding of GAs by sugars can lead to the formation of inert, water-soluble complexes which may constitute storage or transportable forms (Burton 1989).

Ethylene

The plant hormone ethylene is known to play a critical role in many diverse physiological processes. In spite of its chemical simplicity, it is a potent growth regulator, affecting the growth, differentiation and senescence of plants. Ethylene, a volatile hormone, is now considered one of the most important natural plant growth regulators, and the literature abounds with reports of its effects on almost every stage of the life of plants, particularly ripening and senescence (Reid 1987; Alexander and Grierson 2002; Chang and Bleecker 2004). Ethylene has long been recognized as a crucial factor in the storage life of various fruits and vegetables, and was used in cultivation and post-harvest practices long before its role was understood (Abeles *et al*. 1992; Arshad and Frankenberger 2002). The important role that ethylene plays as a regulator of plant growth and development has been translated into a wide variety of applications in crop production, where ethylene is applied either as a gas or in the form of ethylene-releasing compounds (e.g. ethephon) (Arshad and Frankenberger 2002).

The effects of ethylene on potato tuber sprouting have been the subject of extensive studies, however, with some discrepancies in the results reported. Based on the studies of the last four decades, Rylski *et al*. (1974) reported that

short-term ethylene treatment can prematurely terminate tuber dormancy, while continuous treatment results in sprout growth inhibition. Alam *et al.* (1994) indicated that the ethylene responsiveness of potato tubers is cultivar-dependent. Using ethephon as an ethylene-releasing agent, Cvikrova *et al*. (1994) reported conflicting results and observed that these treatments either hastened or delayed sprouting. Other work demonstrated that continuous ethylene treatment is an effective sprout suppressor, but resulted in an undesirable accumulation of reducing sugars, which are detrimental to tuber quality (Prange *et al*. 1998). Cvikrova *et al*. (1994) reported that ethylene production from field-grown tubers is highest immediately after harvest and declines to low levels thereafter. Hence it is unclear why tubers are dormant immediately after harvest. As in other storage and dormant organs, ethylene production in potato tubers is low (Creech *et al*. 1973; Kubo *et al*. 1990; Suttle 2003).

Despite the low rates of production, endogenous ethylene plays a critical role in tuber dormancy, and the concentrations tested have accelerated sprouting. Ethylene also has a dual effect on potato tubers: it markedly shortens the duration of rest, but it inhibits elongation of the sprouts during extended treatment (Rylski *et al*. 1974). Other investigators reported similar results and observed an increase in the rate of ethylene production during the release of tuber dormancy and the onset of sprouting (Poapst *et al*. 1968; Okazawa 1974; Suttle 2003). However, the physiological basis of this increase remains unknown. Moreover, Suttle (1998b) also noted that endogenous ethylene is essential for the full expression of potato microtuber endodormancy and its involvement may be restricted to the initial period of endodormancy development. As stated above, this increase may indicate a significant role for endogenous ethylene in the release of dormancy or could relate to other physiological or biochemical factors associated with early sprout growth. Evidence to support this argument comes from observations of a rapid increase in the respiration rate, reaching 5 to 10 times the rate of untreated tubers for 30 hours following the treatment of potato tubers with ethylene gas (Reid and Pratt 1972). This increase in respiration rate could be one of the numerous potential factors triggering a biochemical signal that induces sprouting. Another effect of exogenous ethylene application is an increase in the concentration of fructose-2,6-biphosphate (Stitt *et al*. 1986).

Interactions of ethylene with other hormones

As described earlier, the gaseous hormone, ethylene, and auxins are closely linked. Application of exogenous auxins stimulates ethylene production (Morgan and Hall 1962) by inducing a gene encoding the rate-limiting enzyme in ethylene biosynthesis (Abel *et al*. 1995). Conversely, ethylene inhibits lateral (Burg and Burg 1966) and basipetal (Suttle 1988) auxin transport. Ethylene and ABA are also known to interact both synergistically and antagonistically in a number of developmental processes (de Bruxelles and Roberts 2001). However, the exact nature of these interactions between the two dormancy promoting hormones in potato tubers and in other plants still remains unclear and requires further attention and characterization.

POTATO TUBER DORMANCY AND CARBOHYDRATE METABOLISM

Throughout the duration of dormancy, despite the absence of visible sprouting, the continuous metabolic activity of the tuber, even at a low rate, leads to changes in the concentrations of sugars within the tissues. Subsequently, during the post-dormant stage, the growing sprouts constitute carbohydrate sinks which are supplied by carbohydrates solely from the mother tuber until the sprouts develop into new plants with autonomous photosynthetic activity.

Tuber respiration during dormancy

During the initial formation of the tuber on the mother plant, a small amount of carbohydrates is consumed by respiration, the level of which depends on the temperature (Burton 1989). During subsequent tuber growth, the carbohydrates that are provided to the tuber by the foliage are transformed into structural, storage or metabolically active forms and the rate of respiration is high. As the tuber completes its development, respiratory activity progressively declines and eventually stabilizes at about the stage when the foliage dies back. Thus, tuber respiration at the time of harvest is in the order of 3-5 mg CO_2 kg^{-1} h^{-1} at 10°C, depending on the cultivar and the prevailing environmental conditions during tuber development. The most important factors influencing the rate of tuber respiration at this time are the degree of maturity (i.e., the physiological age) of the tuber and the severity of mechanical injury incurred during harvest and handling (Burton 1989).

During storage the respiratory activity of tubers is affected primarily by temperature. According to Hooker (1986), the rate of respiration is relatively high (6-8 mg CO_2 kg^{-1} h^{-1}) at temperatures of 0-2°C, lower (3-4.5 mg CO_2 kg^{-1} h^{-1}) at 5-15°C and high (8-11 mg CO_2 kg^{-1} h^{-1}) at 20-25°C.

The post-harvest respiration of tubers during dormancy also depends on the stage of maturation (i.e., the physiological age). At harvest immature tubers have a significantly higher rate of respiration than mature ones, but this rate soon decreases (maybe by as much as 3-5 times) to the level of the mature tubers and thereafter remains constant throughout the duration of dormancy (Burton 1989). During dormancy, the rate of respiration of the buds and closely surrounding tissues, is higher than that of the other tissues of the tuber (Alexopoulos 2006).

The respiration rate of tubers increases at the time of sprouting and reaches the same level as in mature tubers at harvest. However, when tubers are harvested at an immature stage, the rate of respiration at sprouting is less than that at harvest (Burton 1989). According to Schippers (1977), the increase in respiratory activity of tubers during the post-dormant period is detectable once sprouts are 1 cm in length. When the sprouts have acquired a weight equivalent to 1% of the weight of the tuber, the rate of respiration is 1.5 times higher than that during the dormant state (Burton 1989). This is because respiration measurements include the respiration of both the tuber and the sprouts during the post-dormant period. A large proportion of the increase in respiration after dormancy breakage is due to the respiratory activity of the sprouts *per se*, while additionally the presence of sprouts probably enhances the metabolic activity of the tuber as a whole (Burton 1989). Moreover, during the sprouting period, it has been proposed that measurements of respiration should be based on CO_2 evolution rather than O_2 consumption since in aged or aging tubers the respiratory quotient ($RQ = CO_2 / O_2$) is not 1 but may reach a value as high as 4 (Isherwood and Burton 1975).

The increase in respiration is accompanied by a breakdown of starch and a consequent reduction in tuber weight. Water loss from the tuber surface contributes only slightly to the total weight loss due to the prevention of water evaporation by the periderm, the removal of which significantly increases the rate of water loss (Burton 1989).

Small tubers have a relatively higher rate of water loss because of their proportionally higher surface area: volume ratio. In addition, the presence of sprouts increases water loss because the epidermis of the sprouts is 100 times more permeable to water than the tuber periderm. Consequently, when the surface area of the sprouts reaches 1% of the area of the tuber, water loss is doubled (Burton 1955). Burton and Hannan (1957) found a linear relationship between the rate of water loss (up to 12%) and the percent weight of sprouts relative to total tuber weight.

Changes in carbohydrate pattern during dormancy

Sucrose

The sucrose transported to the tuber from the leaves is converted into starch during potato tuber development on the mother plant. The concentration of sucrose decreases progressively in the periderm and skin (Dimalla and van Staden 1977) as well as in the tuber as a whole (Ross and Davies 1992) during storage. According to Solomos and Laties (1975), the effect of ethylene on dormancy breakage probably relates to changes in the sugar content of the tubers. Moreover, Alexopoulos *et al*. (2007b) observed that the exogenous application of GA_3 and especially GA_3 + BA caused an increase in the concentration of sucrose within the region of the tuber buds before the onset of visible sprouting. After dormancy breakage and during sprout development, the concentration of sucrose within the tubers continues to fall (Dimalla and van Staden 1977). However, according to Davies (1990), sprout growth is not affected by the amount of sucrose or by sucrose transport.

Reducing sugars

The concentration of reducing sugars, such as glucose and fructose, within the tuber is relatively low and stable throughout the development of the tuber on the mother plant (Hawker *et al.* 1979; Ross *et al.* 1994), but during storage the concentration of both these sugars increases (Ross and Davies 1992) before the breakage of dormancy (Bailey *et al.* 1978). Dimalla and van Staden (1977) observed that the increase in reducing sugars occurred just prior to the visible emergence of sprouts, and Wiltshire and Cobb (1996) related this fact to the energy requirements of the sprouts that subsequently develop. Exogenous application of GA_3 or GA_3 + BA to tubers grown from TPS also led to an increase in glucose concentration within the tissues close to the tuber buds just before visible sprouting (Alexopoulos *et al*. 2007b).

The treatment of potato tubers with substances that break dormancy, such as chlorethanol, causes an increase in the concentration of reducing sugars but according to Coleman (1987) these substances provoke other changes too.

During the post-dormant period the concentration of reducing sugars (glucose and fructose) in the rind and tissues near to the buds or in the internodes, is lower than that recorded just prior to or during dormancy breakage (Dimalla and van Staden 1977).

Starch

Starch is a polysaccharide that is synthesised in the plastids and may be described as transient or permanent according to the size, shape and synthesis of the starch grains. Generally, starch grains have a three-dimensional, semi-crystalline structure and a size of 1-100 µm or more (Smith *et al.* 1997). Transient starch is synthesised in the photosynthetically active leaves during the day and is transported to other tissues during the night. In permanent starch, which is located in the roots, tubers, fruit and other regions, the starch grains have a specific shape for each plant species and are composed of amylose and amylopectin. Amylose is a straight-chain polysaccharide that forms approximately 30% of starch, while amylopectin is a branched polysaccharide that may constitute as much as 70-80% of the total starch (Smith *et al*. 1997). In potato tubers, starch amounts to 10-25% of the total tuber fresh weight (Cutter 1992) and is synthesised in the plastids of parenchyma cells, for which starch synthesis is their principal metabolic function. The size of the starch grains in potato tubers is generally greater than 100 µm (Cutter 1992).

The synthesis of starch from the sucrose that reaches the tuber from the aerial organs of the plant involves a series of enzyme catalysed reactions, and starch accumulation correlates closely with the growth of the tuber on the mother plant. Although Dimalla and van Staden (1977) reported that the starch content of tubers remains stable throughout the duration of dormancy, more recent studies have shown that the concentration of starch decreases at a low rate (Davies and Ross 1987; Davies and Viola 1988). Burton (1989) calculated that for each g of CO_2 released by tuber respiration, the starch content decreases by 0.61 g, provided that the respiratory quotient (RQ) is stable and equal to one. Thus, with a mean respiration rate of 3-5 mg CO_2 kg^{-1} h^{-1} tubers lose approximately 1 g starch within 2-3 weeks of storage at 10°C.

Davies and Ross (1987) and Davies and Viola (1988) observed a significant reduction in the concentration of starch during the post-dormant period. However, the rate of starch breakdown is similar in all the tissues of the tuber, indicating that the development of sprouts from the buds is not associated with increased starch breakdown (Davies 1990). This supports the suggestion of Sonnewald (2001) that during natural dormancy breakage, the initial growth of sprouts is supported by the soluble sugars already present in the tuber. However, when dormancy is broken by the exogenous application of GA_3 to tubers grown from TPS, a reduction in the number and size of starch grains in the tissues close to the buds is observed shortly prior to sprout emergence (Alexopoulos *et al.* 2008).

During the development of potato tubers on the mother plant, significant changes in the activity of enzymes involved in carbohydrate metabolism are observed, especially in those connected with sucrose metabolism and the synthesis or breakdown of starch. After harvest and throughout storage, simultaneous synthesis and breakdown of starch and related metabolites occurs in so-called 'futile cycles' (Geigenberger *et al.* 1994).

Changes in enzyme activity during dormancy

Sucrose metabolism

The metabolism of sucrose that reaches the potato tubers from the aerial plant occurs either via hydrolysis catalysed by invertase (acid or alkaline) or via breakdown catalysed by sucrose synthase (SuSy) (Ross *et al*. 1994; Appeldoorn *et al*. 1997) (**Fig. 4**).

Invertase

The hydrolysis of sucrose by invertase results in the formation of glucose and fructose via an exothermic, non-reversible reaction (Kruger 1990; Avigad 1982). After the induction of tuber formation and during tuber development on the mother plant, invertase activity declines (Ross *et al.* 1994; Appeldoorn *et al.* 1997) and remains at a very low level (ap Rees and Morrell 1990). Viola *et al.* (2001) related the reduction in invertase activity to a change in the method of uploading sucrose in the developing tuber from apoplastic to symplastic.

During storage of dormant tubers, Ross and Davies (1992), in contrast to Claassens (2002), detected an increase in invertase activity. However, Alexopoulos *et al*. (2008) found that the activity of invertase in the region of the buds of dormant tubers derived from TPS was low and did not increase even when dormancy was broken by the exogenous application of GA_3. Invertase activity after dormancy breakage of microtubers remains low but it is significant in the developing sprouts (Claassens 2002; Davies 1990).

Sucrose synthase

Sucrose synthase (SuSy) catalyses the reversible breakdown of sucrose into fructose and uridine-diphospho-glucose (UDPglucose) (Doehlert 1987; Neuhaus and Stitt 1990):

Sucrose + UDP ⇆ Fructose + UDP-glucose

The products of this reaction may subsequently be used in the pentose phosphate pathway, glycolysis and starch synthesis (Viola 1996).

Sucrose metabolism in developing tubers appears to be catalysed mainly by SuSy, the activity of which increases significantly from the stage of swelling of the stolon tip and relates to the symplastic transport of sucrose to the growing tuber (Viola *et al*. 2001). The reversibility of the reaction and its high level of control constitute an efficient mecha-

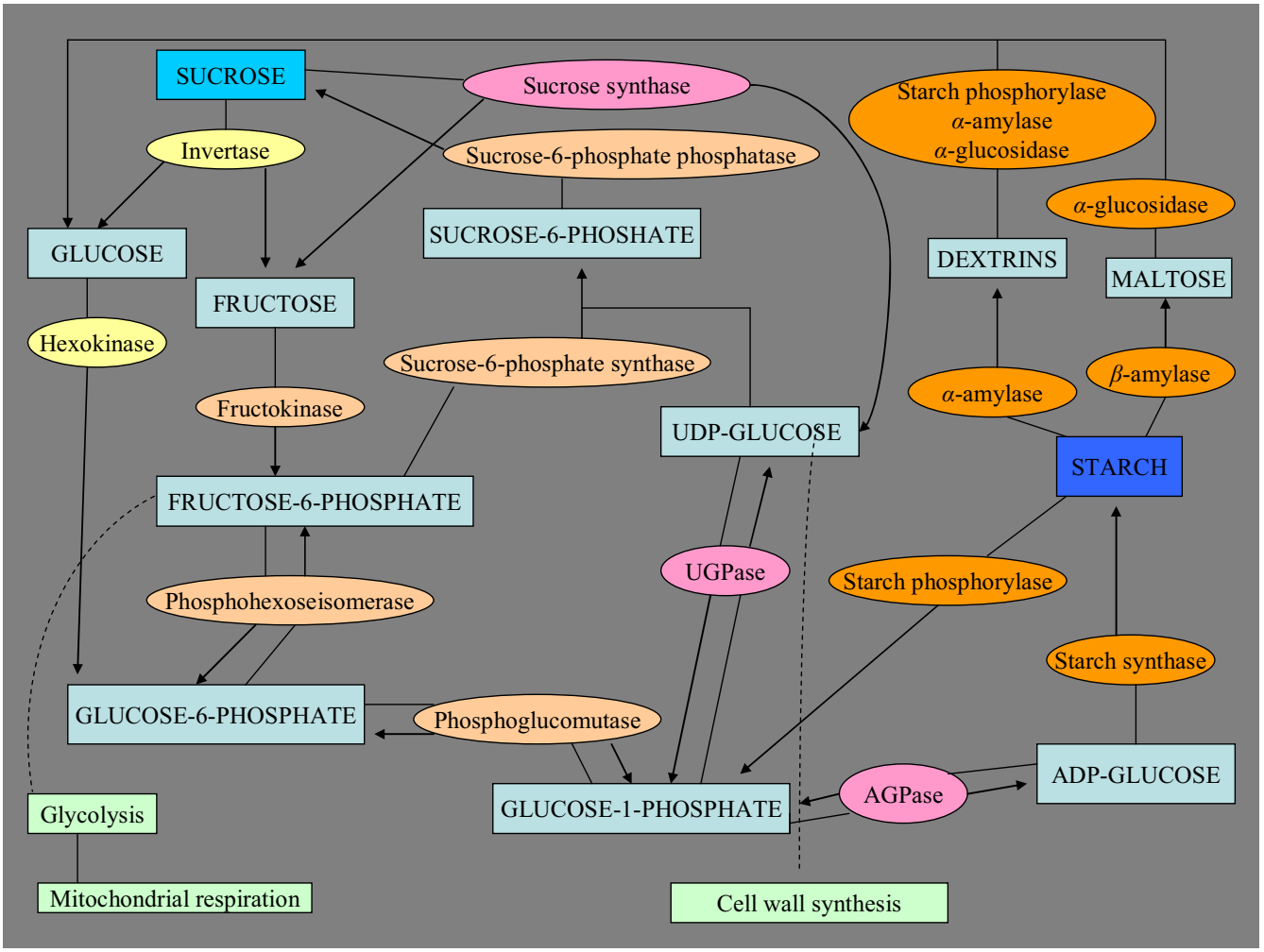

Fig. 4 Pathways of carbohydrate metabolism in potato tubers.

nism of sucrose recycling that permits sucrose breakdown when required, transport to developing tubers and starch synthesis (Appeldoorn *et al.* 1997). Inhibition of SuSy in genetically modified potato plants resulted in the production of tubers with reduced dry weights (Zrenner *et al.* 1995), while the increase in activity of SuSy in developing tubers correlated with a restriction of oxygen levels within the tissues, a condition that causes the inhibition of invertase (Bologa *et al.* 2003).

The activity of SuSy is high immediately after tuber harvest but decreases during storage and remains at a low level in tubers during the post-dormant period (Ross and Davies 1992; Claassens 2002).

Hexokinase

Hexokinase catalyses the phosphorylation of hexoses (glucose, fructose, mannose):

ATP + hexose → ADP + hexose-6-phosphate

For example, in the presence of ATP, hexokinase catalyses the phosphorylation of glucose to glucose-6-phosphate (G-6-P) in the cytoplasm with a parallel release of ADP.

Ross *et al.* (1994) found that hexokinase exhibits a low, stable activity *in vivo* in developing tubers. According to Appeldoorn *et al.* (1997), hexokinase activity shows a strong positive correlation with the reduction in the acid invertase activity of the cell walls, probably due to the decrease in hexoses, which are the substrate for hexokinase.

Renz *et al.* (1993) reported that during the storage of dormant tubers, the activity of hexokinase increases, probably in response to the reduction in activity of SuSy and increase in invertase referred to above. However, Claassens (2002) reported that in potato grown *in vitro*, the activity of hexokinase in the tubers remained very low throughout the whole period of dormancy. Moreover, Alexopoulos *et al.* (2008) did not observe a change in the activity of hexokinase in the region of the buds of tubers before dormancy was broken by the exogenous application of GA_3.

The activity of hexokinase, even if it is low during storage (Claassens 2002), increases during the induction of sprouting and in the post-dormant period, probably as a result of the increase in glucose concentration caused by starch breakdown (Renz *et al.* 1993).

Fructokinase

Fructokinase catalyses the phosphorylation of fructose to form fructose-6-phosphate (F-6-P):

ATP + fructose → ADP + fructose-6-phosphate

This reaction requires energy (ATP), and ADP is released, as in the phosphorylation of glucose. The product of the reaction (F-6-P) can be used as a respiratory substrate or for the biosynthesis of starch and other carbohydrates (Renz and Stitt 1993).

The activity of fructokinase is inhibited by the products of its catalysed reaction (Renz and Stitt 1993). Thus, an increase in the concentration of F-6-P and ADP inhibits fructokinase and causes an increase in fructose concentration in the tissues, with the concomitant inhibition of SuSy and therefore a reduction in the rate of sucrose breakdown. According to Renz *et al.* (1993), fructokinase is more active than hexokinase in developing tubers. This correlates with the demand for phosphorylation of fructose produced by SuSy-catalysed sucrose breakdown.

During the storage of dormant tubers, the activity of fructokinase decreases, but does not fall below the level of hexokinase (Renz *et al.* 1993). In potato tubers produced *in vitro* Claassens (2002) observed that the reduction in fructokinase activity was very high during the first third of the

dormant period, but subsequently stabilised at a low level. However, the activity of this enzyme, in the region of the buds, did not change before the breakage of dormancy by the exogenous application of GA_3 (Alexopoulos *et al*. 2008). After the breakage of dormancy of micro-tubers, the activity of fructokinase remains low without significant change (Claassens 2002).

Uridine-5'-diphospho-glucose pyrophosphorylase (UGPase)

This enzyme catalyses a reversible reaction in which UDP-glucose, produced by the breakdown of sucrose catalysed by SuSy, is transformed into glucose-1-phosphate (G-1-P).

UDP-glucose + inorganic phosphate (Pi) ⇆ G-1-P + UTP

The activity of UGPase is located in the cytoplasm and its role in the carbohydrate metabolism of the potato tuber is significant because UDP-glucose can be used as a precursor in the synthesis of cell walls (Gibeaut 2000) as well as taking part in the synthesis of glycolipids and glycoproteins (Florez-Diaz *et al.* 1997; Bishop *et al.* 2002).

Appeldoorn *et al.* (1997) reported a progressive increase in the UGPase activity of *in vitro* produced potato tubers, but Kleczkowski *et al.* (2004) stated that the activity of this enzyme does not provide a measure of the rate of sucrose breakdown or synthesis. Thus, the experimental data are contradictory and while some researchers consider changes in the activity of UGPase as playing a significant role in carbohydrate metabolism (Spycalla *et al.* 1994; Borovkov *et al.* 1996), others suggest the opposite (Zrenner *et al.* 1993).

UGPase activity increases when the tubers are subject to stress, such as low temperature, excessive humidity or drought (Borovkov *et al.* 1996; Ciereszko *et al.* 2001). Sowokinos (2001) attributed a regulatory role to UGPase in the increase of sugars in tubers during storage under low temperatures, a phenomenon known as 'low temperature-induced sweetening' of potato tubers.

During the storage of potato tubers produced *in vitro* the activity of UGPase presented a slight drop during the first stage of dormancy but thereafter remained at this level throughout the subsequent storage period (Claassens 2002). To the contrary, Appeldoorn *et al.* (1997) observed a progressive increase in UGPase activity throughout the storage of tubers produced *in vitro*. Alexopoulos *et al.* (2008) also found a high level of UGPase activity in the region of the buds during tuber storage, but the activity of this enzyme was not affected by the exogenous application of GA_3.

During the post-dormant period the activity of UGPase did not change significantly in potato micro-tubers (Claassens 2002).

Starch metabolism

Starch biosynthesis in potato tubers involves the conversion of G-1-P into adenine-5′-diphosphoglucose (ADP-glucose), from which amylase and amylopectin are synthesised to form the starch grain. The pathways of starch breakdown are different according to whether it occurs in photosynthetic or non-photosynthetic tissues (Avigad and Dey 1997). In potato tubers, as in seeds, starch accumulation occurs over a long period of time and Smith (1999) reported that during this time the enzymes involved in starch breakdown are inactive. However, Geigenberger *et al.* (1994) stated that there is a simultaneous synthesis and breakdown of metabolites and starch in potato tubers, which occurs in so-called 'futile cycles' (**Fig. 4**).

During the storage of dormant tubers and after dormancy breakage, there is a progressive reduction in starch concentration that correlates with changes in the activities of enzymes involved in starch synthesis and breakdown.

Adenosine-5'-diphospho-glucose pyrophosphorylase (AGPase)

This is considered to be the most significant enzyme associated with starch biosynthesis in potato and catalyses the reversible conversion of G-1-P into ADP-glucose which constitutes the substrate for starch synthesis.

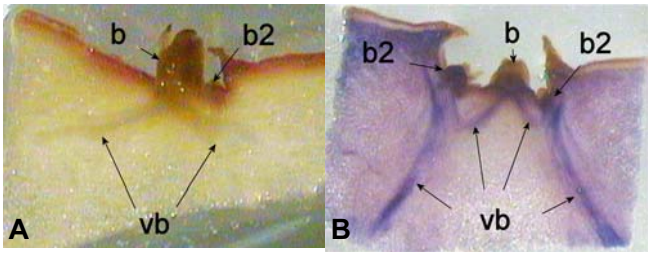

Fig. 5 Model of the histochemical detection of the enzyme AGPase, the activity of which is located in the vascular bundles (vb) beneath the bud (b) or secondary buds (b2). (**A**) No staining in the control reaction (minus the substrate ADP-glucose). (**B**) High staining within the vascular bundles of the bud and in tissue at the base of the bud of tubers produced from TPS.

G-1-P + ATP↔ ADP-glucose + pyrophosphate (PPi)

In tissues where starch is formed, AGPase is located within the plastids (Tetlow *et al.* 2004) and is considered to be the controlling factor for starch synthesis (Smith *et al.* 1997), even though a recent study (Geigenberger *et al.* 2004) has indicated that a reduction in the activity of this enzyme does not affect the rate of starch biosynthesis. The activity of AGPase increases with greater concentrations of sucrose (Geigenberger and Stitt 2000) as well as in the presence of 3-phosphogluconic acid (3-PGA), but it is inhibited by phosphate (Pi) (Sowokinos 1981; Sowokinos and Preiss 1982; Preiss 1988).

Appeldoorn *et al.* (1999) found that the activity of AGPase in developing tubers produced *in vitro*, progressively increased from the start of swelling of the stolon tip and according to Visser *et al.* (1994) this may relate to the increase in starch accumulation.

AGPase activity decreases significantly during the first stage of storage of microtubers and for the duration of dormancy (Claassens 2002). However, Alexopoulos *et al.* (2008) found that the activity of this enzyme is high in the region of the buds of tubers grown from TPS (**Fig. 5**) and it was not affected prior to dormancy breakage by the exogenous application of GA_3. Although the activity of AGPase in tubers does not change at the start of sprouting, it decreases during the post-dormant stage, suggesting that the presence of sprouts inhibits starch biosynthesis (Claassens 2002).

Starch synthase (STS)

Two isotypes of this enzyme, which is located in the plastids, have been identified: the water soluble starch synthases, which are involved in the addition of glucoside residues to the developing amylopectin chain, and the starch grain-bound synthases that contribute to the formation of amylose (Avigad and Dey 1997). In genetically modified potato plants the inhibition of STS leads to the formation of starch without amylose (Visser *et al.* 1991), indicating the significant role that this enzyme plays in starch synthesis in storage organs such as potato tubers (Kruger 1997).

Despite the fact that starch synthesis continues after tuber harvest, since breakdown and synthesis of starch grains occurs via the futile cycles (Geigenberger *et al.* 1994), the activity of STS during dormancy has not been studied thus far. Nevertheless, STS may be expected to decrease during storage and remain at low levels during the post-dormant period.

Starch phosphorylase (STP)

This enzyme catalyses the reversible phosphorylation of starch after which there is a reduction in the length of the starch chain by one molecule of glucose and the formation of G-1-P. In this way the energy of the glucosidic bond is not lost, but preserved in the internal bond giving it the possibility to contribute to the reverse process as well as to conserve energy for the plant cell (Drosopoulos 1998). Despite

the fact that starch phosphorylation occurs in starch synthesis, the primary role of STP in the potato tuber is considered to be starch breakdown (Claassens and Vreugdenhil 2000).

The activity of STP increases with the initiation of swelling of the stolon tip in potatoes grown *in vitro* and Appeldoorn *et al.* (1999) connected this fact to the increased activity of AGPase and the accumulation of starch in the developing tubers, without, however, elucidating if STP is involved solely in starch synthesis or also in starch breakdown at this stage. Morrell and ap Rees (1986) observed a high rate of STP activity in mature tubers, so it is likely that starch breakdown in dormant tubers is regulated mainly by this enzyme, as assumed earlier (Porter 1953). During the storage of tubers at low temperature (4°C) the activity of STP increases significantly and is believed to be responsible for starch breakdown, and this is the reason why low temperatures induce sweetening of tubers (Sowokinos *et al.* 1985).

Bailey *et al.* (1978) noted an increase in STP activity at the start of tuber sprouting, which was followed by a decrease. However, treatment of tubers with GA_3 does not affect STP activity in tuber tissues (Davies 1990).

Amylases

The amylases catalyse the hydrolysis of starch either from the exterior of the molecule (exo-amylases) or from the interior (endo-amylases) (Heldt 1999). Endo-amylase catalyses the hydrolysis of the α-1,4 glycoside bonds at random sites on the starch chain causing it to break into smaller molecules (dextrins) from which maltose, maltotriose and glucose are formed (Taiz and Zeiger 2002). β-amylase (exo-amylase) hydrolyses the α-1,4 glycoside bonds of starch, releasing maltose from the non-reducing end and forming maltose and dextrins. According to Anders *et al.* (1997), the activity of β-amylase occurs after the activation of starch grains by α-amylase.

The contribution of amylases to starch breakdown in dormant potato tubers is thought to be less than that of starch phosphorylase until the breakage of dormancy (Bailey *et al.* 1978). Davies (1990) reported that α-amylase is not detectable in dormant tubers, while Morrell and ap Rees (1986) did not detect either α- or β-amylase in mature dormant tubers. Claassens (2002) found that the activity of α-amylase is consistently less than that of β-amylase in dormant micro-tubers. The activity of both enzymes decreases during storage, but the activity of β-amylase decreases to a larger extent. However, the contribution of α-amylase to this process increases following the termination of dormancy and as starch breakdown proceeds (Davies 1990).

The activity of the amylases increases from the onset of tuber sprouting, but subsequently declines (Bailey *et al.* 1978). Although the involvement of α-amylase in starch breakdown is greater during this period, nevertheless there are reports of 5-10 times higher activity of β-amylase than α-amylase, but according to Davies (1990) this difference may arise from the different methods of enzyme assay. Claassens (2002) observed that during the sprouting of microtubers and the subsequent post-dormant period, there were no changes in amylase activity within the microtuber tissues and β-amylase was about 3-4 times more active than α-amylase. However, in the apical region of the tuber there was a significant increase in the activity of both enzymes (Claassens 2002), which suggests that starch breakdown does not take place at the same rate in all tissues of the tuber (Davies 1990), but it is higher in the region of sprout development.

a-Glucosidase

α-Glucosidase (maltase) hydrolyses the α-1,4 glycoside bonds of oligosaccharides, such as maltose, which is derived from the breakdown of starch under the influence of amylases, to form glucose (Avigad and Dey 1997). Yamasaki and Konno (1985) reported that α-glucosidase can catalyse the hydrolysis of a wide range of carbohydrates, from disaccharides to starch. However, Avigad and Dey (1997) stated that the hydrolysis of polysaccharides by α-glucosidase occurs only at a slow rate, if at all. Although, there are no references concerning the activity of this enzyme during the development of tubers on the mother plant, it is expected to be low because of the low activity of the amylases.

The activity of α-glucosidase depends to a significant extent on the activity of amylases, and due to the continual reduction of amylase activity during the storage of dormant tubers the activity of α-glucosidase is expected to be correspondingly low. Alexopoulos *et al*. (2008) observed low activity of α-glucosidase in the region of the buds of dormant tubers, but there was an increase in α-glucosidase activity just before the breakage of dormancy following the exogenous application of GA_3 indicating a higher rate of starch breakdown, followed by maltose hydrolysis (Davies 1990).

OTHER FACTORS IN POTATO TUBER DORMANCY

Maleic hydrazide (MH)

Maleic hydrazide (1,2–dihydro-3,6-pyridazinedione) has been used as a systemic plant growth regulator, inhibitor, herbicide or as an onion sprout inhibitor since its introduction 40 years ago, but there is only one reference reporting its inhibition of sprouting of potato tubers (Paterson *et al*. 1952). According to these authors, the application of different concentrations of MH (500, 1000, and 2500 ppm) to the foliage of potato cv. 'Irish Cobbler' prevented sprouting, and promising results were obtained in storage tests.

Isopropyl *N*-phenyl carbamate (CIP)

Isopropyl *N*-phenyl carbamate (CIP or propham) or its chlorinated form, CIP Chloride (CIPC or chlorpropham), is a selective systemic herbicide and plant growth regulator belonging to the *N*-phenyl carbamate group of pesticides. In agricultural practice, CIP or CIPC is used for pre-emergence control of grass weeds in a large number of crop and woody nursery stock. It is also used for the control of annual grasses, as a sprouting inhibitor in potato tubers during storage and for sucker control in tobacco (Meister 1992). Despite its extended use on potato tubers, little is known about its metabolism in the cellular environment. Nowak (1977) reported that CIPC inhibited the displacement of nitrogenous constituents from the internal to external portions of potato tubers, prevented the changes in soluble fractions of proteins and inhibited protein synthesis in the apical region. Seetharaman and Mondy (1991) also reported that a low concentration (1%) of CIPC significantly increased the total nitrogen and protein content in stored potato tubers, while a higher concentration (3%) increased total nitrogen but decreased protein. Although the effects of CIP (or CIPC) on physiological aspects are unknown, there are reports of an inhibition of cell division in the region of the tuber buds (Wiltshire and Cobb 1996). More recently, there has been a tendency to replace these toxic substances by others (natural biological compounds or ecological compounds) that are safer for human health and less harmful to the environment, e.g. carvone (Sorce *et al.* 2005).

Oxygen (O_2) and carbon dioxide (CO_2)

Coleman (1998) investigated the possible roles of oxygen and carbon dioxide treatments in the presence or absence of ethylene on tuber dormancy release in potato. Using two gas compositions (60% CO_2 + 20% O_2 and 20% CO_2 + 40% O_2), it was found that the phase of tuber dormancy and previous storage temperatures were important parameters for dormancy release by these gas mixtures (Coleman 1998). The high O_2 concentration caused a decrease in ABA levels within 24 h regardless of previous storage temperatures, although this effect was reversible. Exogenous ethylene also caused a decrease in ABA levels within 24 h. The high O_2

level also enhanced dormancy release and further promoted the decrease in ABA level. On the other hand, high CO_2 treatment led to slight reductions in ABA levels, which were further decreased by ethylene, and a decrease in the concentrations of sugars at 3°C, but an increase in sugars at 13°C (Coleman 1998). Theologis and Laties (1982) studied the behaviour of potato tubers under high O_2 concentrations, and found that the response to ethylene under pure O_2 is much greater than in air. These authors therefore postulated that O_2 primarily influences the stimulatory effect of ethylene on respiration rate, rather than on the respiration process *per se*.

Bromoethane

Bromoethane has been successfully used to break bud dormancy in potato tubers. It is normally applied for 24 h at a concentration of 0.2 ml l^{-1} (Coleman 1984), but higher concentrations must be avoided because rotting occurs (Akoumianakis 1998).

Bromoethane treatment induces changes in ABA levels within the tubers (Destefano-Beltran *et al*. 2006) and increases cell division in the buds between 7-9 days after application (Law and Suttle 2002). In addition, bromoethane application induces a large increase in ethylene release from the tubers, a high rate of tuber respiration (Akoumianakis *et al.* 2008), as well as an increase in the concentrations of glucose, fructose and sucrose (Alexopoulos unpublished data) and the activity of enzymes associated with carbohydrate metabolism (Akoumianakis *et al.* 2008).

According to Akoumianakis *et al.* (2000), bromoethane application causes more rapid and uniform breakage of tuber dormancy in comparison to natural dormancy breakage, which may take a relatively long time for completion (Burton 1989).

Rindite

'Rindite' is the name given to a mixture of ethylene chlorhydrin, ethylene dichloride and carbon tetrachloride (in a ratio of 7:3:1), which may be applied at a rate of 0.5 kg T^{-1} potato tubers, for the breakage of bud dormancy (Akoumianakis 1998). Rindite induces an increase in the rate of tuber respiration and ethylene release prior to the visible appearance of sprouts, but to a lower degree than bromoethane (Akoumianakis 1998). Ethylene chlorhydrin alone causes an increase in the reducing sugar content of the tubers (Coleman 1987).

Rindite, like bromoethane, causes rapid, more uniform breakage of bud dormancy in potato tubers than that occurring during natural dormancy breakage. The sprouts formed do not differ morphologically from those derived from tubers in which dormancy is broken naturally (Akoumianakis 1998). It should be noted, however, that both rindite and bromoethane are highly toxic and extreme care is required during their use (Wiltshire and Cobb 1996; Akoumianakis *et al*. 2000).

Ethanol

The potential effect of ethanol on dormancy breakage in tubers was reported for Jerusalem artichoke (Petel *et al*. 1993), but no details were presented on the mode of action of ethanol in dormancy breakage.

Claassens *et al*. (2005) first showed that dormancy of potato tubers could be broken by alcohols and ethanol blocked gene expression in tuber tissue by the inhibition of alcohol dehydrogenase. By contrast, products derived from alcohol dehydrogenase activity (acetaldehyde and acetic acid) neither induced sprouting nor affected luciferase reporter gene activity in the tuber tissue. These authors suggested that ethanol-induced sprouting may be related to an alcohol dehydrogenase-mediated increase in the catabolic redox charge.

Phenolics

Phenolic compounds have also been thought to have growth-inhibiting activity in various bioassay systems. Although few researchers have paid attention to this aspect, Cvikrová *et al*. (1994) demonstrated that the loss of tuber dormancy is accompanied by a reduction in the phenolic acid content and by an increase in phenolic conjugate levels.

Polyamines (PAs)

The polyamines (PAs) (putrescine, spermidine, and spermine) and their biosynthetic enzymes, such as arginine decarboxylase, ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase, are present in all parts of dormant potato tubers. They are equally distributed among the buds of apical and lateral regions and in the tissues of other tuber regions. Kaur-Sawhney *et al*. (1982) reported that the breakage of dormancy and initiation of sprouting in the apical bud region were accompanied by a rapid increase in PA biosynthetic enzyme activities, and by higher levels of putrescine, spermidine, and spermine in the apical buds. In contrast, these activities remained unchanged in dormant lateral buds and in tissues of other tuber regions. They concluded that the low level of polyamine synthesis during dormancy and its dramatic increase in buds in the apical region at the break of dormancy suggests that PA synthesis is linked to sprouting, perhaps causally. However, the relative roles of individual, PA biosynthetic enzymes in dormant and actively dividing plant tissues are not well understood.

Other chemical substances

The application of chemical substances, such as carbon disulphide, thiourea, sulphur-containing compounds (a solution of wettable sulphur, potassium thiocyanate, sodium sulphate or sodium sulphite), or osmoticants (e.g. polyethylene glycol and sodium chloride solutions) may also induce more rapid breakage of tuber dormancy, which is associated with small changes in tuber respiration rate during the first few days after application (Akoumianakis 1998). Although these substances are less effective than bromoethane or rindite for dormancy breakage, they are nonetheless interesting because they are less harmful to man and the environment.

Electricity

According to Kocacaliskan *et al.* (1989), the application of direct or alternating electrical current to potato tubers may reduce the duration of dormancy as a consequence of the effect of electricity on cell membrane integrity.

CONCLUSIONS AND FUTURE PERSPECTIVES

It is clear that consistent progress has been made in the characterization and understanding of the roles of different endogenous phytohormones in the regulation of potato tuber dormancy. Moreover, changes in carbohydrates and the enzymes implicated in tuber carbohydrate metabolism have been elucidated. However, there remain many unanswered questions relating to the involvement and roles of phytohormones in the physiology and dormancy of the potato tuber and other processes. Much further research is needed to understand the complex systems operating in tuber dormancy control. Indeed, many aspects of plant hormone biology are still unexplored and other endogenous regulators are continually being recognized, such as brassinosteroids which are assumed to play a role in the control and regulation of dormancy.

The great challenge is to identify (i) the biochemical processes regulating the biosynthesis and the activity of these hormones, (ii) the signals triggering their actions, and (iii) their mode of action in different physiological processes, including dormancy. These dormancy control processes, once they have been identified, can contribute to the

development of improved postharvest storage sprout-control technologies. Recently some progress has been made in the characterization of processes by utilizing a wide range of new and emerging experimental techniques, including transcriptomics, metabolomics and flux profiling. Moreover, recent advances in analytical techniques, such as gas and liquid chromatography coupled to mass spectrometry (GC-MS and LC-MS), have given the possibility to analyze a large number of compounds from a single plant sample. Such metabolite profiles can present not only a much broader view on systematic adjustment in metabolic processes, in comparison with conventional biochemical approaches, but also provide an opportunity to reveal new insights on cellular metabolism, even at compartmental levels. Use of these methods would facilitate the systematic analysis of unknown metabolites and the biological interpretation of their relationships, which would provide a basis for integrating metabolite information into the system-level study of plant biology.

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