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The Mechanisms of *in Vitro* Storage Organ Formation in Ornamental Geophytes

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ABSTRACT

In most ornamental geophytes, the rate of conventional vegetative propagation is low. Ten or even twenty years can pass until the commercial release of a new genotype. Therefore, numerous micropropagation methods have been developed to enhance the propagation rate, but also to obtain healthy elite stock material, speed up the breeding process, provide new genotypes on the market and restore endangered geophytes. In a number of geophyte species, formation of bulbs, corms or tubers is an essential step in the micropropagation process because only a storage organ shows high rooting ability and good field performance. In nature, abundance of photosynthesis-derived sugars together with some inducing environmental factors (low temperature, short or long day) trigger a sequence of biochemical, physiological, and finally, morphological events leading to storage organ formation. In *in vitro* conditions, however, due to insufficient light intensity, sugars have to be provided exogenously. *In vivo*, sugars exist as the multifunctional internal factors, while *in vitro*, they act both as internal and external factors. This can lead to certain disturbances in the course of the *in vitro* tuberization process. Therefore, success in storage organ formation often requires administration of the proper growth regulators whose endogenous production is insufficient (cytokinins, abscisic acid, jasmonates, auxins and polyamines), and sometimes other specific compounds such as inhibitors of gibberellin biosynthesis. This review focuses on the recent findings about the tuberization process *in vitro* of ornamental geophytes in relation to the newest knowledge concerning tuber formation in potato as model plant. Also some aspects of storage organ formation *in vitro*, the stages and the factors regulating this process on the morphological, physiological and biochemical levels are discussed in relation to storage organ formation occurring in nature (*in vivo*).

Keywords: abscisic acid, auxin, bulbs, corms, cytokinins, geophytes, gibberellin, growth retardants, *in vitro*, jasmonate, polyamines, tuberous roots, tubers

Abbreviations: **ABA**, abscisic acid; **ACC**, aminocyclopropane-1-carboxylic acid; **AGPase**, ADP-glucose pyrophosphorylase; **BA**, 6-benzyladenine; **CCC**, chlorocholine chloride; **CEPA**, 2-chloroethyl-phosphonic acid (etephon); **DDRT-PCR**, differential display reverse transcription PCR; **GA**, gibberellin; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid, **iP**, isopentenyladenine; **JA**, jasmonic acid; **LOX**, lipoxygenase; **MeJA**, methyl jasmonate; **MS**, Murashige and Skoog (1962) medium; **NAA**, 1-naphthaleneacetic acid; **PBZ**, N-dimethylaminosuccinamic acid (paclobutrazol); **PGR**, plant growth regulator; **PHOR1**, photoperiod-responsive1 protein; **phyB**, phytochrome B; **POTH1**, potato protein transcription factor (potato homeobox1); **SHAM**, salicylhydroxamic acid; **SOD**, superoxide dismutase; **StBEL5**, potato mRNA signal molecule (*Solanum tuberosum* BEL5); **StCDPK1**, *Solanum tuberosum* calcium-dependent protein kinase; **StGA20ox1-3**, *Solanum tuberosum* GA 20-oxidase; **TA**, tuberonic acid; **TAG**, tuberonic acid glucoside; **TDZ**, N-phenyl-N^1-1,2,3-thiadiazol-5-ylurea (thidiazuron); **Z**, zeatin

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Received: 9 February, 2011. Accepted: 20 December, 2012.

Invited Review

INTRODUCTION

Geophytes, plants developing underground storage organs, grow in areas with significant seasonal changes. The ability for storage organ formation developed during evolution, and allows plants to survive harsh seasonal periods, winters with a low temperature or hot and dry periods both in subtropical and temperate climates. At the same time, these organs serve geophytes for propagation. Among the geophytes, many species are utilized as ornamental plants (Rees 1992). The majority of these species belong to 16 families, most of which are monocotyledons. Commercially, they are propagated vegetatively by means of storage organs specific for the species: bulbs, corms, tubers, rhizomes or other perennial parts such as crowns. The process of storage organ formation is also called tuberization (Rees 1992). In this article both terms are used.

The strategy of geophytes to accumulate water and nutrition allows plants to survive adverse conditions and to use the reserves for rapid growth of shoots and flowers when the conditions become favorable. The geophytes accumulate food mainly in the form of carbohydrates combined with storage proteins, minerals and some other compounds. Among the carbohydrates, the most abundant is starch with a few percents of soluble sugars as sucrose, glucose and fructose (Miller 1992). Ranwala and Miller (2008) analyzed carbohydrates in storage organs of 30 ornamental geophytes and found that in all *Allium* species and in *Camassia leichtlinii* starch was absent but fructan was present in a high concentration. This carbohydrate occurs also in large amounts in *Hyacinthus*, *Iris*, *Muscari* and *Scilla*. In *Narcissus*, starch dominates and a few percent of fructan, glucomannan and soluble sugars are present.

Producers of ornamental plants search for new cultivars as well as for new species which until recently have been known as wild plants occurring only in nature. However, commercial release of a new genotype may take 10 or even 20 years. This is because in most ornamental geophytes the natural propagation rate is low. Their reproduction is based on the splitting of storage organs. Each year, only a few daughter bulbs, corms or tubers can be obtained from one mother storage organ. Another problem is that in this type of vegetative propagation, one source of plant material is being used for many years. This involves the risk of accumulation of viruses and fungal or bacterial pathogens, and then their spreading via daughter storage organs (Loebenstein *et al.* 1995; Chen and Ziv 2005). Viral diseases can drastically affect the yield and quality of plants. Therefore, numerous micropropagation methods were developed to both enhance propagation rate, obtain healthy elite stock material, speed up the breeding process and introduce new genotypes onto the market. In the past and recently, several wild geophyte species, all with ornamental potential, such as *Dichelostemma congestum*, *Triteleia* sp., *Dierama* sp., *Watsonia* sp., *Romulea minutiflora*, *R. sabulosa*, S*isyrinchium laxum*, *Tritonia gladiolaris*, *Muscari azureum*, *Brunsvigia undulata*, *Ornithogalum* sp., *Sprekelia formosissima* have been the subject of studies concerning *in vitro* propagation (Ilan *et al.* 1995; Madubanya *et al.* 2006; Ascough *et al.* 2007, 2008b, 2010; Koetle *et al.* 2010; Prado *et al.* 2010; Rice *et al.* 2010; Uranbey 2010; Moyo *et al.* 2011; Karaguzel *et al.* 2012; Swart *et al.* 2012). Authors stated that an efficient micropropagation method is indispensably needed for development of a wild plant as a new ornamental crop. *In vitro* propagation techniques have also been adopted for restoring of endangered geophytes or the preservation and conservation of germplasm for breeding of ornamental plants as wild populations of *Eucomis* L'Herit species, *Curculigo orchioides*, *Lilium ladebourii*, *L. longiflorum*, *L davidii*, *L. martagon*, *Muscari mirum*, *Fritillaria imperialis* or *Tigridia pavonia* (Taylor and Van Staden 2001; Thomas 2007; Azadi and Khosh-Khui 2007; Mohammadi-Dehcheshmeh *et al.* 2008; Pina-Escutia *et al.* 2010; Rong *et al.* 2011; Nasircilar *et al.* 2011; Du *et al.* 2012; Skorić *et al.* 2012). Moreover, *in vitro* techniques have been used for basic research to clarify the mechanism of storage organ formation on the physiological, biochemical, and molecular levels (Xu *et al.* 1998a, 1998b; Viola *et al.* 2001; Chen and Ziv 2005; Rodríguez-Falcón *et al.* 2006; Park *et al.* 2006; He *et al.* 2008; Sarkar 2008; Abelenda *et al.* 2011; Aksenova *et al.* 2012; Raspor *et al.* 2012). Recently, micropropagation methods of ornamental geophytes were reviewed by Ascough *et al.* (2008a, 2009) and De Klerk (2012). These authors pay special attention to storage organ formation and pointed out several advantages resulting from storage organ production *in vitro*. Unlike many geophytes, in the majority of other common ornamental plants, e.g., gerbera, rose or chrysanthemum, the final product of micropropagation is a rooted or unrooted shoot (microcutting) which can be easily acclimatized to *ex vitro* conditions in greenhouse. In a number of geophyte species, formation of bulbs, corms or tubers is an essential stage in the micropropagation process because only the storage organ shows high rooting ability and good field performance, e.g., lily (*Lilium*) and tulip (*Tulipa*) (Langens-Gerrits *et al.* 2003; Ishimori *et al.* 2007; Podwyszyńska and Sochacki 2010). Some geophytes, such as *Gladiolus* or *Narcissus tazetta* can be successfully transplanted to *ex vitro* conditions either as rooted shoots or storage organs (Danthu and Bhojwani 1995; Chen and Ziv 2005). The survival rate is usually higher for bulbs, corms and tubers than for shoots because storage organs have a far more favourable surface-area-to-volume ratio and because they are covered with a protective outer layer which is more resistant to desiccation than the tissue of shoots. Planting storage organs shortens the period needed to obtain first flowering plants, e.g., in *Lilium* to less than one year, in *Nerine* to two years, and in *Tulipa* to two or three years (Nhut 1998; Langens-Gerrits *et al.* 2003; Vishnevetsky *et al.* 2003; Podwyszyńska 2005; Ishimori *et al.* 2007). Moreover, bulbs, tubers or corms are easy for handling and transport. They are also very convenient for planting because they can be sown as seeds. In micropropagation of bulbous geophytes, there is an additional positive aspect of bulb formation. Juvenile micro-bulbs are very useful propagules for cyclic multiplication by single scales or their fragments in *Lilium* (Varshney *et al.* 2000; Langens-Gerrits *et al.* 2003; Kumar *et al.* 2005; Skorić *et al.* 2012), twin-scales in *Narcissus* and *Cyrtanthus* (Hussey 1982; Hong and Lee 2012) or halves or quarters of the bulblets such as in *Amaryllis*, *Nerine*, and *Hippeastrum* (De Bruyn and Ferreira 1992; Vishnevetsky *et al.* 2003; Ilczuk *et al.* 2005) and, interestingly, by the means of pseudo-bulblets from *in vitro* shoot-tip-derived stem nodes in *Lilium longiflorum* (Nhut 1998).

The storage organ production *in vitro* of ornamental geophytes has been the subject of numerous studies which were reviewed by Ascough *et al.* (2008a). Most of the studies focused on optimization of culture conditions. The researchers have examined exogenous factors such as light and temperature as well as explant type and its physiological status, and medium composition (mainly growth regulators and sugar types and levels). The process of storage organ formation in ornamental geophytes has been analyzed on the morphological and physiological levels but only a few reports concerned the gene regulation of the process (Li *et al.* 2002; Li-Nagasuga and Okubo 2005; Maehara *et al.* 2005; Chen and Ziv 2005; Park *et al.* 2006; He *et al.* 2008). *In vitro* tuberization has been most extensively studied in potato, *Solanum tuberosum*, the species which has become the model plant in which tuber formation *in vitro* is best known (Rodríguez-Falcón *et al.* 2006; Dobránszki *et al.* 2008; Sarkar 2008; Abelenda *et al.* 2011; Aksenova *et al.* 2012). Analyzing all the knowledge comprehended in works of above mentioned authors, referring to both ornamental geophytes and the potato as a model plant, it seems that several important aspects of the tuberization, independently of the species, are universal and the process performs similarly in bulbs, corms and tubers. This opinion has been also presented by Park *et al.* (2006) who performed investigations on the *in vitro* formation of lily bulbs and potato

tubers using molecular techniques for gene isolating and transformation (see below). Therefore some aspects of the storage organ development, discussed in this article are referred to the advance findings (knowledge) concerning potato tuber formation *in vitro*. There are the following common events for storage organ formation independent of the species: reorganization of cell growth at the initial stage of tuberization in the response to external and internal factors, subsequent carbohydrate accumulation, and simultaneous dormancy development (in the majority of species).

This review focuses on the recent findings on the tuberization process *in vitro* in ornamental geophytes and supplements the previous excellent review of Ascough *et al.* (2008a). With regard to the limited information on a gene regulation of storage organ formation in ornamental geophytes, this process is discussed including potato, the plant being one of the most important crop in the world, and thus, its tuberization process has been studied most extensively. Some interesting findings on hormonal regulation of the storage organ formation process, non-available for ornamental plants, have been presented in relation to other non-ornamental geophyte crops such as onion (*Allium cepa*), garlic (*Allium sativa*), cassava (*Manihot esculenta*), yam (*Dioscorea* sp.) and cocoyam (*Xanthosoma sagittifolium*). This knowledge can constitute the touchstone for the research on storage organ formation in ornamental geophytes. Also some aspects of *in vitro* tuberization of these plants, the stages and the factors regulating the process are discussed on the morphological, physiological and biochemical levels and referred to storage organ formation occurring in nature (*in vivo*).

TYPES OF STORAGE ORGANS AND THEIR ORIGIN

There are three main types of storage organs. They differ with respect to the origin of the modified organ: stem (tubers, rhizomes and corms), root (tuberous roots), or leaves (bulbs) (Rees 1992).

Corms are modified buds, built by the xylem or phloem parenchyma of the hypocotyl and a few stem internodes. The corms are formed by species belonging to *Iridaceae* (*Crocus*, *Dierama*, *Gladiolus*, *Freesia*, *Ixia*, *Watsonia*), *Colchicaceae* (*Iphigenia*, *Gloriosa*, *Sandersonia*) and *Brodiaeae*.

Tubers derive from the vascular zone of stem parenchyma. The stem tubers usually start off as enlargements of a part of the hypocotyl but also sometimes include the first node or two of the epicotyl and the upper section of the root. The stem tuber has a vertical orientation with one or a few vegetative buds on the top. They are characteristic for tuberous *Begonia* (hypocotyl tuber), *Cyclamen* and *Dioscorea*. The stem tuber of potato derives from the subapical region of the stolon and axillary vegetative buds are located on the entire tuber.

The tuberous roots are modified lateral roots. The enlarged regions of the root-tuber develop at the end or middle of a root, or involve the entire root. Tuberous roots are produced by *Hemerocallis* sp., *Dahlia* sp., and *Ipomoea batatas*.

In bulbs, nutrient reserves accumulate in the modified lower leaves (*Tulipa*, *Fritillaria*, *Lilium martagon*), leaf bases (*Hippeastrum*, *Hyacinthus*, some *Lilium* species), leaf sheath (*Allium cepa*) or both in leaf bases and leaves (*Narcissus*). The leaf bases may resemble scales, or they may overlap and surround the center of the bulb as in the *Hippeastrum*. A modified stem forms the base of the bulb.

Rhizomes are the plagiotropic underground stems formed by *Alstroemeria*, *Convallaria* or *Iris*. There are also examples from ferns, such as *Asplenium bulbiferum*, developing offshoots on top of the fronds, which are also regarded as bulbils. Some orchids produce above-ground storage organs called pseudobulbs.

In general, storage organs developing *in vitro* are built similarly to those forming in nature (*in vivo*), as described e.g. in tulip (Chanteloube *et al.* 1993) or snowdrops (*Galanthus*) (Staikidou *et al.* 2006). There are, however, some differences during storage organ development *in vivo* and *in vitro* such as described e.g. in nerine corm and potato tuber formation. Vishnevetsky *et al.* (2003) compared the morphology and anatomy of axillary buds and daughter bulbs of *in vivo*-grown *Nerine* plants and *in vitro*-originated adventitious buds. Unlike the *in vitro* formed buds, the axillary buds initiated between the outer scales of mother bulbs grown *in vivo* do not form the first leaf during their development. The newly formed *in vivo* buds are connected to the basal plate of the mother bulb with a short stem and consist of the shoot apex and a single scale. The *in vitro* forming buds do not survive if this first leaf is absent because the nutrients are supplied by vascular system of this first leaf connected with the initial explant scale.

In potato, tubers developing *in vitro* from micro-stolons produced by buds on single node cuttings under tuber-inducing conditions cease growing at a diameter of 0.8 cm (Xu *et al.* 1998b). In the *in vitro* conditions, tubers grow fast during the first 8 days due to the longitudinal division of the pith and cortex cells such as those observed in the early stage of *in vivo* tuberization. After the 8th day, the number of cells in the *in vitro* tubers remain constant, but cells continue to enlarge till about the 15th day, and then stop growing. Tubers developing *in vivo* increase their sizes much longer. The authors found, that the larger size of *in vivo* tubers resulted from further cell enlargement and division of perimedullary region, which was absent in the *in vitro* conditions.

STAGES OF STORAGE ORGAN FORMATION

Tuberization is a multilayered developmental process controlled by the interacting environmental, biochemical and genetic factors. In general, this process comprises three stages: 1) induction, 2) initiation, and 3) growth of storage organ (Rees 1992; De Hertogh and Le Nard 1993; Sarkar 2008). The storage organ formation can be considered on morphological, physiological, biochemical and molecular levels.

During induction, the changes triggered by exogenous factors, e.g. low or high temperature, short or long day and/or high sucrose level, are detectable only in biochemical and molecular analysis (activation of receptor proteins, signal molecules, enzymes, transcription factors, gene expression). At initiation stage, the events are visible in cytological analysis (microtubule reorientation, changes in the cell plane division) as well as in biochemical analysis (changes in phytohormone metabolism and commencement of storage carbohydrate biosynthesis). Growth of bulb, corm, tuber or any other storage organ occurs as swelling of bud, leaf, shoot base or root, and the changes are visible in macro- and microscopic observations (in cytological and histological analysis) and are always associated with water and reserves accumulation as well as with dormancy development (in a number of geophytes).

GENERAL SCHEME OF STORAGE ORGAN FORMATION

Success of storage organ formation in *in vitro* conditions requires knowledge about the exogenous and endogenous factors inducing/stimulating the successive phases of this process development and proper manipulating with these factors. The process *in vitro* has been studied in many geophytes, e.g., development of bulbs in lily (Takayma and Misawa 1980; Van Aartrijk 1984; Kim *et al*. 1994; Langens-Gerrits *et al.* 2003; Kumar *et al.* 2005; Park *et al.* 2006; Ishimori *et al.* 2007), tulip (Nishiuchi 1980; Le Nard *et al.* 1987; Taeb and Alderson 1990a, 1990b; Podwyszyńska *et al.* 2004, 2005; Podwyszyńska 2006a, 2006b), onion and leek (*Allium ampeloprasum* var. *porrum*) (Keller 1993; Le Guen-Le Saos *et al.* 2002), garlic (Ravnikar *et al.* 1993; Chung and Nam 2001; Kim *et al.* 2003), corms in gladiolus (*Gladiolus*) (Ziv 1990; Dantu and Bhojwani 1995; Kumar

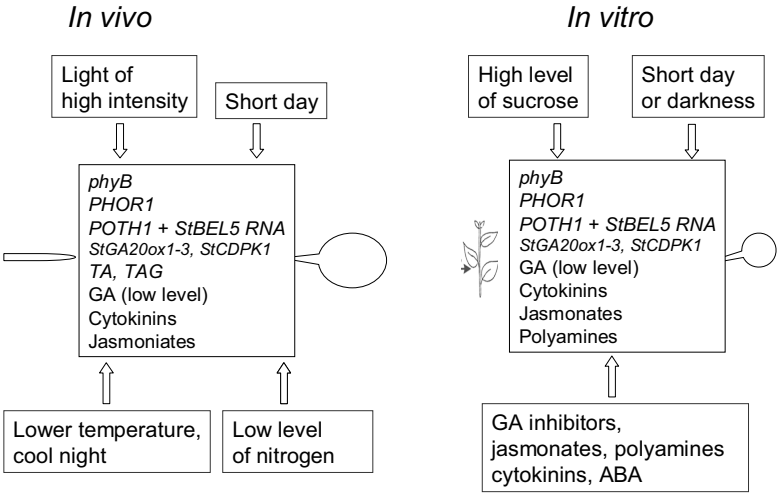

Fig. 1 External and internal factors influencing *in vivo* and *in vitro* tuberization process in potato. Based on the reports of Koda and Okazawa (1983); Xu *et al.* (1998a,b); Mader (1999); Jackson (1999); Rodrígues-Falcón *et al.* (2006); Sarkar (2006); Dobránszki *et al.* (2008); Abelenda *et al.* (2011); Aksenova *et al.* (2012). See "**Abbreviations".**

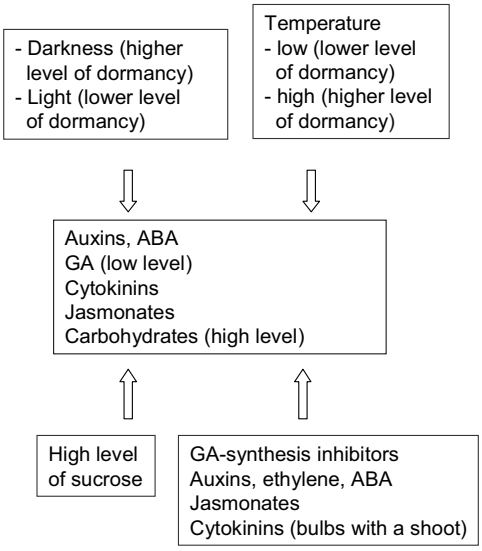

Fig. 2 External and internal factors influencing *in vitro* bulb formation process in lily. Based on the reports of Takayma and Misawa 1980; Van Aartrijk 1984; Kim *et al.* 1994; Langens-Gerrits *et al.* 2003; Kumar *et al.* 2005; Ishimori *et al.* 2007; Park *et al.* 2006, Xu 2007.

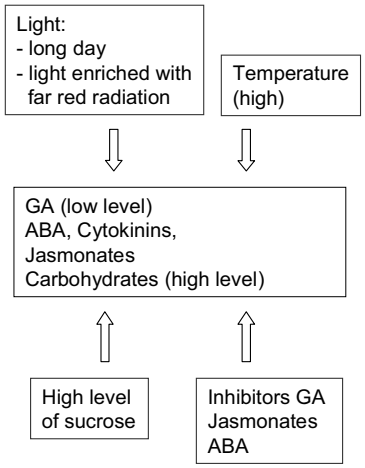

Fig. 3 External and internal factors influencing *in vitro* bulb formation process in onion. Based on the reports of Keller (1993); Le Guen-Le Saos *et al.* (2002).

and Palmi 2010), tubers in potato (Koda and Okazawa 1983; Xu *et al.* 1998a, 1998b; Jackson 1999; Mader 1999; Rodrígues-Falcón *et al.* 2006; Sarkar 2006), and tuberous roots in cassava (Medina *et al.* 2007). The process of storage organ formation proceeds similarly *in vitro* and *in vivo*. There is, however, the general difference in the source of sugars needed for induction (sugars signal molecules) and filling storage organs (sugars as carbon source). In nature, abundance of photosynthesis-derived sugars and inducing environmental factors trigger a sequence of biochemical, physiological, and finally morphological events leading to bulb, corm or tuber formation (Koch 1996). In the *in vitro* conditions, however, due to insufficient light intensity, sugars have to be provided exogenously. Thus, sugars *in vivo* exist as multifunctional internal factors while *in vitro* they are both internal and external factors. This can lead to certain disturbances in the *in vitro* tuberization process. Therefore, success in storage organ formation often requires the proper plant growth regulators (PGRs) whose endogenous production is insufficient such as cytokinins, ABA, jasmonates, auxins, polyamines, and some others specific compounds such as inhibitors of gibberellin biosynthesis.

Various geophytes have their own specific external, inducing factors, being the requisites for storage organ formation, e.g., short day and low night temperature in potato, short day in dahlia (*Dahlia*), long day in some onion genotypes, and low temperature in tulip and hyacinth (*Hyacinthus*). But there are two common factors, *viz.*, a high sucrose concentration and a low level of endogenous gibberellins, which induce this process in the majority of geophytes. If the inhibitory mechanism of gibberellin biosynthesis does not act properly during *in vitro* conditions, application of gibberellin inhibitors is recommended. Based on the available information reported on storage organ formation *in vivo* and *in vitro*, the schemes of storage organ formations are presented for geophytes differing in external inducing factors: potato (**Fig. 1**), lily (**Fig. 2**), onion (**Fig. 3**), garlic (**Fig. 4**) and tulip (**Figs. 5, 6**).

BIOCHEMISTRY OF THE EARLIEST STAGE OF STORAGE ORGAN FORMATION

There are few reports on genes regulating storage organ formation in ornamental geophytes. Studies on characterization of genes related to *in vitro* bulb formation of hyacinth and lily were performed in Japan (Li *et al.* 2002; Li-Nagasuga and Okubo 2005; Maehara *et al.* 2005). Okubo (2000) has proposed the hypothesis that the induction of bulb formation and dormancy are the same phenomenon and that ABA is involved. The relationship between these

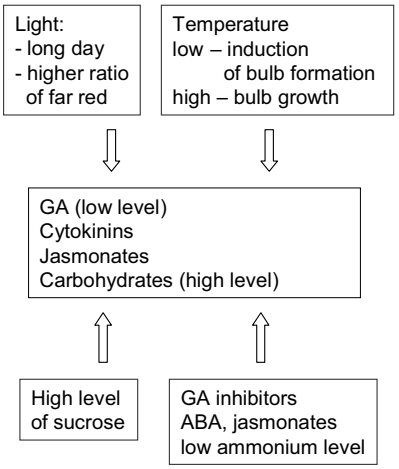

Fig. 4 External and internal factors influencing *in vitro* bulb formation process in garlic. Based on the reports of Ravnikar *et al.* (1993); Chung and Nam (2001); Kim *et al.* (2003).

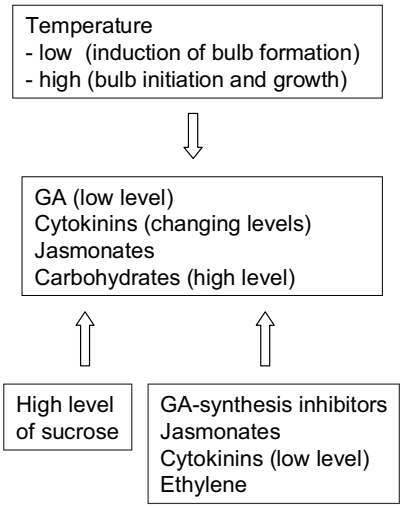

Fig. 5 External and internal factors influencing *in vitro* bulb formation process in tulip. Based on the reports of Nishiuchi (1980); Rice *et al.* (1983); Le Nard *et al.* (1987); Taeb and Alderson (1990a, 1990b); Kuijpers and Langens-Gerrits (1997); Podwyszyńska and Sochacki (2010).

processes was investigated using molecular analysis, *viz.*, differential display reverse transcription PCR (DDRT-PCR) (Li-Nagasuga and Okubo 2005; Maehara *et al.* 2005). In lily, bulb formation *in vitro* on microbulb scales was induced by a high temperature (25°C) and ABA. In hyacinth, bulbs were induced by a low temperature treatment lasting 8 weeks followed by culture at high temperature (25°C) and ABA. Several genes were isolated that were expressed at low temperature and/or after addition of ABA during the induction stage of bulb development. Three of them showed 100% homology with the starch phosphorylase gene of sweet potato (*Ipomoea batatas*). Starch phosphorylase is involved in starch cleavage to sucrose and other soluble sugars. The other isolated genes had a high homology, over 60%, with genes related to ABA induction of seed dormancy of several species. In hyacinth, one cDNA fragment showed high homology with the gene that regulates gibberellin biosynthesis in *Pisum sativum*, suggesting that gibberellin biosynthesis occurred in the very early stage of hyacinth bulb formation. Summarizing, among the genes expressed under bulb-forming conditions, eight were in

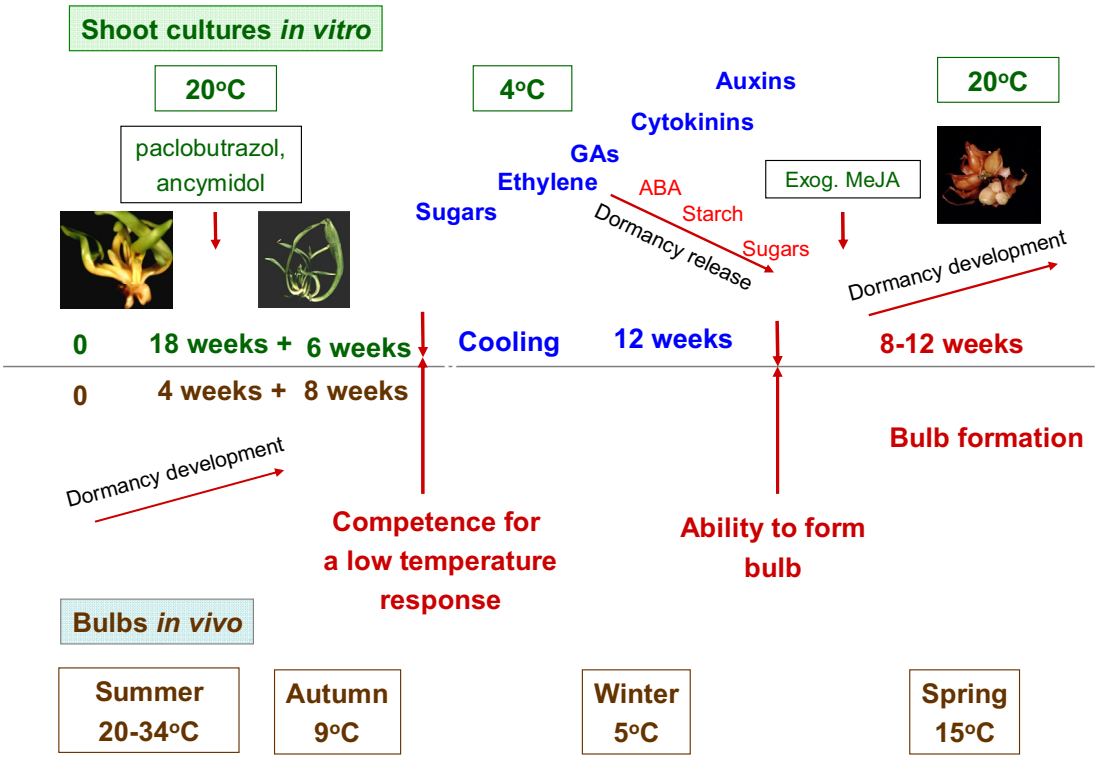

Fig. 6. Course of bulb formation process in tulip in the *in vivo* and *in vitro* conditions. Based on the reports of Le Nard and De Hertogh (1993); Saniewski *et al.* (2000); Xu (2007); Saniewski and Okubo (2005); Nishiuchi (1980); Le Nard *et al.* (1987); Taeb and Alderson (1990a, 1990b); Ohyama *et al.* (2006); Podwyszyńska (2006a, 2006b); Podwyszyńska *et al.* (2004, 2005). **Bulb development *in vitro*** (in the cycle multiplied adventitious shoots) consists of three phases and lasts 9-10 months: 1) the last multiplication subculture is prolonged from 8 to 14 weeks, TDZ is replaced in a the medium with iP, and at the 8th week, the liquid medium with PBZ is added (dormancy development in shoots); 2) cooling of shoots for 12-14 weeks at high sucrose level in a medium (dormancy breaking); 3) bulb development during 8-12 weeks in higher temperatures (dormancy development); the rooting of bulbs and the dormancy release proceeds simultaneously in an *ex vitro* conditions at lower temperatures. **Bulb development *in vivo***: 1) axillary bud development during the spring; 2) dormancy development in buds during the summer (before the harvest and during bulb storage; 3) breaking dormancy and partial bulb development (during the winter or dry cooling of bulbs for 8-12 weeks; 3) bulb development during the spring.

common for *Lilium speciosum* and *Hyacinthus orientalis*. All these genes were postulated to be involved in the induction of bulb formation.

The genes of starch phosphorylase and starch synthase were isolated from hyacinth and sequenced (Addai 2010). High expression of these genes as well as increased activity of starch phosphorylase were detected in *in vivo* cooled bulbs. The author reports that sucrose, a mobile form of carbohydrates, is rapidly transported from scales of cooled hyacinth bulbs to the developing organs. Similarly, the starch phosphorylase is involved in starch degradation at the very beginning of potato *in vitro* tuber formation from axillary buds in a single node system (Appeldoorn *et al.* 2002). Taken together all these findings, it can be concluded that the starch phosphorylase gene is one of the genes closely related to the earliest stages of storage organ formation. Thus, expression of this gene results in high activity of starch phosphorylase in an induced tissue from which the storage organ develops, and is followed by the starch degradation to soluble sugars which are then synthesized to sucrose that is exported to the newly developing storage organ.

Interesting information on genes regulating the bulbing process in lily was reported by Park *et al.* (2006). The DDRT-PCR analysis was used for isolation of genes expressed during *in vitro* bulblet formation of the lily oriental hybrid 'Marcopolo'. The authors isolated two genes related to proteolysis and three genes related to antioxidant mechanism in the plant. One of the three latter genes, Cu/Zn lily-superoxide dismutase (LSOD) (able to scavenge reactive oxygen in stress environment), isolated from lily, was introduced into potato plants via *Agrobacterium tumefaciens* transformation in order to confirm that this gene could play more common role in storage organ formation. Thus, *in vitro* tuberization of the potato single node cuttings was promoted in SOD-overexpressed transgenic lines, whereas delayed in SOD inhibited antisense transgenic lines. In lily *in vitro,* LSOD expression was the highest during the first 12 days under inducting conditions, at the bulb induction time, suggesting that one of the functions of SOD genes may be involved in the early stage of bulb development. The authors conclude that lily and potato may have a common controlling mechanism for storage organ formation.

The best known molecular mechanism controlling induction of storage organ formation is in potato, the multiple signal transduction pathways, mediated by phytochrome B, described in excellent reviews of Rodrígues-Falcón *et al.* (2006) and Sarkar (2008). The main environmental factors inducing potato tuberization are: short day, high irradiance and low night temperature. The perception of the short day factor is mediated by the phytochrome photoreceptor (phyB) and increased gibberellin biosynthesis in the above ground plant part. They subsequently induce a systemic signal which occurs in the leaf veins. This signal molecule is transported to the stolon and induces tuberization. The model proposes that short day promotes the accumulation of this mRNA signal molecule (StBEL5) in the leaf veins. It is transported via the phloem to the stolon, and regulates in tandem with the specific protein transcription factor (POTH1) the transcription of various genes influencing tuberization. The complex StBEL5-POTH1 represses the expression of the GA_{20}-oxidase gene. The decrease in this enzyme downregulates active gibberellin (GA) synthesis in stolons. Thus, the StBEL5-POTH1 complex influences also an increase in cytokinin level during tuberization. The decreasing concentration of active GA and presence of cytokinins in the region of tuberization are main hormonal factors in the induction of *in vitro* tuber formation (Xu *et al.* 1998a). The role of cytokinins in storage organ formation is reviewed below. Furthermore, these hormonal changes resulted in expansion and radial cell division of the cells located in the pith and cortex and subsequent random cell division in subapical part of a stolon. GA is considered to play a dominant role in tuberization. In turn, ABA and high sucrose concentration decreased the level of GA and stimulated tuberization. The authors suggest that the stimulatory effect of ABA is due to its antagonistic effect with GAs. The important role of the GA decrease in potato tuberization corresponds with the finding that an inhibitor of GA synthesis, uniconazol, caused reorientation of the stolon cell cortical microtubule to longitudinal and initiating radial division of these cells, resulting in swelling of the stolon (Fujino *et al.* 1995). It seems that the sharp decrease in GA content, can be the universal (common) hormonal factor which induces the process of storage organ formation in many geophytes. This hypothesis corresponds with the numerous reports on the stimulation of storage organ formation in other geophytes, including ornamental ones, by inhibitors of gibberellin biosynthesis (see below in the further part of this review).

There are some other aspects of signal transduction pathways possibly involved in the control of the potato tuberization process presented by Sarkar (2008). These aspects are: the evidence that flowering and tuberizing signals might be similar; a cross-talk with an oxidative burst-mediated redox signaling pathway; calmodulin (Ca-binding modulator protein) acting as signal molecules; aquaporins (proteinaceous membrane water channels responsible for cell-to-cell transcellular water flow) as molecules regulating cell division and expansion; and lipoxygenase (LOX) cascade. The latter process has been well documented in potato and narcissus (*Narcissus*), and is described in the further part of this review.

MULTIFUNCTIONAL ROLE OF SUGARS IN TUBERIZATION AND CARBOHYDRATE METABOLISM

It is widely accepted that sucrose as endogenous and/or exogenous factor, influences the storage organ formation, both *in vivo* and *in vitro*, in different modes namely as signal molecule, as carbon source for storage carbohydrates biosynthesis, and as osmolarity factor.

Sugars as signal molecules

Among numerous ornamental geophytes reviewed, the elevated sucrose level to 6%, and in some cases to 8% or even 10% induced *in vitro* storage organ formation in the species from the following genera: *Lilium*, *Tulipa*, *Narcissus*, *Hyacinthus*, *Gladiolus*, *Eucrosia*, *Calochortus*, *Crinum*, *Cyclamen*, *Crocus*, *Dierama*, *Iris*, *Hippeastrum*, *Cyrtanthus* and *Romulea* (Takayama and Misawa 1980; Taeb and Alderson 1990a; Ascough *et al.* 2008a, 2009; Sultana *et al.* 2010; Hong and Lee 2012; Swart *et al.* 2012). The improvement of storage organ formation by increased sucrose concentration (5%) was also obtained in *Brodiaea* species, *Ornithogalum virens*, and *Watsonia vanderspuyiae* (Ilan *et al.* 1995; Naik and Nayak 2005; Ascough *et al.* 2008b). Potato tuberization is also induced by a high level of sucrose (Xu *et al.* 1998a). The sucrose as metabolic, endogenous (*in vivo*) and exogenous/endogenous (*in vitro*) signal coordinates gene expression during storage organ initiation and growth. Sucrose-dependent gene regulation in tuberization of potato was reviewed by Rodrígues-Falcón *et al.* (2006). Thus, the switch from apoplastic to symplastic sucrose unloading during potato tuberization is correlated with a decrease in hexose and increase in sucrose levels in the subapical cells of stolon, resulting in increased starch synthesis (Ross *et al.* 1994). The genes of ADP-glucose pyrophosphorylase (AGPase), with a key regulatory function in starch synthesis, and sucrose synthase are activated by sucrose. High sucrose was correlated with higher expression of the cell-cycle D-type cyclin transcripts (Riou-Khamlichi *et al.* 1999). Rodrígues-Falcón *et al.* (2006) suggested that sucrose may in this way influence the cell division at stolon swelling. Without doubt, the high sucrose concentration in the medium of *in vitro* culture is one of the most important factors inducing storage organ formation.

The hexoses, such as glucose, fructose, or mannose also

influence storage organ development. Bach *et al.* (1992) reported that the most effective carbohydrate for the *in vitro* bulb induction of hyacinth, was a high concentration of both sucrose and glucose. *Curculigo* sp. bulblets production *in vitro* was the highest with sucrose (400 mM) and mannose (200 mM) which were superior to glucose (100-700 mM) tested as a carbon source in Murashige and Skoog (1962) medium (MS) containing thidiazuron (TDZ) and indole-3-butyric acid (IBA) (Thomas 2007). In micropropagated tulips, the sugar type used during the shoot multiplication strongly affected further bulb development (Podwyszyńska 2001). In three of the five studied cultivars, enhanced bulbing was noted for shoots which were previously multiplied on medium containing sucrose and in two remaining cultivars, glucose alone or combined with fructose positively affected bulb formation. Recently, the formation of renewal bud, a storage organ in herbaceous peony propagated *in vitro*, was induced by removing the leaves and increasing glucose concentration in medium to 6-9% (Gabryszewska 2010). The author suggests that glucose can influence the storage organ formation as signal molecule since hexoses are considered as a regulator of many biological processes (Koch 1996).

Sucrose as osmoticum and carbon source

The roles of osmolarity and type of carbon source were studied in relation to *in vitro* bulb formation in two *Narcissus* cultivars (Staikidou *et al.* 2005). The sugars, sucrose, glucose and fructose (66-526 mM), and the sugar alcohols, mannitol and sorbitol at 30 g l^{-1}, were added to the medium at different combinations. Glucose or fructose at 263 mM, or sucrose at 132 mM supplied to the medium approximately 19 g l^{-1} of the metabolizable carbon. Increasing the medium osmolarity using sugar alcohols in the presence of 30 g l^{-1} sucrose did not stimulate bulblet formation in any cultivar. However, increasing the medium osmolarity and carbon supply with metabolisable carbon sources stimulated bulblet production, independently of the sugar type. The largest bulbs were obtained at the highest carbon level of 37.9 g l^{-1}. The authors concluded that the carbon supply was a more important determinant of bulblet development than osmotic potential of the culture medium. The application of sugar alcohols as osmoregulators is associated with a risk of phytotoxicity which can influence organogenic processes (Thompson *et al.* 1986; Staikidou *et al.* 2005). Therefore, separating carbon source and osmolarity effect is difficult.

Starch biosynthesis

The synthesis of storage carbohydrates, including starch, is highly associated with the late stages of storage organ formation. As reviewed by Rodrígues-Falcón *et al.* (2006), in these stages of potato tuberization genes involved in starch synthesis are highly upregulated. Thus, the expression of genes involved in sucrose cleavage is accompanied by a switch from apoplastic to symplastic sucrose unloading. This coincides with a biochemical switch from an invertase-sucrolitic pathway to a sucrose synthase-sucrolytic pathway in the tuberization region (Viola *et al.* 2001). Sucrose synthase and fructokinase increase dramatically and acid invertase is downregulated. Plastidic phosphoglucomutase is strongly upregulated, indicating active import of glucose-6-phosphate into the amyloplasts. AGPase, and transcripts for soluble starch synthase, granule-bound starch synthase, and starch branching enzyme are highly upregulated during the tuber development.

In bulbs of *Narcissus tazetta* used as explants source, the effect of storage duration (0-10 months) at 30°C, followed by 6-week-cooling at 15°C period was studied with respect to the regeneration capacity of the explants and AGPase activity (Chen and Ziv 2005). A short storage period and cooling of donor bulbs markedly increased the regeneration capacity of explants taken from these bulbs. A high regeneration potential was strongly correlated with high AGPase activity and high starch concentration in initial explants, twin-scales and flower stem slices. Furthermore, cooling of donor bulbs was prerequisite both for increased AGPase and satisfactory regeneration. Authors suggested that starch-rich explants were an excellent source of energy for developing meristems.

Recently, high activities of AGPase and starch synthase were found in the newly formed organs in hyacinth bulbs *in vivo* (Addai 2010). It was accompanied by starch accumulation in these developing organs.

In conclusion, the high activity of AGPase occurring in hyacinth and narcissus bulbs and in potato tubers under inducing conditions, correlated with the commencement of starch accumulation, seems to be one of the biochemical markers of the initial stage of bulb formation.

MORPHOLOGICAL CHANGES AT INITIAL STAGE

On the morphological level, the tuberization process begins with the inhibition of longitudinal growth of the organ that develops into a storage organ, such as bud, shoot, stem, leaf, root or their parts. At the cellular level, induction is characterized by a reorientation of the direction of both cell division and cell radial expansion in the region of subsequent swelling. This process has been precisely described in potato based on cytological and cytochemical analyses of *in vitro* developing potato tubers from axillary buds on single nodes (Xu *et al.* 1998b; Vreugdenhil *et al.* 1999).

Cell biology during narcissus *in vivo* bulb development was analyzed by Zaffryar *et al.* (2007). Thus, amyloplasts gradually increased and were the largest at dormancy. Simultaneously, microtubules changed their organization. At the start of bulb growth, in February, they were oblique. In May, they formed a network with round "holes". In early summer, they formed parallel arrays developing a specific association with amyloplasts, which was correlated with entering dormancy. Such a unique amyloplast-cytoskeleton association was characteristic for dormancy initiation, being its cytological marker.

At the biochemical level, microtubule reorientation during storage organ initiation stage, is always associated with carbohydrate and storage proteins accumulation. The tubule structure reorganization can be treated as cytological marker of the second stage of storage organ formation. These microtubule changes, associated with this process were reported to be stimulated by jasmonates, inhibitors of gibberellin biosynthesis, a decrease in endogenous GA content, and a high sucrose concentration (Fujino *et al.* 1995; Koda 1997; Xu *et al.* 1999b).

GIBBERELLINS AND INHIBITORS OF GIBBERELLIN BIOSYNTHESIS

The important role of a decrease of GA during storage organ formation corresponds with numerous evidences that inhibitors of GA synthesis stimulate the formation of bulbs, corms and tubers in many geophytes, including ornamentals. Growth retardants are used mainly to optimize storage organ induction and multiplication in liquid systems with a potential application for micropropagation of geophytes, e.g. nerine (*Nerine*) or garlic, in bioreactors (Ziv 1989, 1990; Ziv *et al.* 1994; Kim *et al.* 2003; Ziv 2005). The growth retardants are used in such liquid systems to stimulate bulb/corm/tuber multiplication as well as to reduce leaf and root growth, and to prevent undesirable phenomena encountered in liquid cultures, like hyperhydricity and leaf damage, and finally to improve acclimatization efficiency (Ziv 2005). Ziv (1989) obtained enhanced cormlet proliferation in liquid cultured gladiolus buds by application of the following growth retardants: 2.5 mg l^{-1} daminozide (B-9), 2.0 mg l^{-1} ancymidol, 1.0 mg l^{-1} paclobutrazol (PBZ) and 1.0 mg l^{-1} uniconazole (Majic-S3307D). Among them, PBZ appeared to be the most effective. It was suggested that the production of cormlets in liquid medium could be a consequence of leaf growth inhibition. Similarly, PBZ at 10 mg

l^{-1} promoted gladiolus corm formation in liquid shake culture in the presence of high sucrose (6%) (Steinitz *et al.* 1991). In the presence of this GA inhibitor, leaf and root elongation were strongly reduced. The authors concluded that PBZ shifted assimilate partitioning preferentially to the corm. A similar interaction between PBZ and sucrose and their beneficial effects on *in vitro* cormel formation in six gladiolus cultivars were noted by Nagaraju *et al.* (2002). In Easter lily (*Lilium longiflorum*) liquid culture, application of PBZ and ancymidol at 1.7 or 3.4 μM facilitated development of plantlets with a higher bulb-to-leaf ratio and made it amenable to culture in bioreactor system (Thakur *et al.* 2005). The treated plantlets developed larger bulbs. In the micropropagated lily hybrid 'Star Gazer', addition to the medium of alar, cycocel or PBZ at 1 mg l^{-1} caused an increase of bulb number and size (Kumar *et al.* 2005). Flurprimidol (0.01^{-1} mg l^{-1}) and PBZ (0.01^{-1} mg l^{-1}) appeared to be effective also in promoting tuberization in *Gloriosa rothschildiana* (Kozak 2002). In *Hippeastrum* x *chmielii*, flurprimidol application (0.1 or 1.0 mg l^{-1}) both in a liquid and solid medium, resulted in a doubled propagation rate (Ilczuk *et al.* 2005). This growth retardant enhanced bulb size, reduced leaf elongation, and enhanced root formation. *In vitro* bulb development of shallot (*Allium cepa* L. Aggregatum Group), under inducing conditions (long day with light enriched in far-red radiation and high sucrose concentration) was also promoted by the growth retardants ancymidol, flurprimidol and PBZ, added to the medium at 10 μM; whereas exogenous gibberellin inhibited this process (Le Guen-Le Saos *et al.* 2002). Furthermore, ancymidol caused a 66% decrease in sucrose content in leaf bases but significantly increased the contents of glucose, fructose and fructan. The latter is the major carbohydrate reserve in *Allium* sp. (Ranwala and Miller 2008). These alterations in carbohydrates resulted from a three-fold rise in sucrose uptake. It was suggested that the growth retardants acted together with long day, being the bulb inducing factor in shallot, and decreased GA content. This, in turn, could cause reorientation of microtubules leading to the radial cell expansion and swelling of the leaf base. Recently, Zheng *et al.* (2012) reported that PBZ and another growth retardants, chlorocholine chloride (CCC), promoted carbohydrate accumulation in bulbs of *Lilium* Oriental hybrids 'Sorbonne' formed *in vitro*. CCC increased also the number and weight of bulbs in micropropagated *Hippeastrum hybridum* (Sultana *et al.* 2010). In *Dierama luteoalbidum*, addition of PBZ (5 or 10 mg l^{-1}) to a medium containing high sucrose concentration (6-8%) reduced the corm formation period from six to three months (Madubanya *et al.* 2006). In tulip, PBZ stimulated direct bulb regeneration on initial explants (Kuijpers and Langens-Gerrits 1997). Also in the tulip micropropagation system based on cyclic multiplication of adventitious shoots, treatment with PBZ or ancymidol (1-2 mg l^{-1}) given before cooling (inducing factor), markedly improved subsequent bulb formation (Podwyszyńska (2006b). The numbers of bulbs obtained from shoots treated with the GA inhibitors were two fold higher in all three studied cultivars than those obtained from untreated shoots.

On the other hand, there are also some evidences that GA addition stimulated the storage organ formation. In *Watsonia vanderspuyiae*, addition of GA_3 at 1 mg l^{-1} increased corm formation at 25°C from 35 to 70% (Ascough *et al.* 2008b). In this study, however, the highest tuberization efficiency, reaching 95%, was obtained at low temperatures, *viz.*, 10 or 20°C. This indicated that GA_3 could partly replace the effect of low temperature. In tulip *in vitro* cultures, the response of shoots to GA_3 treatment was similar. Rice *et al.* (1983) enhanced bulb formation in tulip *in vitro* by soaking adventitious non-cooled shoots in GA_3 solutions. In contrast, the results of Podwyszyńska and Ross (2003) showed that all of the treatments with GA_3 (1, 10 and 100 mg l^{-1}) given before or after the three-month-cooling period strongly inhibited bulb development, and instead markedly stimulated shoot multiplication and growth.

Rebers *et al.* (1995) showed that in *in vivo* bulbs of tulip, at planting just after cooling, sprouts of non-cooled bulbs contained significantly more GA_4 and GA_1 than those from precooled bulbs. In the cooled bulbs, the increase in gibberellin levels (GA_4 and its metabolite GA_{34}) occurred two weeks after planting. The floral stalks of precooled bulbs contained 2-3 times more gibberellins than those of non-cooled bulbs. The authors supposed that this increase in gibberellins might result from their synthesis in developing roots, since the removal of roots inhibited GA-dependent tulip stem elongation (Kawa-Miszczak *et al.* 1992). Furthermore, in tulip *in vivo*, flower stem elongation and daughter bulb formation are induced by a low temperature treatment lasting 12-16 weeks (Le Nard and De Hertogh 1993; Saniewski *et al.* 2000; Saniewski and Okubo 2005; Ohyama *et al.* 2006). Similarly, bulbing of *in vitro* propagated tulip is highly dependent on low temperature treatment (Nishi-uchi 1980; Rice *et al.* 1983; Le Nard *et al.* 1987). It was also demonstrated that the interacting factors, low temperature and gibberellins, increased the sensitivity of tulip flower stem tissue to auxin, thereby causing its elongation (Rietveld *et al.* 2000). The authors showed that only after a proper cold treatment and the resulting shift in the auxin response the stem is able to elongate at a proper rate, but only in the presence of a certain amount of gibberellins. They concluded that cold and gibberellins influenced the auxin response in separate ways, but with similar results. Considering the events described above, it seems likely, that the stimulatory effects of GA_3 (used at relatively low concentrations) on storage organ formation, observed under non-inducing conditions in *Watsonia vanderspuyiae* and *Tulipa* could partly result from an increased sensitivity to exogenous or endogenous auxins. The auxins are known to stimulate bulb formation in a number of geophytes (Ascough *et al.* 2008a).

Summarizing, there is some evidence that the tuberization process, associated with a local GA decrease at the site of the storage organ development is preceded by an increase of GA in other plant organs such as leaves in potato (Rodrígues-Falcón *et al.* 2006), and roots and flower shoots in tulips (Rebers *et al.* 1995), or in the organs forming storage organ such as basal leaf sheaths of *Allium* sp. (Nojiri *et al.* 1993; Yamazaki *et al.* 2002). Consequently, during the *in vivo* bulb development in *Allium wakegi*, the concentrations of the analyzed gibberellins (GA_1, GA_3, GA_4, GA_{12}, GA_{15}, GA_{19} and GA_{20}) in the basal leaf sheaths increased transitory shortly before they began to swell, but decreased rapidly during bulb development (Yamazaki *et al.* 2002). The concentration of ABA, however, in these basal leaf sheaths began to increase about one month before they began to swell, reaching a maximum just after bulb harvesting. In potato, the gibberellin-dependent mobile signal, derived from the induced leaves is transported to stolons where it locally inhibits GA synthesis, thereby inducing tuber development (Rodrígues-Falcón *et al.* 2006). Recently, the marked decrease in endogenous GA levels was detected both in the below- and above-ground tissues of the potato plants induced by short day and low night temperature (13°C) (Malkawi *et al.* 2007). Besides in the above-ground tissue, GA content was markedly higher than in the below-ground part during the entire tuberization process. This GA decrease was associated with a gradual increase of JA and a sharp rise of *cis*-zeatin content followed by its fast drop just before the onset of swelling. Perhaps, a similar mechanism by which GA influences storage organ formation occurs also in tulip and green onion and presumably it exists commonly in some other geophytes. So, a common mechanism of gibberellin action in the tuberization process is postulated. Thus, an increased biosynthesis of GA in certain organs (tulip roots or onion leaves), induced by a species specific factor, such as low temperature in tulip or long day in onion, results in the production of an unknown signal. This can be transported to a region of bulb initiation and cause a decrease in GA content, triggering storage organ development.

CYTOKININS

Cytokinins play a crucial role in regulation of many developmental and physiological processes, including proliferation and differentiation of plant cells, seed germination, apical dominance, flower and fruit development, leaf senescence, and transduction of nutritional signals. Their metabolism, action and translocation were recently reviewed (Sakakibara 2006; Werner and Schmülling 2009). Naturally occurring cytokinins are adenine derivatives carrying an isoprene-derived or aromatic side chain at N6 terminus. Isoprenoid cytokinins have been more frequently found in plants than aromatic ones. Within the former cytokinin type, the most common ones are *trans*-zeatin (*t*Z), isopentenyl adenine (iP), *cis*-zeatin (*c*Z), dihydrozeatin (DZ), and their sugar conjugates. The numerous reports on storage organ formation in ornamental geophytes reviewed by Ascough *et al.* (2008a) indicate that cytokinins have a promoting role in this process. Thus, cytokinins, 6-benzyladenine (BA) or kinetin, stimulated the development of tubers in *Ceropegia* and *Cyclamen*, bulbs in *Lilium longiflorum* and *Crinum*, and rhizomes in *Alstroemeria*. The authors report that cytokinins are usually added in combination with auxins, mainly NAA. The direction of organogenesis, to shoots or storage organs, often depends on the ratio of cytokinins to auxins. A high cytokinin/auxin ratio improved the bulb production in *Hyacinthus* and *Crocus* but a low one increased bulb formation in *Hippeastrum*. However, evidence for the role of cytokinin in storage organ formation is contradictory. Exogenous cytokinins did not promote, rather inhibited potato tuber growth *in vitro* (Sarkar *et al.* 2006). They also suppress *in vitro* tuberization of yam (Forsyth and Van Staden 1984; Donnelly *et al.* 2003) and gladiolus (Dantu and Bhojwani 1995).

Recently, Ishimori *et al.* (2007) found that BA and low temperature promote phase transition from juvenile to vegetative adult in bulbs of *Lilium* x *formolongi* 'White Aga' cultured *in vitro*. Two-month low temperature (8°C) incubation with BA (4.4 μM) was given after a one-month incubation at 25°C, and followed by two-month final culture at 25°C. Treatment with BA or low temperature only was less effective. In lily bulblets, such phase transition results in development of an elongated stem and thereby such bulbs grow faster after planting in soil, reaching larger sizes in the first growing season *ex vitro* (Langens-Gerrits *et al.* 2003). Histological analysis revealed that under synergistic action of cytokinin and low temperature, the shoot primordia were significantly enlarged and characterized by the development of tunica-corpus structure with increased mitotic activity compared to the shoot primordia in juvenile bulbs (for more information see chapter of De Klerk 2012).

In micropropagated tulips, cytokinins applied during the stage of shoot regeneration seemed to influence subsequent bulb formation (Le Nard *et al.* 1987). The shoots obtained in the presence of iP produced much more bulbs than those regenerated with BA. It was suggested that BA as the more stable cytokinin could accumulate in shoots and subsequently inhibit bulb development. Similarly, in cyclic multiplication of adventitious tulip shoots in the presence of TDZ, the difficulties with bulb formation met in some cultivars might be attributed to a TDZ after-effect (Podwyszyńska *et al.* 2005). This phenylurea-type cytokinin is very stable (Mok and Mok 2001), so it was supposed that TDZ could affect metabolism of endogenous cytokinins towards a disturbing bulb development in tulip. Experiments showed that using iP instead of TDZ in the medium during the last multiplication subculture prior to cooling increased the bulbing efficiency by 30% (Podwyszyńska *et al.* 2005). Analysis of endogenous cytokinins revealed that the easy-bulbing cultivar 'Prominence' had higher levels of active cytokinins compared to the cultivar 'Blue Parrot' that showed poor bulb formation. This was true for the entire duration of the micropropagation process except for the last phase of bulb formation (swelling of shoot bases), which occurred 6 weeks after the end of cold treatment. At this phase, in the poorly bulbing cultivar, shoots pretreated with iP had a total amount of active cytokinins twice as high as the easy bulbing cultivar. Moreover, it was shown that in 'Prominence' *trans*-zeatin isomers were generally predominant, whereas the major zeatin compounds in 'Blue Parrot' were *cis*-isomers. This cultivar contained also higher amounts of O-glucosides, a storage form of cytokinin. It is suggested that genotype dependent low capacity for bulb formation might be related to the accumulation of inactive *cis*-isomers and O-glucosides in the poorly bulb-forming genotype. O-glucosides are stable compounds, considered as storage forms of cytokinins, *cis*-isomers are relatively stable (less amenable to degradation by oxidase/dehydro-genase), and both O-glucosides and *cis*-isomers are metabolised to active cytokinins (Sakakibara 2006). Moreover, in 'Prominence' shoots derived from iP-medium (forming bulbs with the high efficiency of 96.0%), the active cytokinin content increased after cooling but at the time of shoot base swelling it decreased sharply. On the contrary, in 'Blue Parrot' shoots (with low bulbing efficiency of 40%), the active cytokinin levels increased all the time after cooling. It is concluded that the high efficiency of bulb formation in tulip can be associated with a transient increase in active cytokinins contents in response to the cold treatment, and a subsequent decrease of their concentrations at the time when shoot bases began to swell. These results are consistent with those obtained for potato plants in which the marked rise in cytokinin content was followed by its sharp decline just before swelling of stolons in the plants induced by short day (Malkawi *et al.* 2007).

All these findings indicate that cytokinins in tulip *in vitro*, similarly as in potato tuberization *in vivo*, may be involved in the induction or initiation of bulb formation. These PGRs could promote formation of scale primordia of the future bulbs but at high levels they can inhibit subsequent bulb growth.

Rodrígues-Falcón *et al.* (2006) based on the reviewed reports, concluded that in potato, POTH1 proteins seem to promote meristem activity by increasing cytokinin and lowering gibberellin levels being the requisite for meristem development. The authors considered that cytokinins may function to control tuber enlargement and growth at early stages of development, but would not signal transduction to a tuber induction. Cytokinins are possibly involved in cell proliferation during the early phases of tuber growth by stimulating cyclin D gene expression (Riou-Khamlichi *et al.* 1999). These PGRs might also control the sink strength by activating the expression of genes implicated in assimilate partioning, such as invertases, sucrose synthase, and hexose transporter genes (Roitsch and Ehness 2000).

JASMONATES

Jasmonates, jasmonic acid (JA) and methyl jasmonate (MeJA), have been considered to play an important role in the process of storage organ formation (Saniewski and Puchalski 1987; Ravnikar *et al.* 1993; Koda 1997; Rohwer and Erwin 2008). Jasmonates as a class of PGRs are involved in several developmental processes as well as in plant responses to biotic and abiotic stress. The authors have emphasized that jasmonates are important endogenous factors controlling tuberization. They showed the potential for horticultural application of these PGRs. The biosynthesis of jasmonates in the process of lipoxygenase (LOX) cascade, and involvement of these PGRs in potato tuber formation was reviewed by Sarkar (2008). Thus, lipoxygenases catalyze the oxygenation of polyunsaturated fatty acids, such as linoleic and linolenic acids to a number of hydroperoxy linolenic acids from which JA is synthesized and then metabolized to tuberonic acid (TA) and tuberonic acid glucoside (TAG). TA and TAG have strong tuber-inducing activities (Koda 1997). In potato, LOX is activated by low temperature (inducing tuberization), resulting in the accumulation of JA metabolites (Nam *et al.* 2007). TAG, the mobile form of jasmonates, is synthesized in leaves and

transported to the stolons and is considered as a possible tuber-inducing substance. It is postulated that LOX-derived metabolites may promote tuberization by antagonizing the effect of GA, similar to the mode of action exerted by ABA (Sarkar 2008). The proposed mechanism controlling tuber formation in potato is consistent with recent findings concerning corm formation in gladiolus (He *et al.* 2008). High activity of LOX was detected in gladiolus during the initiation and enlargement of cormels on stolons *in vivo* (in the field) as well as in corms developing in shoot cultures *in vitro*. This high activity of the enzyme in developing cormels was strongly correlated with an increased concentration of jasmonates, sucrose, starch and cellulose. LOX was suggested to regulate growth and development of gladiolus corm by affecting the jasmonate biosynthesis, resulting in carbohydrate accumulation. The authors supported their concept showing that the treatment with an inhibitor of JA biosynthesis, salicylhydroxamic acid (SHAM), suppressed LOX gene expression, reduced LOX activity, decreased the concentrations of JA and carbohydrates in the treated shoots, and inhibited the corm formation. Furthermore, the inhibitory effect of SHAM was overcome by application of MeJA to the medium. The optimum concentration of MeJA for promoting *in vitro* gladiolus corm formation in the presence of high sucrose concentration was 0.5 mM. The stimulatory effect of jasmonates on *in vitro* storage organ formation was confirmed in other numerous studies. The improvement of bulb formation was obtained in micropropagated shoots of *Narcissus triandus* (Santos and Salema 2000). The highest number of bulbs and their best quality were found when JA was applied alone. The combination of JA with NAA or the use NAA exclusively resulted in a low bulbing efficiency and a poor bulb quality, bulbs were elongated instead of roundish. In *Allium sativum*, however, the highest number of bulbs developing in shoot cultures was noted on medium containing 10 µl l^{-1} JA combined with 0.1 mg l^{-1} NAA (Kim *et al.* 2003). JA in the presence of high sucrose concentration (8%) improved the garlic bulb formation (Žel *et al.* 1997). This PGR (10 µM) in the absence of kinetin increased tuber number in yam (Ovono *et al.* 2007). The effect of JA and MeJA (2.5-10 µM) combined with BA (22 and 44 µM) under optimum tuber-inducing treatment with 8% sucrose was examined on the *in vitro* tuberization of potato single-node cuttings in three cultivars differing in maturity levels (Sarkar *et al.* 2006). A jasmonate promoting effect on tuber growth and starch accumulation was apparent only in the early cultivar. Furthermore, a cytokinin presence was found to detrimentally affect the tuber growth of all three potato cultivars and jasmonates counteracted this effect. Recently, MeJA treatment has been recommended for improvement of bulb formation in tulips propagated *in vitro* by the means of cyclic multiplication of adventitious shoots (Podwyszyńska and Sochacki 2010). In tulip micropropagation, the stage of bulb formation is prerequisite for acclimatization of micropropagules to *ex vitro* conditions. MeJA should be used at the stage of shoot swelling commencement, approximately six-eight weeks after three-month-cooling, inducing bulb development. Addition of MeJA at 25-50 µl l^{-1} to medium containing 7% sucrose resulted in increased bulb formation efficiency in the recalcitrant tulip cultivar 'Blue Parrot' from 25% to 60% (Podwyszyńska 2006a). Such a treatment improved also bulb quality. It is noteworthy that the application of MeJA at the earlier stage of tulip bulb development, three weeks after cooling when swelling of shoot bases was not apparent, did not influence the bulbing process (Podwyszyńska and Ross 2003).

Anatomical analysis of tuberizing *Gloriosa rothschildiana* shoots, performed in order to determine the mechanism of jasmonate action, revealed that MeJA (25 mg l^{-1}) caused an increase in the number and the diameter of veins in developing vascular tissue, corresponded with better tuber formation (Weryszko-Chmielewska and Kozak 2002). Besides, with ABA treatment, the strong reduction of vessel number as well as poor *Gloriosa* tuber formation was noted. This observation corresponds with the results concerning the potato tuberization *in vivo* (Cenzano *et al.* 2003). JA stimulated tuber development in stolons being in the second (initial) or third (swelling) tuberization stage through the strong impact on meristem development. This PGR markedly enhanced the meristematic cell sizes, reduced the length of leaf primordia, and caused early vascular tissue differentiation. The mode of jasmonate action in the process of *in vitro* storage organ formation was extensively described by Koda (1997) in several geophytes, *Solanum tuberosum*, *Dioscorea batatas*, *Helianthus tuberosus*, *Allium sativum*, and *A. cepa*. The author reported that JA and MeJA exerted their tuber-inducing effects by initiating the expansion of cells that resulted from both an increase in osmotic pressure due to the accumulation of sucrose and changes in cell wall architecture by increased extensibility of the cell wall. Cell expansion resulting in tuberization induced by JA, always involves reorientation of cortical microtubules. The author suggested that JA controls the direction of cell expansion by changing the arrangement of microtubules. Moreover, Nojiri *et al.* (1992), analyzing the level of endogenous JA and MeJA in *Allium cepa*, revealed that these compounds occurred in the tissue in the phase of swelling of leaf sheaths forming bulb. It occurred at 4th week after the application of a long day, the bulb inducing factor in this species. Interesting results were obtained by Jásik and De Klerk (2006). Gaseous MeJA (30-1000 µl l^{-1}) reduced the size and number of bulblets in *Lilium longiflorum* Thunb. and Asiatic hybrid *Lilium* 'Connecticut King' explants, but the effects on *Lilium speciosum* Thunb. were minimal. In all three lily species, MeJA suppress leaf blade formation and interestingly, inhibited dormancy development, resulting in reduction of cold requirement for bulblet sprouting (3% sucrose). In contrast, MeJA at 1.0 µM stimulated bulb growth in lily 'Mona' *in vitro*, and similarly as above, reduces leaf blade development (Jin 2009). Unfortunately, in *Watsonia vanderspuyiae* and *Tritonia gladiolaris*, MeJA tested at the concentration of 2.2 mg l^{-1} with 3% sucrose did not influence corm formation (Ascough *et al.* 2008a, 2011).

Considering all the reports, it can be concluded that jasmonates rather stimulate the growth of storage organs but they are not the primary inducing factors. In commercial micropropagation, the timing of jasmonate application as well as optimizing its concentration for particular species, should be established experimentally.

OTHER HORMONAL FACTORS

ABA and dormancy development

In plants, dormancy is a period of arrested growth. Plants develop dormancy to survive harsh conditions in climates with marked changes in temperature, light and water conditions, with cold or dry seasons. In many cases, storage organ formation is associated with dormancy entering. The physiological aspects of dormancy in ornamental geophytes from the horticultural point of view have been described by Kamerbeek *et al.* (1970), Rees (1992), and Le Nard and De Hertogh (1993). Dormancy development and breaking in storage organs formed *in vitro* in several ornamental geophytes was reviewed by Ascough *et al.* (2008a). During their formation *in vitro,* bulbs, corms and tubers usually develop dormancy. Such dormant organs are not able to properly develop aerial parts, even when planted in favorable conditions. Dormant bulbs require a few or more weeks of low temperature to break dormancy, enabling normal growth after planting in soil: *Lilium speciosum* 4-6 w., *L. rubellum* 14 w., and *Tulipa* sp. 12-14 w (Xu *et al.* 2007; Podwyszyńska and Sochacki 2010).

An increase of ABA content is considered to be the most important regulator in dormancy development. Okubo (2000) proposed the concept that the induction of bulb formation and bulb dormancy in bulbous plants are processes which develop simultaneously and in which ABA is

involved. This concept is supported by evidences that ABA induces *in vitro* bulb formation in lily (Gerrits and De Klerk 1992; Kim *et al.* 1994) and hyacinth (Li *et al.* 2002). Furthermore, fluridone, an inhibitor of ABA synthesis completely inhibits bulb formation in lily, and this effect is reversed by adding ABA simultaneously (Kim et *al.* 1994). Recently, a high concentration of endogenous ABA was detected *in vivo* in non-cooled bulbs of lily, hyacinth and tulip; and a gradual decrease of this PGR was noted during dormancy release under low temperature treatment (Xu *et al.* 2006; Xu 2007). The correlation between the decrease of endogenous ABA and dormancy release was also found in the *in vitro* bulbing of tulip (Podwyszyńska *et al.* 2004) and *in vivo* bulb development of *Allium wakegi* (Yamazaki *et al.* 2002). But relationship between the storage organ formation, dormancy development and increased ABA biosynthesis is not so simple. It was found in lily, that formation of bulbs at lower temperature of 15°C results in development of non-dormant bulbs (Langens-Gerrits *et al.* 2003).

Dormancy release is mainly associated with increasing levels of sugars (sucrose, glucose and fructose) as was shown recently in *Lilium rubellum* bulbs (Xu *et al.* 2006). It is also correlated with the endogenous content increase of auxins, cytokinins and gibberellins (Le Nard and De Hertogh 1993; Saniewski *et al.* 2000; Yamazaki *et al.* 2002; Xu 2007).

Some other aspects of the dormancy in ornamental geophytes propagated *in vitro*, the dormancy types, levels, development and release, are discussed in a separate chapter in this volume (De Klerk 2012).

Polyamines

Polyamines (putrescine, spermidine and spermine) occur ubiquitously in plants and are considered as PGRs influencing several physiological and developmental processes (Kaur-Sawhney *et al.* 2003). They are involved in senescence, somatic embryogenesis, stem elongation and flowering, root growth and tuber development. However, the mechanism of their action remains to be elucidated. Polyamines are compounds having aminogroups. Arginine and ornithine are precursors of putrescine. Spermidine is synthesized from putrescine and spermine from spermidine with s-adenosylmethionine in the presence of the spermine synthase. There are several evidences for their role in tuberization. First, their role was found in potato (Kumar *et al.* 1996; Mader 1999). Recently, their beneficial action was reported in *in vitro* formation of gladiolus corms and yam tubers (Kumar and Palni 2010; Ovono *et al.* 2010). The incorporation of spermidine in gladiolus culture medium resulted in the highest number of corms (Kumar and Palni 2010). In yam, both polyamines (putrescine, spermidine and spermidine) and their precursors (arginine and ornithyne) added to a medium at various concentrations positively influenced tuber formation from nodal cuttings. All these compound markedly accelerated tuber development, and significantly enhanced tuber sizes. More than two-fold larger corms were obtained with putrescine applied at the lowest tested concentration of 3 10^{-7} M, arginine at 10^{-5} M and 10^{-4} M and ornithine at 10^{-5} M. The treatment with putrescine (10^{-5} M) resulted in a dramatic increase of endogenous auxin content (ten times) and endogenous putrescine in tubers compared to control. Analysis of endogenous polyamine contents revealed that their levels in yam explants gradually increased during dormancy development. All these findings indicate that polyamines are needed for storage organ formation. These growth regulators are suggested to be involved in cell division during early phases of storage organ formation (Pedros *et al.* 1999). The increased auxin content in the putrescine treatment suggests their interaction in storage organ formation.

Auxins

Auxins are essential for both cell division and cellular expansion (Woodward and Bartel 2004). Auxins may promote shoot elongation, and induce formation and organization of phloem and xylem. They are a key hormonal factor inducing adventitious root formation. Auxins and other plant hormones interact to determine patterns of plant development. Although auxins are not considered as important factors controlling storage organ formation *in vivo*, there are numerous evidences that exogenous auxins induce this process *in vitro*. They promoted bulb formation on initial explants as well as secondary bulb formation in several ornamental geophytes such as hyacinth and some lily species (Pierik and Steegmans 1975; Niimi and Onozawa 1979; Van Aartrijk and Blom-Barnhoorn 1981). As reviewed by Ascough *et al.* (2008a), from the various auxins usually 1-naphthylacetic acid (NAA) is recommended for induction of the storage organ formation. Besides, in some geophytes, auxins are more effective in stimulating tuberization when they are combined with cytokinins and high sucrose concentration, as in tuberous roots of cassava (Medina *et al.* 2007). In tulip, their positive effect was not as apparent as that observed for MeJA (Podwyszyńska 2006a, 2006b). Auxins used in a medium before or after cooling of tulip shoots enhanced the frequency of bulbing, but the majority of bulbs was malformed and not covered with brown tunic. In potato, auxins did not induce tuber development, but rather reduced their growth, so that smaller tubers were formed (Xu *et al.* 1998a). The mechanism of auxin action in tuberization is unknown. There is some evidence indicating auxin interaction with ethylene, since auxins stimulate ethylene production in *Lilium.* Van Aartrijk (1984) found that ethylene biosynthesis played a key role in the process of adventitious bud formation from bulb-scale explants of *Lilium speciosum*. This process was regulated by auxin and its basipetal transport, as influenced by, e.g. 2,3,5-triiodobenzoic acid (TIBA) and/or ethylene.

As mentioned above, there are some evidences showing that other PGRs and environmental factors influence the endogenous auxin level in developing storage organs, e.g. the polyamine-derived increased auxin content in developing yam tubers (Ovono *et al.* 2010), or the enhanced tissue sensitivity to auxin in tulip bulbs correlated with low temperature treatment or gibberellin application. Furthermore, cooling induces storage organ formation in a number of geophytes. Recently, Xu (2007) found in tulip *in vivo* bulbs, using the immunolocalization procedure, that IAA was induced by low temperature or by GA_3 application, suggesting that GA_3 had an effect similar to low temperature in promoting IAA activity. However, the IAA signal was stronger in cooled tissue than in those not cooled and treated with GA_3.

All these evidences indicate that auxins may influence storage organ formation in concert with other PGRs (gibberellins, cytokinins, polyamines and ethylene), and other factors (sucrose and low temperature) by stimulating cell division and expansion in the developing storage organs.

Ethylene

This gaseous plant hormone is produced by all plant tissues and induces leaf senescence, fruit ripening and other developmental processes such as callus or root formation. Ethylene biosynthesis is stimulated, among others, by auxins and wounding, such as excision of explants. Ethylene stimulated *in vitro* bulb formation in tulip from shoots regenerated directly on initial explants (Taeb and Alderson 1990b) as well as in the system based on cyclic shoot multiplication (Podwyszyńska 2006a). In the latter case, 1-aminocyclopropane-1-carboxylic acid (ACC; an ethylene precursor) and, 2-chloroethyl-phosphonic acid (etephon, CEPA; a compound releasing ethylene) promoted bulb formation. However, the application of these compounds was not as effective as a treatment with MeJA. The increased bulb

numbers with ACC and CEPA were obtained only when these compounds were applied twice, *viz.*, at the 4th and the 8th week after shoot cooling (in the 8th week, shoot base swelling occurs). Ethylene also stimulated *in vitro* bulblet and tuber formation in onion and potato at a high sucrose concentration (Keller 1993; Vreugdenhil and Van Dijk 1989). In dahlia *in vivo*, tuberous root formation is promoted by short day conditions (Halevy and Biran 1975). This is associated with increased endogenous levels of ABA and ethylene. Endogenous ethylene peaked between the second and the third week after the start of short days, one week before the onset of tuberization, and then decreased to the low level found in plants growing under long days. Furthermore, the authors found that ethephon and exogenous ABA stimulated the tuberous root formation whereas GA_3 and long day were inhibitory. The authors suggested that ethylene may have different effects at different stages of development, promoting the initiation of tuberization and inhibiting the later stages of tuber filling. But the key internal factors in dahlia tuberization are ABA and GAs.

Other compounds possibly involved in storage organ formation

The shift in flavonoids (quercetin and kaempferol) content in tulip bulbs after low temperature treatment was detected by Saniewski and Horbowicz (2004). It was suggested that flavonoids as endogenous auxin transport inhibitors or factors influencing activity of several enzymes may play an important role in dormancy release and daughter-bulb enlargement *in vivo* in mother bulb of tulip.

NITROGEN LEVEL

The mechanism by which high nitrogen levels inhibit storage organ formation is not yet known. Possibly nitrogen influences the metabolism of phytohormones, altering the ratio between GA and ABA levels (Krauss 1985). It is also suggested that cytokinin biosynthesis is affected by nitrogen sources (Sakakibara 2006). An alternative hypothesis is that the ratio of carbohydrate to nitrogen is important in the tuberization process (Koda and Okazawa 1983). High levels of carbohydrates in the form of sugars and starch favor the formation of storage organs, whereas high nitrogen levels are known to promote shoot growth. The latter would utilize much of the available carbohydrate and would thereby reduce the amount available for tuber formation (Jackson 1999). Results consistent with this hypothesis have been reported by Koda and Okazawa (1983) who found that potato *in vitro* tuberization was inhibited by increased nitrogen levels only at low sucrose concentration of 2% but not at concentrations higher than 4%. Moreover, the form of nitrogen was a very important factor in tuberization. Enrichment of nitrate exerted only a slight effect on tuberization, whereas reduced nitrogen, ammonium and casamino acids had a detrimental effect on tuber formation. In general, the standard medium for potato tuberization on single-node cuttings contains high sucrose concentration (8%) and reduced nitrogen salts to one-tenth of the MS standard concentration (Xu *et al.* 1998a, 1998b). On the other hand, Zakaria *et al.* (2007) revealed in their studies on potato *in vitro* tuberization at a high sucrose concentration (8%), that the number and size of microtubers were the highest at the standard nitrogen level (60 meq) of MS medium and at two-fold elevated potassium level (40 meq) (meq, molar equivalent). In garlic *in vitro* mass propagation, it is recommended to reduce the level of ammonium as nitrogen source to ½ or ¼ of MS full strength medium used for bulb formation in liquid medium containing a high sucrose concentration (Chung and Nam 2001). Such reduction in nitrogen level significantly enhanced the percentage of shoots forming bulbs and increased bulb fresh weight. Corms of *Brodiaea* species (wild plants with ornamental potential) on hormone-free medium containing 5% of sucrose and reduced level of ammonium nitrate (to 5.1 mM) showed the increased dormancy, which was important for the satisfactory acclimatization to greenhouse conditions (Ilan *et al.* 1995). In turn, the *in vitro* tuberization of cocoyam was induced by BA (30 μM), high sucrose concentration (8%) and short day (Omokolo *et al.* 2003). Tuberization of this species could be also induced on BA-free medium, by varying the NO_3^-:NH_4^+ ratio under a short day and with a high sucrose level. The most favorable ratios for cocoyam tuber formation were 1:1 and 2:1. In *Ornithogalum virens*, *in vitro* bulb development induced at the base of regenerated shoots on the basal MS medium with enhanced sucrose concentration (4.5-9%), was improved by decreasing the salt strength of MS medium to half (Naik and Nayak 2005). Presumably, the beneficial effect of low mineral salt concentration on storage organ formation can be attributed to nitrogen. Lowering the level of mineral salts in a growing medium causes a simultaneous decrease in nitrogen, the macroelements applied at the highest concentration in MS medium. Such a lowering of mineral salts in a medium improved also the *in vitro* formation of bulbs in *Cipura paludosa* or corms in *Crocus*, *Gladiolus* and *Gloriosa* (Sengupta and Sen 1988; Ziv 1989; Kozak 2003; Raja *et al.* 2007). The above information on nitrogen effects on storage organ formation is analogous to the action of this macroelement in rooting process in the *in vitro* conditions. In many micropropagated plant species, rooting is promoted by lowering the nitrogen level in the medium and a high ratio of carbon to nitrogen is beneficial for this process (Hyndman *et al.* 1982). Jackson (1999) concluded that nitrogen is probably not involved in the induction of tuberization but that it is able to repress the tuber formation once induction has taken place.

PROSPECT FOR STORAGE ORGAN FORMATION IN ORNAMENTAL GEOPHYTES

Storage organ formation is a critical stage in micropropagation of ornamental geophytes. Currently, several studies to identify genes, signal molecules, proteins and other substances (e.g. flavonoids) which could be involved in storage organ formation, have been undertaken in ornamental geophytes. Endogenous factors specific for the competence of storage organs formation have been determined. Some attempts aim at increasing efficiency of the storage organ formation through timing of treatments with some PGRs (e.g. jasmonates), the biosynthesis inhibitors of several PGRs, and other substances regarded as cofactors of tuberization process (polyamines). Precise timing of PGRs application based on the molecular analyses of gene expressions and the endogenous PGRs and carbohydrate status during the succeeding phases of tuberization process may further improve formation of bulbs, tubers and corms. Another approach, earning special attention, is slow growth of storage organs in *in vitro* conditions (compared to *ex vitro*) that is suggested to be related to poor translocation of nutrients from medium to the developing organs (De Klerk 2012). This author discusses this problem is in another chapter of this volume.

Certain studies are devoted to the manipulation with environmental factors such as light intensity and quality, and temperature, considered as environmentally friendly methods which may increase efficiency of storage organ formation. Such attempts might lead to accelerating and higher synchronization of storage organ formation and dormancy release.

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