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In Vitro Tuberization in Hormone-Free Systems on Solidified Medium and Dormancy of Potato Microtubers

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ABSTRACT

The use of *in vitro* tubers (microtubers) as the final products of potato micropropagation, in addition to or rather than *in vitro* plantlets has several advantages in seed tuber production. Moreover, microtubers are important in germplasm storage and they are used as experimental tools in basic research and in *in vitro* selection of agronomically important characters. Their reliable production has, therefore, been widely studied under different *in vitro* growing conditions, with varying efficacy regarding the number, size and weight of microtubers. Microtubers exhibit different periods of dormancy depending on the genotype and on the *in vitro* conditions during tuberization. Generally, the use of growth regulators for tuberization does not allow the maximal expression of the tuberization capacity that is determined by the genotype, and therefore, the production of microtubers in sufficient quantity and size. As a result, there are later problems in breaking tuber dormancy. Although the growth responses between *in vitro* and field-grown tubers are very similar, there is the possibility for the production of *in vitro* tubers without using growth regulators by modifying *in vitro* environmental factors, such as light, temperature and mineral nutrition, among others. This review discusses the results of *in vitro* potato tuberization performed in hormone-free systems on solidified medium, including the effects of environmental factors, composition of medium, genotype and explants. Furthermore, we assess the effects and post-effects of tuberization conditions on the dormancy and sprouting characteristics of microtubers originating from these hormone-free systems.

Keywords: genotype, light intensity, photoperiod, sprouting

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Received: 19 May, 2008. Accepted: 6 October, 2008.

Invited Review

INTRODUCTION

Potato is the third most important crop in the world according to their production area (CIP 2008). There are two main problems associated with conventional seed-potato production: (i) low multiplication rate in the field, and (ii) high susceptibility of potato to viral, bacterial and fungal diseases. Moreover, the risk of viral, bacterial and fungal diseases increases with each multiplication step in the field, because diseases may be transferred to progeny through the tubers during vegetative propagation. However, *in vitro* micropropagated materials can be used to overcome both these problems associated with production practices and result in high quality, virus-free seed-potato production (Struik and Lommen 1991).

The final products of potato micropropagation are either plantlets or microtubers (*in vitro* tubers). The use of microtubers in the storage and exchange of germplasm and seed-potato production is advantageous (Hussey and Stacy 1981; Tovar *et al.* 1985; Seabrook *et al.* 1993; Ranalli *et al.* 1994) because microtubers can be stored longer, and are easier to handle and to transport than plantlets (Struik and Lommen 1991).

Economical use of microtubers is possible if the *in vitro* tuberization rate is satisfactorily high and reliable (at least one microtuber per explant, or higher) and if the size of developed microtubers is sufficiently large. Microtubers larger than 2 mm can be further propagated, but only microtubers larger than 4 mm are suitable for long-term storage. It is necessary to increase the tuber size because the larger the microtuber, the lower the loss during storage (Tábori *et al.* 1999a), and the better is initial vigour, emergence and performance (Wiersema *et al.* 1987; Ranalli *et al.* 1994).

Apart from being used as propagation material, microtubers are useful in other applications, including germplasm storage and exchange (Tovar *et al*. 1985; Donnelly *et al*. 2003), or as experimental research tools in the areas of plant metabolism, germplasm selection and evaluation, transformation, somatic hybridization or molecular farming (reviewed by Coleman *et al*. 2001), and for *in vitro* selection of agronomically important characters, such as maturity, abiotic stress tolerance, among others (Lentini and Earle 1991; Gopal and Minocha 1997; Gopal and Minocha 1998; Gopal *et al.* 1998; Veramendi *et al*. 2000; Donnelly *et al.* 2003; Gopal and Iwama 2007).

TUBERIZATION *IN VITRO*

Tuberization of potato is a survival mechanism and a complex developmental process. The formation of tubers, as a vegetative storage organ in potato, is under hormonal regulation. The developmental process is influenced by environmental factors, which presumably take effect through the hormonal balance of the plant. Potato plants are able to sense changes in environmental conditions that are required to trigger tuberization, after the plant attains adequate physiological development. Leaves play an important role in the perception of environmental stimuli and in the synthesis of endogenous plant growth regulators (PGRs) (Kahn *et al.* 1983; Koda *et al.* 1988; Seabrook *et al*. 2004; Peres *et al*. 2005), therefore their adequate developmental state is of great importance in the subsequent tuberization process.

The following short summary of the hormonal regulation of tuber formation is based upon the review of Vreugdenhil and Struik (1989). Four developmental steps are necessary for tuber formation in potato plants: (1) stolon induction and initiation from axillary buds, (2) stolon growth and branching, (3) cessation of longitudinal growth of the stolon, (4) tuber induction and initiation. The major regulators during the whole process of tuberization are the gibberellins (Xu *et al.* 1998; Vreugdenhil and Sergeeva 1999) and in some steps they have permission role. Their high level promotes stolon initiation and stolon growth; thereafter, it should decrease to allow tuber initiation. The participation of other hormones is necessary for the fine control of the developmental process. For stolon initiation and elongation, low cytokinin level is necessary but tuber initiation could occur if the gibberellin to ethylene ratio is low, and also if their levels remain low but the level of the cytokinins increases as well as the export of jasmonic acid from the leaves (van den Berg and Ewing 1991). Jasmonic acid is the key controller of tuber induction and initiation, in addition to the main controller, the gibberellins (Koda *et al.* 1991; Kiyota *et al.* 1996; Pruski *et al.* 2003a, 2003b; Cenzano *et al.* 2007). Other factors, such as theobroxide, are also known to influence tuberization. It induces potato tuberization *in vitro* and *in vivo* by triggering jasmonic acid production (Gao *et al*. 2005).

The most important environmental stimulus that affects the growth, morphogenesis and tuberization of potato is light, including photoperiod, light intensity, spectral wavelength, and others (Seabrook 2005). Other environmental factors, such as temperature, nitrogen nutrition, could also significantly modify the developmental process.

Hormonal regulation of tuber induction and initiation and the possible ways of *in vitro* tuber induction, which is based upon the hormonal and environmental control of tuber development, are summarized in **Fig. 1**. There are two possible ways of inducing tuberization *in vitro*: (i) using PGRs added to the medium, and (ii) modification of environmental factors, such as photoperiod, light intensity, in order to modify the hormonal balance of *in vitro* plantlets towards tuberization, or the two ways can be combined.

The majority of published works on *in vitro* tuberization of potato have focussed on using PGRs for tuber induction. Different regulating substances, such as auxins (Mangat *et al*. 1984), anti-gibberellins (Tovar *et al.* 1985; Estrada *et al.* 1986; Harvey *et al.* 1991; Langille and Hepler 1992; Levy *et al.* 1993; Šimko 1993; Vreugdenhil *et al*. 1994; Hussain *et al.* 2006), cytokinins (Wang and Hu 1982; Tovar *et al.* 1985; Slimmon *et al*. 1989; Lentini and Earle 1991; Levy *et al.* 1993; Šimko 1993; Veramendi *et al.* 2000; Hussain *et al.* 2006), coumarin (Stallknecht and Farnsworth 1982), and jasmonic acid (JA) (Pelacho and Mingo-Castel 1991; Castro *et al*. 2000; Pruski *et al*. 2001, 2002) have been investigated. However, generally less than one microtuber per explant could be produced with the use of exogenously applied PGRs, except when JA was applied to potato stolons cultured *in vitro* and 100% tuberization could be achieved (Pelacho and Mingo-Castel 1991). On the contrary, Zhang *et al*. (2006) observed that JA did not promote *in vitro* tuberization. Presumably, the use of these PGRs did not allow the expression of the tuberization capacity determined by the genotype.

Several authors have studied the effects of environmental factors, such as light (Slimmon *et al*. 1989; Lentini and Earle 1991; Pelacho and Mingo-Castel 1991; Perl *et al.* 1991; Nowak and Asiedu 1992; Seabrook *et al*. 1993; Pelacho *et al*. 1994), or temperature (Nowak and Colborne 1989; Harvey *et al.* 1992), on *in vitro* tuberization, however, the effects of these factors were examined in the presence of PGRs in the medium. Thus, the environmental factors had permitting effects rather than regulating ones during tuberization, and the PGRs applied in the medium had a regulating role.

Recently, there have been reports concerning the effects of environmental factors, such as light, temperature and that of mineral nutrition, or other special *in vitro* conditions, without using exogenous PGRs. In these *in vitro* systems, the potential effect(s) of exogenous PGRs on the environmental stimuli can be avoided. In the following subsections the results of *in vitro* tuberization studies performed in these hormone-free systems are reviewed and, if required, are compared to the results arising from studies using PGRs for *in vitro* tuberization.

Environmental factors

Light (photoperiod, light intensity, wavelength) and temperature are the most often studied environmental factors in

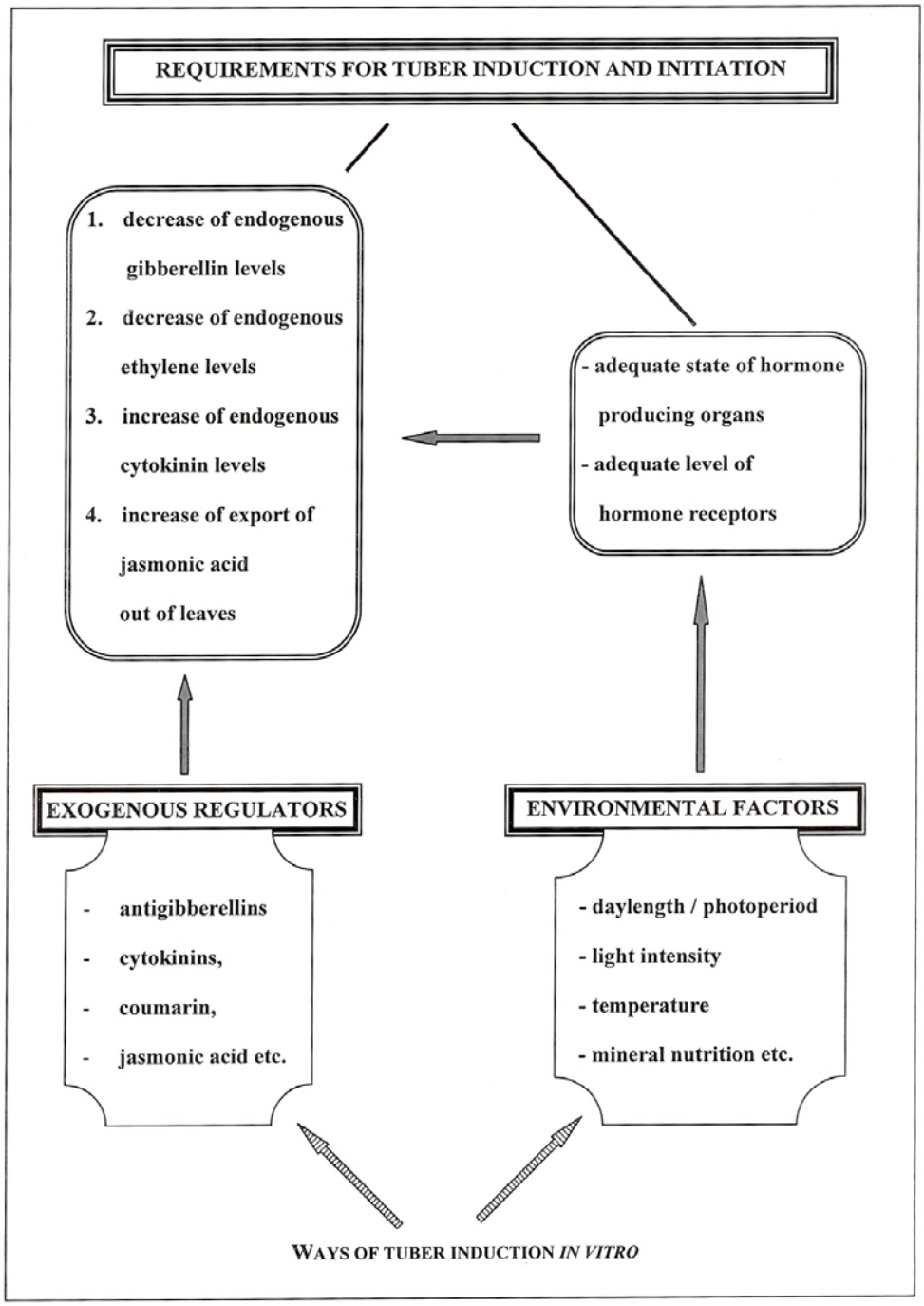

Fig. 1 Hormonal regulation of tuberization of potato and possible ways of tuber induction *in vitro*.

relation to *in vitro* tuberization. Their effects have been studied both in PGR-containing and PGR-free tuberization systems.

Light

The effect of light has a typical phytochrome response and it can affect morphogenesis through photoperiod, light intensity, or spectral wavelength. Studies on the effects of photoperiod on *in vitro* tuberization started some time ago in the 1970s when experiments with *in vivo* potato plants suggested that tuberization is under the control of photoperiod (Ewing 1978). Subsequently, there have been several lines of evidence that a morphogenetic light effect is involved in tuber formation also under *in vitro* conditions (Perl *et al.* 1991; Nowak and Asiedu 1992; Seabrook *et al.* 1993; Vecchio *et al.* 2000; Seabrook 2005). However, most of the published results have originated from a tuberization system that used PGRs, where the photoperiod had a permitting rather than a regulating effect.

The first publication that mentioned the possibility of obtaining microtubers without using PGRs emerged from Hussey and Stacy (1984); however, little attention was paid to this feasibility for fairly long period of time. Garner and Blake (1989) examined the factors necessary for the development of microtubers on medium free of PGRs. Beside the sucrose and nitrogen content of the medium, they studied the effect of environmental stimuli, namely the day length, on the microtuber producing capacity of microplants. Following the application of three different combinations of long days (16 h), short days (8 h) and darkness (0 h), Garner and Blake (1989) concluded that short days advanced microtuber formation, while darkness had little effect on microtuber development. Although tuber weight was significantly reduced when darkness was applied immediately after long days, however, it stimulated rapid microtuber development when preceded by short days. Darkness without a preceding short-day-treatment promoted premature senescence of microplants and therefore reduced the bulking potential of microtubers. The time at which *in vitro* plantlets were transferred to short days from the long days affected the final yield of *in vitro* tubers; consequently, long days before tuber induction are necessary to encourage vigorous leaf and stem development. The tuberization rate obtained

Fig. 2 Microtubers originating from different light treatments in three potato cultivars: 'Boró', 'Gülbaba' (Gb), 'Desiree' (Des). The numbers refer to the photoperiod treatments: 1, tuber initiation and development occurred under short days for 13 weeks; 2, the tuberization occurred mostly under short days interrupted by a two-week-long dark period after 2 week-long short days; 3, cultures were exposed to short days for 2 weeks and then to total darkness till the end of the experiment.

by their method was at least as high (or higher), than in any method described earlier that used PGRs (Garner and Blake 1989).

Charles *et al*. (1992) examined the effects of quality and intensity of light besides the effect of different photoperiods on *in vitro* potato plants. Three developmental pathways were distinguished: (1) activating growth, but retarding the aging of plantlets by a 12 h/day photoperiod, white fluorescent lighting or low light intensity, (2) opposing growth, but inducing tuberization by accelerating aging by short days (8 h), high light intensity or Grolux fluorescent lighting, or (3) promoting the development of vigorous plantlets with large leaves, but accelerating aging by long days (16 h) and high light intensity. Photoperiod was shown to be the main factor controlling plant development and other environmental parameters interacted with it.

In experiments performed in our laboratory, *in vitro* tuberization responses of eleven potato cultivars were evaluated under different photoperiods and light intensities (Dobránszki and Mándi 1993; Dobránszki 1996, 1997; Dobránszki *et al.* 1999a; Dobránszki 2000, 2001). Tuberization was induced on medium with a layer of 8% sucrose solution poured onto 4-week-old plantlet cultures grown under long days (16 h). Five different combinations of short days and total darkness were applied after the application of short days for various periods. Three light intensities (5.5, 55, 111 µmol m^{-2} s^{-1}) were applied under short days. Light applied after the tuber induction phase delayed or inhibited tuber initiation at higher light intensities. However, darkness following the induction stage accelerated and synchronised tuber initiation after high light intensity but there was a tight exponential correlation between the duration of darkness and the light intensity applied previously in the short days. If darkness was applied after low light intensities, it had only a synchronizing effect. Our results indicated that a determined quantity of light illumination was necessary to induce tuberization; however, beyond which light effects were unfavourable.

Light also affected the morphology of *in vitro* tubers (**Fig. 2**). Tubers developed mainly under light conditions formed either directly under or partly in the medium, or under the plastic caps of the jars at the end of long stolons. Tuber developed in the medium had prominent lenticels, their shape resembled field tubers and their colour varied from green to blackish green due to the light illumination. Their diameter varied between 0.7-1.8 cm, but their skin often split open and prevented their further use. At a given light intensity, an extension of darkness in the photoperiod treatment resulted in an increase in the rate of sessile tubers, which might indicate a stronger tuberization stimulus (McGrady *et al.* 1986). These tubers were positioned high above the surface of the medium and had a round-shape with true-to-type skin colour from yellowish-white to dark rose-red. However, their diameter was smaller (up to 1.0 cm) compared to microtubers produced mainly under light conditions and the tuber loss caused by water-loss was much less. Similar morphological characters of microtubers were obtained by other research groups (Slimmon *et al.* 1989; Nowak and Asiedu 1992; Seabrook *et al.* 1993; Vecchio *et al.* 2000; Seabrook 2005).

The number of tubers larger than 2 mm in diameter per plantlet varied from 1.20 to 1.52 depending on the cultivar, which means that high *in vitro* tuber production could be achieved without using PGRs because the tuberization rate exceed 1.00 in the best photoperiod – light intensity combination.

Temperature

The effects of temperature on potato tuber development are very complex (Struik *et al.* 1989); its effects on *in vitro* tuberization have hardly been studied. Martinez and Tizio (1990) reported that low temperature (4°C) delayed tuber initiation but improved the tuber weight. During benzyl aminopurine-induced tuberization, high temperature (26°C) strongly inhibited tuberization and significantly reduced the weight of microtubers (Harvey *et al.* 1992).

Composition of medium

Apart from PGRs there are some other medium constituents that greatly affect *in vitro* tuber development in PGR-free systems, such as sucrose, nitrogen content, and gelling agents.

Sucrose

Sugars, such as sucrose, play a role in coordinating the occurrence or timing of developmental processes in plants. Much evidence has been published concerning the existence of sugar-specific signalling pathways and sugar response pathways interact with other pathways, such as those for phytohormone and for light responses. High level of soluble sugars can cause the formation of extra organs in different plant species, such as tubers in potato (Gibson 2005).

Sucrose is essential *in vitro* as an energy source or as an osmotic potent agent and at a higher concentration it serves as a signal for microtuber formation (Perl *et al*. 1991; Donnelly *et al.* 2003), resulting in an increase in the number of tubers in potato culture (Gibson 2005). The importance of sucrose concentration for *in vitro* tuberization has been described in tuberization systems that used PGRs (Wang and Hu 1982; Hussey and Stacy 1984; Gopal *et al*. 2004; Hussain *et al*. 2006) and also in PGR-free systems (Garner and Blake 1989; Forti *et al.* 1991; Alix *et al*. 2001). In contrast with the 2-3% sucrose content of the shoot multiplication medium, a high sucrose concentration (8%) is used in microtuberization media independently from the presence or the lack of PGRs. According to Garner and Blake (1989), the use of 8% sucrose in PGR-free medium advanced tuber initiation, and increased the number and weight of microtubers compared to a lower (4%) sucrose concentration. However, further increases in the concentration up to 12% caused a delay in tuber initiation and resulted in smaller tubers.

Nitrogen

Total availability and the source of nitrogen in the medium strongly influenced the development of microtubers. Zarrabeitia *et al.* (1997) observed a "carry-over" effect of nitrogen content in the micropropagation medium on subsequent tuberization using kinetin for tuber induction in three cultivars of the four cultivars studied. In their system, lower nitrogen advanced subsequent tuber initiation. Lê (1999) studied microtuberization with and without exogenous PGRs and found that a high concentration of nitrogen (60 mM) was favourable to yielding larger microtubers.

In PGR-free medium reduced total availability of nitrogen or increasing the ratio of ammonium to nitrate reduced the size and number of *in vitro* tubers (Garner and Blake 1989; Charles *et al*. 1992). The after-effect of the nitrogen supply of *in vitro* plantlets on tuberization was studied by us (Dobránszki *et al*. 2005; Dobránszki and Magyar-Tábori 2009) using PGR-free medium and three potato varieties. Under long days, the lower nitrogen content and reduced nitrate to ammonium rate were favourable for the development of large tubers (> 6 mm in diameter). Under short days the total number of tubers increased with an increase in nitrogen content of the medium in two cultivars but with a reduction in nitrogen in one cultivar, while the number of large tubers was not significantly influenced by nitrogen supply. These observations are similar to the results of Krauss and Marshner (1982) obtained in water culture experiments. The optimum nitrogen treatment was genotype specific and the requirements for nitrogen also depended on the photoperiod used *in vitro*. Similarly, we studied the effect of other macroelements, such as phosphorus and sulphur, on *in vitro* tuberization but no effect could be detected.

Gelling agents

Agar-agar is generally the gelling agent used during shoot multiplication and *in vitro* tuberization of potato. Nowak and Asiedu (1992) compared the effects of Gelrite and agar-agar on tuberization induced by kinetin in six cultivars. Earlier and more uniform tuberization occurred on Gelrite than on agar-agar solidified medium.

Recently, fermentors and bioreactors have been adopted for commercial-scale microtuberization. In these systems plant material surrounded (continuously or at intervals) with liquid nutrient solution or the vessels contain different layers of plant material on porous substrates that are subjected to nutrient mists and aeration cycles of varying duration (Akita and Takayama 1994a, 1994b; Teisson and Alvard 1999; reviewed by Donelly *et al*. 2003; Nhut *et al*. 2006).

Others

Bizarri *et al*. (1995) indicated that medium containing activated charcoal resulted in the higher rate of tuberization and the larger *in vitro* tubers independent of the PGR content of the medium.

Genotype

The genotype-dependence of tuber production is well-known (Ranalli *et al*. 1994). Lentini and Earle (1991) developed an *in vitro* tuberization system to screen potato genotypes for maturity but they could only distinguish early genotypes. Veramendi *et al.* (2000) were able to distinguish genotypes with early, mid and late maturity by a bioassay using microtubers developed on kinetin-containing tuberization medium. On the contrary, other research groups (Garner and Blake 1989; Nowak and Asiedu 1992; Seabrook *et al*. 1993; Dobránszki and Mándi 1993; Dobránszki 1996) did not find a relationship between the maturity of tested genotypes and their *in vitro* tuberization responses.

Therefore, we attempted to clear up the controversy over the effect of genotype on tuberization in *in vitro* tuberization studies. Tuberization responses of eleven potato genotypes (cultivars/breeding lines) of different genetic origin and maturity groups were investigated under different photoperiodic treatments and various light intensities as described earlier. To avoid the potential effects of PGRs on the response to environmental light effects and on the natural balance of endogenous hormones of plantlets, no PGRs were added to the medium. For evaluation of data multivariate methods (Cluster and discriminant analysis) were used to determine the cause variable and to control the exactness of artificial grouping.

According to the results of multivariate analyses, the genotype was the variable causing tuberization responses *in vitro*. The most important factors were the parent-offspring relationship and the genetic presence or the effects of wild *Solanum* species which depended on the number and the phase of hybridization and on the interbred species. Thus, the genetic origin of a clone played a basic role in tuberization under *in vitro* conditions tested but environmental stimuli, such as photoperiod or light intensity, modified its effect. The interaction between genotype and light effect was thus proven (Dobránszki *et al*. 1998; Dobránszki and Ferenczy 1998; Dobránszki *et al*. 1999a; Dobránszki 2000). Similar to other studies (Garner and Blake 1989; Nowak and Asiedu 1992; Seabrook *et al.* 1993), a relationship was not found between the maturity of potato genotypes examined and their tuberization responses. However, an *in vitro* tuberization system free of PGRs can be used to screen for

the *in vitro* tuberization capacity of genotypes.

Explants used for tuberization

The productivity of *in vitro* tuberization systems is strongly affected by explants used for tuberization. The physiological age, type of explants and explant density significantly influence tuber development.

Physiological age

The first detailed report on the role of the physiological age of an explant (plantlet) used for tuberization in the subsequent *in vitro* tuberization in a PGR-free system was published by Garner and Blake (1989). They concluded that the number of weeks before transfer of plantlets to short days affected the success of tuberization and at least 4 weeks were necessary in 16 h days for the most rapid microtuber development and for the fresh weight of microtubers to increase. In our experiments tuberization responses of *in vitro* plantlets of different physiological ages (exposure to long days before tuberization for 3, 4, 5 and 6 weeks) and of various genotypes (from early, mid and late maturity groups) were examined (Dobránszki *et al.* 1999b). The physiological state of *in vitro* plantlets affected the tuberization pattern, especially the size distribution of microtubers. Considerable differences in tuber size and tuber number were observed between the genotypes. To determine the physiological age of plantlets, the stem length together with the estimated leaf area of the third leaf were suitable. It was concluded that morphological characters of plantlets can be used to predict the appropriate physiological state for reliable microtuberization, which is genotype-dependent.

Culture density

Forti *et al.* (1991) concluded that culture density could affect the percentage tuberization in a genotype-dependent way if single-node cuttings were used for tuberization. In our experiments *in vitro* plantlets of four varieties were grown at different plant densities (20, 30, 40 plantlet per jar, 400 ml Kilner jar) and the response of varieties differed depending on the culture density. In general, the time taken to initiate tubers was less at higher plantlet densities. The number of tubers per plantlet was not affected by plantlet density but the highest rate of usable (> 4 mm in diameter), uniform microtubers developed in the highest plant densities (Tábori *et al*. 2000a).

Type of explant

In vitro tuberization in PGR-free systems was generally induced on whole plantlets (Garner and Blake 1989; Dobránszki and Mándi 1993; Dobránszki *et al.* 1999a), on nodal segments (Forti *et al.* 1991) or on layered *in vitro* shoots with various number of nodes (Leclerc *et al*. 1994; Magyar-Tábori and Dobránszki 2002; Dobránszki and Magyar-Tábori 2004).

According to the experiments of Leclerc *et al.* (1994), layered shoots with 6 nodes tuberized more rapidly and produced significantly larger but fewer *in vitro* tubers than nodal cuttings. They proposed that the difference in the number of microtubers per original shoot was due to the expression of correlative inhibition in layered shoots, even though it may be altered by changing the orientation by 90°. If shoots were dissected into nodal cuttings, their hormonal balance was disrupted thereby promoting tuberization of each nodal cutting. Similar results concerning explant type were obtained by Pelacho *et al.* (1999) on PGR-free medium by using organic acids.

In vitro tuberization was induced on layered explants with a number of nodes (1-5 nodes per explant) on PGR-free medium with high sucrose (8%) content. The total number of nodes per jar was the same in each treatment (Magyar-Tábori and Dobránszki 2002; Dobránszki and Magyar-Tábori 2004). The type and size of the explant markedly influenced both the number and the size of microtubers developed. When the position of plantlets was vertical in the control treatment, the highest number of tubers per jar were produced, which may be explained by the use of fully developed plantlets with a high leaf area for perception of environmental stimuli, such as photoperiod, and with a well-balanced hormone state of the whole plantlet. However, the size and weight of microtubers was low. Changing the orientation of the shoots by 90° caused fewer tubers per jar and the number of tubers decreased as the size of explants increased. However, the size and weight of microtubers increased as the size of layered shoots increased, which may be caused by improved nutrition of buds due to their better contact with the medium. Cutting of plantlets into nodal cuttings (i.e. explants with 1 node) resulted in the cessation of correlative inhibition among buds (Levy *et al.* 1993), promoted the induction and initiation of more microtubers than on explants with 2-5 nodes. However, the size of microtubers was smaller. This result is similar to the finding of Leclerc *et al.* (1994). The majority of microtubers (49.4%) were 6-8 mm in the case of the smallest explants (with 1 node). When explants with 2 to 5 nodes were used, most microtubers were 8-10 mm but an increase in explant size, increased the number of microtubers produced with a larger diameter up to 16 mm. The average fresh weight of tubers also increased with an increase in the size of the explants (**Fig. 3**). Based on experiments in our laboratory with three cultivars, the use of explants with two nodes is recommended for economically reliable microtuber production. In these experiments, the

Fig. 3 Microtubers originating from different explants. 'K' refers to the control treatment when 8% sucrose solution was poured onto the plantlet and the numbers indicate the explant size (i.e. the number of nodes) when explants were layered onto high-sucrose-medium (8%). (Diameter of Petri-dishes on the picture is 80 mm). From **Magyar-Tábori and Dobránszki** (2002) *International Journal of Horticultural Science* **8**, 33-36, ©2002, with kind permission from Agroinform Publishing House.

average fresh weight of microtubers was sufficiently high (208-250 mg, depending on the cultivar), the number of large sized microtubers was very high (38-55% were larger than 8 mm) as well as the total number of tubers per jar (15.9-20.1) (Magyar-Tábori and Dobránszki 2002; Dobránszki and Magyar-Tábori 2004).

Beside plantlets, nodal segments or layered shoots, microtubers can also be used as explants for *in vitro* tuberization. Khuri and Moorby (1996) produced plantlets ready for microtuberization and *in vitro* tubers developed were morphologically identical to those from nodal segments.

Histology of microtubers

Potato plants form tubers as a storage organ. The sink capacity of a storage organ is important economically and influences the final size of the organ. The economical use of microtubers mainly depends on their size because larger microtubers have higher initial vigour, emergence and performance and they are able to produce a larger crop than small tubers (Wiersema *et al.* 1987; Ranalli *et al.* 1994; Tábori *et al.* 1999a). The size of microtubers can be increased by manipulating the factors mentioned in the previous sections; consequently, some microtubers that developed were larger but their final diameter seldom exceeded 10 mm (Tovar *et al.* 1985; Slimmon *et al.* 1989; Struik and Lommen 1991; Charles *et al.* 1992; Seabrook *et al.* 1993). Considering the pattern of tuberization, it is well known that tuber growth continues after stolon swelling, especially in the perimedullary region of tubers. However, this tissue region does not develop or develops slightly in *in vitro*-grown tubers and it may be hypothesized that this limits the final size of *in vitro* tubers around 10 mm (Struik *et al*. 1999). Microtubers, like conventional tubers, consist of three main tissue regions: the cortex, perimedulla and pith. Liu and Xie (2001) measured the different tuber regions and their correlation with each other in microtubers. They measured the number and volume of the cells in the different tuber tissues during tuber growth and established that cell number and volume increased simultaneously and that the relationship between them could be described by a power function; like the relationship between cell division and expansion to tuber weight, $Y = aW^b$, where Y = cell number or cell volume, and W = tuber fresh weight. The volume of fresh microtubers and their tissues were calculated by applying the data to the formula for an ellipsoid: $V = 4/3\pi 1/8 l w^2 = 0.52 l w^2$ (l = length of tuber, w_1 = width of pith tissue, w_2 = total width of the perimedulla and pith tissues, w_3 = total width of the cortex, perimedulla and pith tissues). Calculations of the volume of tuber tissues are derived as cortex was: $V_{co} = 0.52l(w_3^2-w_2^2)$, perimedulla: $V_{pe} = 0.52l(w_2^2-w_1^2)$, pith: $V_{pi} = 0.52lw_1^2$. There was a linear relationship between tuber volume and fresh weight. They concluded that microtuber size could be increased by the regulation of environmental factors applied during the tuberization process.

The production of large sized microtubers in three potato cultivars were investigated in our laboratories and the effects of the applied tuberization conditions on the proportion of microtuber tissues, especially on the perimedullary region were studied (Magyar-Tábori and Dobránszki 2002; Dobránszki and Magyar-Tábori 2004) based on the mathematical calculation described by Liu and Xie (2001). *In vitro* tubers were produced on PGR-free medium, from which 69-79% had a diameter larger than 6 mm, 53-55% were larger than 8 mm and 11-29% of the microtubers produced were larger than 10 and up to 16 mm in diameter. Microtubers produced on PGR-free medium had a well-developed perimedullary region, which seemed to be important in the final size of tubers until microtubers reached 12 mm in diameter. Presumably, its increase could be one of the important factors influencing the capacity of microtubers to act as a sink for assimilates, as in the case of field-grown tubers. However, in microtubers with diameter larger than 12 mm, the volume rates of the perimedullary region did not increase further and the maximal tuber size was 16 mm (w_3). It seems that this is the maximum tuber size that could be reached on PGR-free medium, if an economically sufficient multiplication rate and tuber number in a jar were necessary.

DORMANCY AND SPROUTING CHARACTERISTICS OF MICROTUBERS

Dormancy is defined as "the physiological state of the tuber in which autonomous sprout growth will not occur, even when placed under ideal natural conditions for sprouting (darkness, temperature 15-20°C, relative humidity about 90%)" (Reust 1986). This innate dormancy period can be followed by an enforced dormancy period when bud growth is suppressed (Jefferies and Lawson 1991). The dormant period can be considered to begin at tuber initiation, and end when buds are capable of growth (Wiltshire and Cobb 1996). Since tuber dormancy begins at tuber initiation and it can be observed *in vitro*, the true differences in the dormant period can be detected (Tábori *et al.* 1998). Generally the dormant period is considered to have ended when 80% of microtubers have at least one sprout longer than 2 mm (Leclerc *et al*. 1995). A time elapse to 50% of sprouted tubers can also be used to determine the dormant period. In the case of a normal distribution, the mean equals the median and therefore the mean duration of dormancy equals the moment at which 50% of tubers have ended dormancy (van Ittersum 1992). In our experiments microtubers were produced under highly standardized conditions and were uniform in size, a normal distribution was therefore assumed (Tábori *et al.* 2000b).

The length of the dormancy period is under genetic and environmental control (Sonnewald 2001). Mapping of a backcross population derived from haploid potato (*Solanum tuberosum*) and diploid wild species (*Solanum berthaultii*) showed that at least eight quantitative trait loci (QTLs) were associated with tuber dormancy (Simko *et al*. 1997). The most prominent QTL for dormancy was detected on chromosome II and explained 7.1% of the variance (Sliwka *et al*. 2008). Moreover, Agrimonti *et al*. (2007) identified the G1-1 gene as a genetic marker for breaking dormancy in potato tubers.

The physiological regulation of potato tuber dormancy and related research were reviewed by Suttle (2004b). Growth promoters and inhibitors play an important role in regulating the dormancy of potato tubers. Ethylene and abscisic acid are considered to be sprout inhibitors, while cytokinins and gibberellins stimulate growth (Sonnewald 2001).

Auxins

Endogenous auxins presumably do not play a role in tuber dormancy (Suttle 2004b). However, Sorce *et al.* (2000) detected a significant increase of indole-3-acetic acid (IAA) concentration from harvest to the end of dormancy, regardless of storage temperatures. When freshly harvested tubers were immersed in IAA solution (10^{-5}-10^{-7} M), it increased the duration of dormancy by 30%. The prolongation of dormancy by IAA may be related to an increase in ethylene production in meristems (Dogonadze *et al.* 2000). Treatment of tubers with biologically active auxins resulted in a transient, dose-dependent increase in ethylene production and inhibition of sprout growth (Suttle 2003). Similarly, sprouting was inhibited by solutions containing greater than 0.1 mg ml^{-1} IAA, and it reduced sprouting by 30-45% compared to the control (Slininger *et al*. 2004).

Ethylene

Applications of different ethylene antagonists Suttle (1998a) showed that endogenous ethylene was required only during the early or induction phase of tuber endodormancy. Additionally, the study demonstrated that endogenous ethylene

plays an essential role in the regulation of potato microtuber endodormancy. Dogonadze *et al*. (2000) also found that ethylene is one of the phytohormones that regulate the dormancy of potato tubers. In comparison to tubers stored in the air control the sprouting was delayed in a dose-related manner on tubers from the ethylene treatments (Daniels-Lake *et al.* 2005). However, Pruski *et al.* (2006) observed that shoot emergence from the ethylene-treated tubers occurred significantly earlier.

Abscisic acid (ABA)

High levels of endogenous ABA have been linked to prolonged dormant periods in field-grown tubers (Coleman and King 1984). A positive correlation was also observed between tissue levels of ABA and the microtubers dormancy (Leclerc *et al*. 1995). Suttle and Hultstrand (1994) demonstrated that endogenous ABA is essential for both the induction and maintenance of potato microtuber dormancy. 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 (Destefano-Beltrán *et al*. 2006a). A relationship between the ABA level and dormancy was demonstrated through polygene mapping (Simko *et al*. 1997). ABA synthesis and metabolism occurred in all tuber tissues examined (meristems, surrounding periderm and underlying cortical tissues) and are presumably controlled at the level of *StNCED* (biosynthesis) and *StCYP707A* (catabolism) gene activities (Destefano-Beltrán *et al*. 2006b).

Cytokinins

Turnbull and Hanke (1985) studied the changes in tissue sensitivity to cytokinin and found that cytokinins may be the primary factor in the switch from innate dormancy to the non-dormant state in potato tuber buds. Accordingly, the loss of endodormancy was reported to precede significant increases in the endogenous levels of different cytokinins in dormant tuber. Injection of several endogenous cytokinins resulted in the rapid and complete termination of tuber endodormancy (Suttle 1998b). However, immediately after harvest and during the initial period of storage, tubers entered dormancy and exogenous cytokinins were completely ineffective in breaking tuber dormancy. Thereafter, dormant tubers exhibited a gradual increase in sensitivity to both cytokinins (Suttle 2001).

Gibberellins (GA)

Suttle (2004a) showed that endogenous GAs do not play a role in potato tuber dormancy release, but in the regulation of subsequent sprout growth. Similarly, treatments of microtubers with exogenous gibberellic acid (GA_3) did not break dormancy, but stimulated sprouting at the end of the dormancy period (Desiré *et al*. 1995b). In contrast, Alexopoulos *et al*. (2007) concluded that GA_3 alone or in combination with benzyl adenine promotes dormancy breakage and sprouting.

The dormant period of microtubers was not influenced by the presence of PGRs in the microtuberization medium (Leclerc *et al*. 1995). In contrast, Gopal *et al*. (2004) found that the microtuber dormancy period was shortened by 3-4 weeks with the addition of ABA to the medium, but that this effect was not detected in all genotypes. The addition of growth retardants to the medium (ancymidol or paclobutrazol at 10^{-5} M) inhibited premature sprouting of potato microtubers (Harvey *et al.* 1991).

Although the physiological mechanism of potato dormancy has been widely studied, very little information is available on the dormancy of potato microtubers. It is important to extend our knowledge about the dormant period of microtubers, since termination of dormancy by chemical (GA_3) is often problematic (Tovar *et al*. 1985). Incomplete breaking of dormancy can cause low production of seed tubers (Pruski *et al.* 2003b).

Microtubers remained dormant for a minimum of 12-15 weeks after initiation (Suttle 1998a). Microtubers had longer dormant periods when harvested after 28 days compared with 56 days of incubation, when the dormant period was measured from harvest to sprouting. However, these differences were not significant when the dormant period was measured from the time of microtuber initiation to sprouting (Leclerc *et al*. 1995). Similarly, the dormant period of microtubers measured from initiation to sprouting were not affected by the duration of the incubation (growth) period, as observed with field-grown tubers (Cho *et al*. 1983). This discrepancy could be explained by the lack of variation in the incubation temperature of the *in vitro* system, while field-grown tubers are subjected to temperature fluctuations, especially later in the season (Leclerc *et al*. 1995).

Van Ittersum and Struik (1992) studied the relationship between stolon and several tuber characteristics and the duration of tuber dormancy. They found that tuber weight was the most significant factor affecting dormancy. Tuber weight was rather closely (negatively) correlated with dormancy, and the length/width ratio of the tuber and the duration of dormancy were negatively correlated. The heavier the tubers, the shorter the duration of dormancy, but the relation was not linear. Similarly, as tuber size increased, tubers broke dormancy earlier and produced more sprouts (Wurr *et al.* 2001). The size of the microtuber was also found to have a significant effect on the duration of dormancy: smaller microtubers (≤250 mg) had a longer dormant period than those greater than 250 mg. The longer dormancy periods of small microtubers may reflect differences in tuber age at the time of harvest (Leclerc *et al.* 1995). In our experiments conducted with microtubers, we could not find any significant differences between different size-groups. This lack of difference may be attributable to the very small range in size (some mm) or to other factors (e.g. too large a loss in the case of smallest tubers or early sprouting of some immature tubers in this fraction) (Tábori *et al.* 1999a). Moreover, van Ittersum (1992) found that the relation between dormancy and tuber weight can be cultivar dependent. However, the number of sprouts was affected by the size of the microtuber: the larger the tuber the greater the number of sprouts that began to grow (Wiersema *et al.* 1987; Tábori *et al.* 1999a).

The duration of dormancy is also significantly related to the date of tuber initiation and to the position of the tuber on the plant during its growth. The later a tuber was initiated, the longer its duration of dormancy in days after haulm cutting. However, a delay in tuber initiation did not result in the same delay in the end of dormancy (van Ittersum and Struik 1992).

Effects of environmental factors during tuberization

Light

Microtubers induced in total darkness had much longer dormancy than those produced under short day illumination (Tovar *et al.* 1985; Coleman and Coleman 2000). Microtubers produced under long days showed rapid sprouting, whereas under short days sprouting was delayed (Vecchio *et al*. 2000). We studied the sprouting pattern of microtubers produced under three different photoperiodic conditions: (i) plantlets were exposed to short days all through for 13 weeks [FP-1] or (ii) to complete darkness for 2 weeks, preceded by short day exposure of 2 weeks, and followed by another short day exposure for further 9 weeks [FP-2] or (iii) first to short days for 2 weeks, then to total darkness to the end of the experiments for 11 weeks [FP-3]). These treatments showed a slight (but in a few cases statistically significant) influence on dormancy. When short days were interrupted by 2 weeks of darkness in the FP-2 treatment, the dormant period was shortened, while 11 weeks darkness applied after a short day exposure of 2 weeks in the FP-3

treatment extended the dormant period as compared those microtubers that developed all throughout in the FP-1 treatment of short days. Expression of these effects depended on genotype and storage temperature (Tábori *et al.* 1999a). The tuber induction treatments influenced the time of tuber initiation, thus they could indirectly cause differences in duration of dormancy. The shortening effect of FP-2 was slightly moderate when dormancy was measured from tuber initiation. This indicates that the shortening effect of FP-2 was mainly attributable to the earlier tuberization (Tábori *et al.* 1998). However, when dormancy was considered from tuber initiation, the elongation effect of FP-3 was considerably more indicating a direct effect on dormancy. This effect may be due to a decrease in temperature accompanying dark treatments (24°C in day-time vs. 15°C at night-time). A similar effect of dark was observed by Tovar *et al.* (1985).

The number of sprouts was also affected by tuber induction treatments. In general, we observed the highest number of sprouts on tubers developed under short day illumination without a dark period, and the lowest number of sprouts was in microtubers produced mainly in darkness following a 2-week short day period. It can be partly explained by changes in tuber shape, which was influenced by photoperiod treatments resulting in longer tubers in the FP-1 treatment. Thus, even if the smallest diameter was the same, the tubers from FP-1 were larger. The differences could also be associated with an advanced age of microtubers (Tábori *et al.* 1999a). Kumar and Knowles (1993) found that the loss of apical dominance is associated with the advanced age of tubers, which can result in more sprouted shoots. Similarly, Gopal *et al.* (1997) found that the number of buds increased when tuberization was induced by short day illumination as compared to tuberization under darkness. Pruski *et al.* (2002) found that microtubers derived from the short photoperiod treatment were green and seemed to be less juvenile than the tubers from the dark treatment.

Light intensity applied through short day illumination influences the dormant period significantly. In general, the lower the light intensity, the longer was the dormant period for tested cultivars. Light intensity had the most significant influence on dormancy when tuber induction was carried out under the FP-2 treatment (short day illumination interrupted by 2 weeks of darkness) (Tábori *et al.* 2000b).

Temperature

Exposure of cultures to low temperature (4°C) during tuberization delayed tuber initiation and showed post-effects on dormancy in *in vitro* experiments (Martinez and Tizio 1990). Thieme (1988/89) also observed that very low temperatures (9°C) during tuberization may prolong the dormant period of microtubers considerably. Low temperatures may affect dormancy length via increased ABA content as compared to hot conditions (van den Berg *et al.* 1991).

Explant

When tuberization occurred in plantlets developed on recycled microtubers, the microtubers possessed a similar period of dormancy as those from nodal segments (Khuri and Moorby 1996). In our laboratory, we conducted experiments with the cv. 'Desiree' on the tuberization capacity of shoot explants with a varying numbers of nodes (1-5). Based on comparisons of these results to dormancy of microtubers developed on whole plants (64 days at 17°C storage temperature), we found that the dormant period of microtubers produced on explants with 1 and 2 nodes was significantly shorter (36 and 44 days, respectively). Although the dormant period of tubers formed on explants with 3, 4 and 5 nodes were also shorter (59, 48 and 46 days, respectively), the differences were not significant.

Genotype

The length of dormancy largely depends on the genotype *in vivo* (Vecchio *et al*. 2000; Hossain *et al*. 2002; Pande *et al*. 2007). The dormant period of microtubers was also found to be cultivar-specific (Ranalli *et al*. 1994) and a significant correlation was observed between *in vitro* and *in vivo* dormant periods (Leclerc *et al*. 1995). In our experiments, we also found that the length of dormancy of microtubers depended on genotype, but not on maturity. Duration of dormancy was shorter in the case of 'Cleopatra' (early) and 'Boró' (late) (73 and 82 days on average of photoperiod and storage temperature treatments, respectively), than in the case of 'Desiree' (mid-late) and 'Gülbaba' (mid-early) (102 and 138 days, respectively (Tábori *et al*. 1999a).

Effects of post-harvest conditions

Temperature

Because of the active metabolic state of dormant tubers (Desiré *et al*. 1995a; Espen *et al*. 1999), they are subject to changes in the physical environment, particularly temperature, which is the most important physical factor affecting dormancy. Within a range of 3 to 20°C, tubers stored at lower temperatures have a longer period of innate dormancy than those stored at higher temperatures. Similarly, after innate dormancy, low temperatures can enforce dormancy, and sprout growth increases at higher temperatures (Wiltshire and Cobb 1996). During storage at 2°C for 61 days, no sprouting was observed on microtubers, but after similar storage period at 8°C, virtually all microtubers had formed small sprouts (Claassen *et al*. 1992). In our experiments also storage of microtubers at low temperatures prolonged the rest-period considerably. Difference was larger between tubers stored at 5 and 10°C than between tubers stored at 10 and 20°C. The effect of low temperatures was larger for cultivars with longer dormancy (Tábori *et al*. 1999a). Desiré *et al*. (1995) found that the duration of dormancy was 22 weeks in cold storage (4°C) in the case of 'Desiree' microtubers, which corresponded to our results obtained for 'Desiree' stored at 5°C.

Storage period (20 or 60 days at 4°C) did not affect sprouting (Vecchio *et al*. 2000). The growth rate of sprouts was very low for 'Desiree' microtubers after a short period (from 25 to 46 days) of cold storage (3°C), while vigorous sprout-growth was observed after 60 and 74 days of cold storage (Tábori *et al*. 1999b). In contrast, dormancy of microtubers decreased more than a half with reduced time of storage at 3°C (28, 56, 84 and 105 days) (Ranalli *et al*. 1994).

Storage of microtubers under different temperatures led to different kinds of losses: in a refrigerator (4°C) more than 40% physical damage was observed, while under higher temperatures 24% tubers weight loss occurred after 60 days (Hossain *et al*. 2002). In our experiments, the loss of tubers largely depended on the size, and considerable loss in the smallest tubers (2-4 mm) may be due to their immature state and the increased surface: volume ratio (Tábori *et al*. 1999a). Weight loss during storage in microtubers was accompanied by turgor loss, brownish discolouration, etc. as was also observed by Lommen (1993) in minitubers (first *ex vitro* generation of *in vitro* plantlets or microtubers). Moreover, microtubers which lost over 40% of their weight during the first week of storage had longer dormancy than others (Akita and Takayama 1994a).

Light

The dormancy period was much longer in microtubers stored under light than those stored in the dark (Gopal *et al*. 2004). Under light-storage, the microtuber dormancy period increases in microtubers produced on medium supplemented with 60 g/l sucrose, but it decreased with further increase in sucrose content (Gopal *et al*. 2004). Interactions between several factors (genotypes, ABA content, sucrose and storage illumination, and others) were observed (Ranalli *et al*. 1994; Tábori *et al*. 2000b; Gopal *et al*. 2004).

Chemicals

Postharvest application of rindite (fumigation with 2 ml rindite for 48 h or 4 ml for 24 h) significantly reduced the dormancy period of potato microtubers of 'Atlantic', 'Superior', 'Lemhi Russet', 'Red Dale' and 'Kennebec'. Kim *et al*. (1999) recommended that microtubers should be treated at least 2 weeks after harvest when the skin of microtubers is mature. Treatment of tubers with bromoethane, seemed to be similarly efficient, but was less toxic (Coleman 1984). Destefano-Beltrán *et al*. (2006a) also used bromoethane to induce rapid and synchronous sprouting of dormant tubers. Treatment of microtubers with GA_3 was more effective than rindite (Pruski *et al.* 2003b). Hossain *et al*. (2002) also found that treatment of microtubers with 8 mg/l GA_3 and storage at 30°C combined with 12 h illumination was effective in breaking dormancy. Treatment of microtubers with ethanol (0.5 % for 3 days) also broke dormancy (Claassens *et al*. 2005). Breaking of dormancy could be also achieved by applying an electrical current of 50 and 100 V for 5 days (Kocaçalişkan *et al*. 1989).

Others

Microtubers produced in liquid medium had a relatively shorter dormancy than those produced on solid medium (Teisson and Alvard 1999). High sprouting (≥ 90%) and survival rate (≥ 89%) could be obtained within 30 days in microtubers originating from hydroponic culture system (Nhut *et al*. 2006). Moreover, when the volume of tuber induction liquid medium was increased from 30 ml to 60 ml, the dormant period decreased by more than a half from 43.1 days to 12.9 days (Stahlshmidt de Fernandez *et al*. 1995). Application of a high concentration of sucrose (140 g/l) in tuberization medium shortens the microtuber dormancy period (Desiré *et al*. 1995b).

Microtubers with a high Ca^{2+} content produced under a long photoperiod showed the highest sprouting percentage and the quickest average time of sprouting. The high amount of K^+ found in Desiree in the presence of CCC [(2-chloroethyl) trimethylammonium chloride], irrespectively of photoperiods, is linked with an extended dormancy (Vecchio *et al.* 2000).

CONCLUDING REMARKS

Tuberization of potato is a complex developmental process involving interactions between environmental, biochemical and genetic factors. Morphological and biochemical processes are regulated by specific gene expression patterns (Bachem *et al*. 2000). The most important environmental signals for tuberization involve short-day photoperiod, high irradiance and low temperature. Perception of environmental stimuli occurs in the leaves and is mediated by phytochrome-B and GAs and then the produced systematic signal transmitted to the stolons to initiate tuberization (Sarkar 2008).

This literature review confirmed the strong analogy in the growth responses between the field-grown and *in vitro* tubers produced in PGR-free systems, which allows the use of these systems in basic research, such as plant metabolism, gene regulation, and germplasm selection, among others.

It was proved (Dobránszki *et al.* 1999a) that a determined quantity of light illumination is necessary to induce reliable *in vitro* tuberization, indicating a phytohcrome-mediated multiple signal transduction pathway of tuberization.

Moreover, it was demonstrated that it is possible to produce a high number of large tubers, which enables economical seed-potato-production. Strong genotype-dependence was detected and statistically proven, both in the tuberization responses and in the dormancy and sprouting characteristics of microtubers.

Commercial production of seed potato is still based mainly on the use of *in vitro* plantlets (Pruski *et al.* 2003b); however, microtubers have been integrating in seed potato programme since 1990 years (Struik and Lommen 1991; Khuri and Moorby 1996; Lazányi *et al*. 1998; Lê 1999). Use of microtubers in the production of minitubers is limited because of their low yielding capacity, mainly if they planted in field. There are some reports about improving the field or greenhouse performance of microtubers (Struik and Lommen 1999; Rolot and Seutin 1999; Vanderhofstadt 1999; Pruski *et al.* 2003b).

Reviewed results originating from PGR-free systems promote the application of microtubers in seed-potato system and their commercial use, which has been becoming more and more important around the world (Donnelly *et al.* 2003). The main problem of application of microtubers is related to their dormancy and sprouting.

Dormancy of microtubers produced in PGR-free systems is influenced by the same factors as reported for field-grown tubers, such as cultivar, environmental conditions during tuberization and storage. The dormant period can be predicted, and thus the timing of tuber production and sprouting can be planned. Sprouting of microtubers could be synchronized and therefore their use in seed-potato-production can be profitable.

REFERENCES

Agrimonti C, Visioli G, Bianchi R, Torelli A, Marmiroli N (2007) G1-1 and *Le*G1-1/*Le*G1-2 genes are involved in meristem activation during breakage of dormancy and early germination in potato tubers and tomato seeds. *Plant Science* **173**, 533-541

Akita M, Takayama S (1994a) Stimulation of potato (*Solanum tuberosum* L.) tuberization by semicontinuous liquid medium surface level control. *Plant Cell Reports* **13**, 184-187

Akita M, Takayama S (1994b) Induction and development of potato tubers in a jar fermentor. *Plant Cell, Tissue and Organ Culture* **36**, 177-182

Alexopoulos AA, Akoumianakis KA, Vemmos SN, Passam HC (2007) The effect of postharvest application of gibberellic acid and benzyl adenine on the duration of dormancy of potatoes produced by plants grown from TPS. *Postharvest Biology and Technology* **46**, 54-62

Alix MJ, Savvides S, Blake J, Herrmann R, Hornung R (2001) Effects of illumination source, culture ventilation and sucrose on potato (*Solanum tuberosum*) microtuber production under short days. *Annals of Applied Botany* **139**, 175-187

Bachem C, van der Hoeven R, Lucker J, Oomen R, Casarini E, Jacobsen E, Visser R (2000) Functional genomic analysis of potato tuber life-cycle. *Potato Research* **43**, 297-312

Bizarri M, Borghi L, Ranalli P (1995) Effects of activated charcoal effects on induction and development of microtubers in potato (*Solanum tuberosum* L.). *Annals of Applied Biology* **127**, 175-181

Castro G, Abdala G, Agüero C, Tizio R (2000) Interaction between jasmonic and gibberellic acids on *in vitro* tuberization of potato plantlets. *Potato Research* **43**, 83-88

Cenzano A, Abdala G, Hause B (2007) Cytochemical immuno-localization of allene oxide cyclase, a jasmonic acid biosynthetic enzyme, in developing potato stolons. *Journal of Plant Physiology* **164**, 1449-1456

Charles G, Rossignol L, Rossignol M (1992) Environmental effects on potato plants *in vitro*. *Journal of Plant Physiology* **139**, 708-713

Cho JL, Iritani WM, Martin MW (1983) Comparison of methods for measuring dormancy of potatoes. *American Potato Journal* **60**, 169-177

CIP (2008) Available online: http://www.cipotato.org/pressroom/press_releases_detail.asp?cod=53

Claassen PAM, Calker MH van, Marinus J (1992) Accumulation of sugars in microtubers of potato node cuttings (cv. Kennebec) during cold storage. *Potato Research* **35**, 191-194

Claassens MMJ, Verhees J, van der Plas LHW, van der Krol, AR, Vreugdenhil D (2005) Ethanol breaks dormancy of the potato tuber apical bud. *Journal of Experimental Botany* **56**, 2515-2525

Coleman WK (1984) Large scale application of bromoethane for breaking potato tuber dormancy. *American Potato Journal* **61**, 587-589

Coleman WK, King RR (1984) Changes in endogenous abscisic acid, soluble sugars and proline levels during tuber dormancy in *Solanum tuberosum* L. *American Potato Journal* **61**, 437-449

Coleman WK, Coleman SE (2000) Modification of potato microtuber dormancy during induction and growth *in vitro* or *ex vitro*. *American Journal of Potato Research* **77**, 103-110

Coleman WK, Donnelly DJ, Coleman SE (2001) Potato microtubers as research tools: A review. *American Journal of Potato Research* **78**, 47-55

Daniels-Lake BJ, Prange RK, Nowak J, Asiedu SK, Walsh JR (2005) Sprout development and processing quality changes in potato tubers stored under ethylene: 1. Effects of ethylene concentration. *American Journal of Potato Research* **82**, 389-397

Desiré S, Couillerot JP, Hilbert JL, Vasseur J (1995a) Protein-changes in *Solanum tuberosum* during storage and dormancy breaking of *in vitro* microtubers. *Plant Physiology and Biochemistry* **33**, 479-487

Desiré S, Couillerot JP, Vasseur J (1995b) Dormancy and sprouting of *in vitro* potato (*Solanum tuberosum* L.) microtubers: Effects of sucrose concentration from tuberization medium, storage period at 4°C and gibberellic acid treatment. *Acta Botanica Gallica* **142**, 371-378

Destefano-Beltrán L, Knauber D, Huckle L, Suttle JC (2006a) Chemically forced dormancy termination mimics natural dormancy progression in potato tuber meristems by reducing ABA content and modifying expression of genes involved in regulating ABA synthesis and metabolism. *Journal of Experimental Botany* **57**, 2879-2886

Destefano-Beltrán L, Knauber D, Huckle L, Suttle JC (2006b) Effects of postharvest storage and dormancy status on ABA content, metabolism, and expression of genes involved in ABA biosynthesis and metabolism in potato tuber tissues. *Plant Molecular Biology* **61**, 687-697

Dobránszki J, Mándi M (1993) Induction of *in vitro* tuberization by short day period and dark treatment of potato plants *in vitro* on medium free of growth regulators. *Acta Biologica Hungarica* **44**, 411-420

Dobránszki J (1996) Effects of dark treatment on tuber initiation and development of induced potato plantlets cultured *in vitro*. *Acta Agronomica Hungarica* **44**, 377-386

Dobránszki J (1997) Effects of light on *in vitro* tuberization of potato cultivars with pure *Solanum tuberosum* origin. *Acta Agronomica Hungarica* **45**, 383-397

Dobránszki J, Tábori KM, Ferenczy A (1998) Clearing up of the background of genotype-dependence in *in vitro* tuberization responses by multivariate analyses. *Beiträge zur Züchtungsforschung – Bundesanstalt für Züchtungsforschung an Kulturpflanzen* **4**, 37-39

Dobránszki J, Ferenczy A (1998) Multivariate analyses in relation to *in vitro* tuberization of potato. *Kertészeti Egyetem Közleményei - Publicationes Universitatis Horticulturae Industriaeque Alimentariae* **LVII**, 51-58

Dobránszki J, Magyar-Tábori K, Ferenczy A (1999a) Light and genotype effects on *in vitro* tuberization of potato plantlets. *Potato Research* **42**, 483-488

Dobránszki J, Magyarné Tábori K, Ferenczy A (1999b) Effects of physiological age of *in vitro* plantlets on tuberization pattern. *14th Triennial Conference of the European Association for Potato Research*, May 2-7, Sorrento, Italy, pp 34-35

Dobránszki J (2000) Effects of light and genetic origin on *in vitro* tuberization of potato. *Acta Agronomica Hungarica* **48**, 1-10

Dobránszki J (2001) Effects of light on *in vitro* tuberization of Desiree and its relatives. *Acta Biologica Hungarica* **52**, 137-147

Dobránszki J, Magyar-Tábori K (2004) Effects of tuberization conditions on the microtuber yield and on the proportion of microtuber tissues. *International Journal of Horticultural Science* **10**, 91-96

Dobránszki J, Magyar-Tábori K, Hudák I (2005) Effect of nitrogen supply and day length on *in vitro* tuberization. *16th Triennial Conference of the European Association for Potato Research*, July 17-22, Bilbao, Spain, pp 793-796

Dobránszki J, Magyar-Tábori K (2009) Influence of nitrogen supply of potato plantlets on *in vitro* tuberization pattern under inductive and non-inductive conditions. *Potato Research* in press

Dogonadze MZ, Korableva NP, Platonova TA, Shaposhnikov GL (2000) Effects of gibberellin and auxin on the synthesis of abscisic acid and ethylene in buds of dormant and sprouting potato tubers. *Applied Biochemistry and Microbiology* **36**, 507-509

Donnelly DJ, Coleman WK, Coleman SE (2003) Potato microtuber production and performance: a review. *American Journal of Potato Research* **80**, 103-115

Espen L, Morgutti S, Abruzzese A, Negrini N, Rivetta A, Quattrini MM, Cocucci M, Cocucci SM (1999) Changes in the potato (*Solanum tuberosum* L.) tuber at the onset of dormancy and during storage at 23°C and 3°C. I. Biochemical and physiological parameters. *Potato Research* **42**, 189-201

Estrada R, Tovar P, Doods JH (1986) Induction of *in vitro* tubers in a broad range of potato genotypes. *Plant Cell, Tissue and Organ Culture* **7**, 3-10

Ewing EE (1978) Critical photoperiods for tuberization: a screening technique with potato cuttings. *American Potato Journal* **55**, 43-43

Forti E, Mandolino G, Ranalli P (1991) *In vitro* tuber induction: Influence of the variety and of the media. *Acta Horticulturae* **300**, 127-132

Gao X, Wang F, Yang Q, Matsuura H, Yoshihara T (2005) Theobroxide triggers jasmonic acid production to induce potato tuberization *in vitro*. *Plant Growth Regulation* **47**, 39-45

Garner N, Blake J (1989) The induction and development of potato microtubers *in vitro* on media free of growth regulating substances. *Annals of Botany* **63**, 663-674

Gibson SI (2005) Control of plant development and gene expression by sugar signalling. *Current Opinion in Plant Biology* **8**, 93-102

Gopal J, Minocha JL (1997) Effectiveness of selection at microtuber crop level in potato. *Plant Breeding* **116**, 293-295

Gopal J, Minocha JL (1998) Effectiveness of *in vitro* selection for agronomic characters in potato. *Euphytica* **103**, 67-74

Gopal J, Minocha JL, Sidhu JS (1997) Comparative performance of potato crops raised from microtubers induced in the dark versus microtubers induced in light. *Potato Research* **40**, 407-412

Gopal J, Minocha JL, Dhaliwal HS (1998) Microtuberization in potato (*Solanum tuberosum* L.) *Plant Cell Reports* **17**, 794-798

Gopal J, Chamail A, Sarkar D (2004) *In vitro* production of microtubers for conservation of potato germplasm: effect of genotype, abscisic acid, and sucrose. *In Vitro Cellular and Developmental Biology – Plant* **40**, 485-490

Gopal J, Iwama K (2007) *In vitro* screening of potato against water-stress mediated through sorbitol and polyethylene glycol. *Plant Cell Reports* **26**, 693-700

Harvey BMR, Crothers SH, Evans NE, Selby C (1991) The use of growth retardants to improve microtuber formation by potato (*Solanum tuberosum*). *Plant Cell, Tissue and Organ Culture* **27**, 59-64

Harvey BMR, Crothers SH, Watson S, Lee HC (1992) Heat inhibition of tuber development in potato (*Solanum tuberosum* L.): effects on microtuber formation *in vitro*. *Potato Research* **35**, 183-190

Hossain MJ, Sultana N, Ahmed AU (2002) Postharvest sprouting physiology and dormancy breaking of potato microtuber. *Bangladesh Journal of Botany* **31**, 77-82

Hussain I, Chaudhry Z, Muhammad A, Asghar R, Naqvi SMS, Rashid H (2006) Effect of chlorocholine chloride, sucrose and BAP on *in vitro* tuberization in potato (*Solanum tuberosum* L. cv. Cardinal). *Pakistan Journal of Botany* **38**, 275-282

Hussey G, Stacy NJ (1981) *In vitro* propagation of potato (*Solanum tuberosum* L.). *Annals of Botany* **48**, 787-796

Hussey G, Stacy NJ (1984) Factors affecting the formation of *in vitro* tubers of potato (*Solanum tuberosum* L.). *Annals of Botany* **53**, 565-578

Jefferies RA, Lawson HM (1991) A key for the stages of development of potato (*Solanum tuberosum*). *Annals of Applied Biology* **119**, 387-399

Kahn BA, Ewing EE, Senesac AH (1983) Effects of leaf age, leaf area, and other factors on tuberization of cuttings from induced potato (*Solanum tuberosum*) shoots. *Canadian Journal of Botany* **61**, 3193-3201

Khuri S, Moorby J (1996) Nodal segments or microtubers as explants for *in vitro* microtuber production of potato. *Plant Cell, Tissue and Organ Culture* **45**, 215-222

Kim HS, Jeon JH, Choi KH, Joung YH, Joung H (1999) Effects of rindite on breaking dormancy of potato microtubers. *American Journal of Potato Research* **76**, 5-8

Kiyota H, Saitoh M, Oritani T, Yoshihara T (1996) Synthesis and potato tuber-inducing activity of methyl 5',5',5'-trifluorojasmonate. *Phytochemistry* **42**, 1259-1262

Kocaçalişkan Î, Küfrevioğlu Î, Keha EE, Çaliskan S (1989) Breaking of dormancy in potato tubers by electrical current. *Journal of Plant Physiology* **135**, 373-374

Koda Y, Omer EA, Yoshihara T, Shibata H, Sakamura S, Okazawa Y (1988) Isolation of a specific tuber-inducing substance from potato leaves. *Plant Cellular Physiology* **29**, 1047-1051

Koda Y, Kikuta Y, Tazaki H, Tsujino Y, Sakamura S, Yoshihara T (1991) Potato inducing activities of jasmonic acid and related compounds. *Phytochemistry* **30**, 1435-1438

Krauss A, Marshner H (1982) Influence of nitrogen nutrition, daylength and temperature on contents of gibberellic and abscisic acid and on tuberization in potato plants. *Potato Research* **25**, 13-21

Kumar GNM, Knowles R (1993) Involvement of auxin in the loss of apical dominance and plant growth potential accompanying aging of potato seed tubers. *Canadian Journal of Botany* **71**, 541-550

Langille AR, Hepler PR (1992) Effect of three anti-gibberellin growth retardants on tuberization of induced and non-induced Kathadin potato leaf-bud cuttings. *American Potato Journal* **69**, 131-141

Lazányi J, Mándi M, Tóth IJ, Dobránszki J (1998) Potato breeding at the Research Centre of Debrecen Agricultural University. *Beiträge zur Züchtungsforschung – Bundesanstalt für Züchtungsforschung an Kulturpflanzen* **4**, 105-109

Lê CL (1999) *In vitro* microtuberization: an evaluation of culture conditions for the production of virus-free seed potatoes. *Potato Research* **42**, 489-498

Leclerc Y, Donnelly DJ, Seabrook JEA (1994) Microtuberization of layered shoots and nodal cuttings of potato: The influence of growth regulators and incubation periods. *Plant Cell, Tissue and Organ Culture* **37**, 113-120

Leclerc Y, Donnelly DJ, Coleman WK, King RR (1995) Microtuber dormancy in three potato cultivars. *American Potato Journal* **72**, 215-223

Lentini Z, Earle ED (1991) *In vitro* tuberization of potato clones from different maturity groups. *Plant Cell Reports* **9**, 691-695

Levy D, Seabrook JEA, Coleman S (1993) Enhancement of tuberization of axillary shoot buds of potato (*Solanum tuberosum* L.) cultivars cultured *in vitro*. *Journal of Experimental Botany* **44**, 381-386

Liu J, Xie C (2001) Correlation of cell division and cell expansion to potato microtuber growth *in vitro*. *Plant Cell, Tissue and Organ Culture* **67**, 159-164

Lommen WJM (1993) Post harvest characteristics of potato minitubers with different fresh weights and from different harvests. II. Losses during storage. *Potato Research* **36**, 273-282

Magyar-Tábori K, Dobránszki J (2002) How to produce large sized microtubers of potato cv. Desiree. *International Journal of Horticultural Science* **8**, 33-36

Mangat BS, Kerson G, Wallace D (1984) The effect of 2,4-D on tuberization and starch content of potato tubers produced on stem segments cultured *in*

vitro. *American Potato Journal* **61**, 355-361
Martinez L, Tizio R (1990) Post-effects of low and high-temperature periods and CCC on *in vitro* tuberisation of potato. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales* **184**, 251-258
McGrady JJ, Struik PC, Ewing EE (1986) Effects of exogenous applications of cytokinin on the development of potato (*Solanum tuberosum* L.) cuttings. *Potato Research* **29**, 191-205
Nhut DT, Nguyen NH, Thuy DTT (2006) A novel *in vitro* hydroponic culture system for potato (*Solanum tuberosum* L.) microtuber production. *Scientia Horticulturae* **110**, 230-234
Nowak J, Asiedu SK (1992) Gellint agent and light effects on *in vitro* tuberization of potato cultivars. *American Potato Journal* **69**, 461-470
Nowak J, Colborne D (1989) *In vitro* tuberization and tuber proteins as indicators of heat stress tolerance in potato. *American Potato Journal* **66**, 35-44
Pande PC, Singh SV, Pandey SK, Singh B (2007) Dormancy, sprouting behaviour and weight loss in Indian potato (*Solanum tuberosum*) varieties. *Indian Journal of Agricultural Science* **77**, 715-720
Pelacho AM, Mingo-Castel AM (1991) Effects of photoperiod on kinetin-induced tuberization of isolated potato stolons cultured *in vitro*. *American Potato Journal* **68**, 533-541
Pelacho AM, Martín-Closas L, Campabadal C, Torres A, Farran I, Mingo-Castel AM (1994) *In vitro* tuberization of potato: Effect of several morphogenic regulators in light and darkness. *Journal of Plant Physiology* **144**, 705-709
Pelacho AM, Martín-Closas L, Sanfeliu JLI (1999) *In vitro* induction of potato tuberization by organic acids. *Potato Research* **42**, 585-591
Peres LEP, Carvalho RF, Zsögön A, Bermúdez-Zambrano OD, Robles WGR, Tavares S (2005) Grafting of tomato mutants onto potato rootstocks: An approach to study leaf-derived signalling on tuberization. *Plant Science* **169**, 680-688
Perl A, Aviv D, Willmitzer L, Galun E (1991) *In vitro* tuberization in transgenic potatoes harboring β-glucuronidase linked to a patatin promoter: effects of sucrose levels and photoperiods. *Plant Science* **73**, 87-95
Pruski K, Duplessis P, Lewis T, Astatkie T, Nowak J, Struik PC (2001) Jasmonate effect on *in vitro* tuberization of potato (*Solanum tuberosum* L.) cultivars under light and dark conditions. *Potato Research* **44**, 315-325
Pruski K, Astatkie T, Nowak J (2002) Jasmonate effects on *in vitro* tuberization and tuber bulking in two potato cultivars (*Solanum tuberosum* L.) under different media and photoperiod conditions. *In Vitro Cellular and Developmental Biology – Plant* **38**, 203-209
Pruski K, Astatkie T, Duplessis P, Lewis T, Nowak J, Struik PC (2003a) Use of jasmonate for conditioning of potato plantlets and microtubers in greenhouse production of minitubers. *American Journal of Potato Research* **80**, 183-193
Pruski K, Astatkie T, Duplessis P, Stewart L, Nowak J, Struik PC (2003b) Manipulation of microtubers for direct field utilization in seed production. *American Journal of Potato Research* **80**, 173-181
Pruski K, Prange RK, Daniels-Lake BJ, Nowak J, Astatkie T, Ronis DH (2006) Growth-room and field studies with seed tubers treated with ethylene and 1-methylcyclopropene (1-MCP) during storage. *American Journal of Potato Research* **83**, 149-160
Ranalli P, Bizzari M, Borghi L, Mari M (1994) Genotypic influence on *in vitro* induction, dormancy length, advancing age and agronomical performance of potato microtubers (*Solanum tuberosum* L.). *Annals of Applied Biology* **125**, 161-172
Reust W (1986) EAPR working group 'physiological age of potato'. *Potato Research* **29**, 268-271
Rolot JL, Seutin H (1999) Soilless production of potato minitubers using a hydroponic technique. *Potato Research* **42**, 457-469
Sarkar D (2008) The signal transduction pathways controlling in planta tuberization in potato: an emerging synthesis. *Plant Cell Reports* **27**, 1-8
Seabrook JEA, Coleman S, Levy D (1993) Effect of photoperiod on *in vitro* tuberization of potato (*Solanum tuberosum* L.). *Plant Cell, Tissue and Organ Culture* **34**, 43-51
Seabrook JEA, Douglass LK, Arnold DA (2004) Effect of leaves on microtubers produced from potato single-node cuttings *in vitro*. *American Journal of Potato Research* **81**, 1-5
Seabrook JEA (2005) Light effects on the growth and morphogenesis of potato (*Solanum tuberosum*) *in vitro*: A review. *American Journal of Potato Research* **82**, 353-367
Ŝimko I (1993) Effects of kinetin, paclobutrazol and their interactions on the microtuberization of potato stem segments cultured *in vitro* in the light. *Plant Growth Regulation* **12**, 23-27
Simko I, McMurry S, Yang HM, Manschot A, Davies PJ, Ewing EE (1997) Evidence from polygene mapping for a causal relationship between potato tuber dormancy and abscisic acid content. *Plant Physiology* **115**, 1453-1459
Slimmon T, Souza Machado V, Coffin R (1989) The effect of light on *in vitro* microtuberization of potato cultivars. *American Potato Journal* **66**, 843-848
Slininger PJ, Burkhead KD, Schisler DA (2004) Antifungal and sprout regulatory bioactivities of phenylacetic acid, indole-3-acetic acid, and tyrosol isolated from the potato dry rot suppressive bacterium *Enterobacter cloacae* S11:T:07. *Journal of Indian Microbiology and Biotechnology* **31**, 517-524
Sliwka J, Wasilewicz-Flis I, Jakuczun H, Gebhardt C (2008) Tagging quantitative trait loci for dormancy, tuber shape, regulatory of tuber shape, eye depth and flash colour in diploid potato originated from six *Solanum* species. *Plant Breeding* **127**, 49-55
Sonnewald U (2001) Control of potato tuber sprouting. *Trends in Plant Science* **6**, 333-335
Sorce C, Lorenzi R, Ceccarelli N, Ranalli P (2000) Changes in free and conjugated IAA during dormancy and sprouting of potato tubers. *Australian Journal of Plant Physiology* **27**, 371-377
Stahlshmidt de Fernandez O, Martínez L, Guiñazú M (1995) Qualitative and quantitative production of potato microtubers (*Solanum tuberosum* L.) in two-phase system. *Biocell* **19**, 57-63
Stallknecht GF, Farnsworth S (1982) General characteristics of coumarin-induced tuberization of axillary shoots of *Solanum tuberosum* L. cultured *in vitro*. *American Potato Journal* **59**, 17-31
Struik PC, Geertsema J, Custers CHMG (1989) Effects of shoot, root and stolon temperature on the development of the potato (*Solanum tuberosum* L.) plant. III. Development of tubers. *Potato Research* **32**, 151-158
Struik PC, Lommen WJM (1991) Production, storage and use of micro- and minitubers. In: MacKerron DKL, Edmond HD, Hall D, Kirkman MA, Lang RW, Mackay GR, McRae DC, Oxley SJP (Eds) *Proceedings of the 11th Triennial Conference of EAPR*, Edinburgh, 8-13th July 1990, pp 122-133
Struik PC, Lommen WJM (1999) Improving the field performance of micro- and minitubers. *Potato Research* **42**, 559-568
Struik PC, Vreugdenhil D, van Eck HJ, Bachem CW, Visser RGF (1999) Physiological and genetic control of tuber formation. *Potato Research* **42**, 313-331
Suttle JC (1998a) Involvement of ethylene in potato microtuber dormancy. *Plant Physiology* **118**, 843-848
Suttle JC (1998b) Postharvest changes in endogenous cytokinins and cytokinin efficacy in potato tubers in relation to bud endodormancy. *Physiologia Plantarum* **103**, 59-69
Suttle JC (2001) Dormancy-related changes in cytokinin efficacy and metabolism in potato tubers during postharvest storage. *Plant Growth Regulation* **35**, 199-206
Suttle JC (2003) Auxin-induced sprout growth inhibition: role of endogenous ethylene. *American Journal of Potato Research* **80**, 303-309
Suttle JC (2004a) Involvement of endogenous gibberellins in potato tuber dormancy and early sprout growth: a critical assessment. *Journal of Plant Physiology* **161**, 157-164
Suttle JC (2004b) Physiological regulation of potato tuber dormancy. *American Journal of Potato Research* **81**, 253-262
Suttle JC, Hultstrand JF (1994) Role of endogenous abscisic-acid in potato microtuber dormancy. *Plant Physiology* **105**, 891-896
Tábori KM, Dobránszki J, Ferenczy A (1998) Dormancy of potato microtuber originating from hormone-free system. *Beiträge zur Züchtungsforschung – Bundesanstalt für Züchtungsforschung an Kulturpflanzen* **4**, 181-182
Tábori KM, Dobránszki J, Ferenczy A (1999a) Some sprouting characteristics of microtubers. *Potato Research* **42**, 611-617
Tábori KM, Dobránszki J, Ferenczy A (1999b) Pre-sprouting of potato microtubers and its effect on shoot emergence. *14th Triennial Conference of the European Association for Potato Research*, May 2-7, Sorrento, Italy, pp 460-461
Tábori KM, Dobránszki J, Ferenczy A (2000a) Effects of culture density on growth and *in vitro* tuberization capacity of potato plantlets. *Acta Agronomica Hungarica* **48**, 185-189
Tábori KM, Dobránszki J, Ferenczy A (2000b) Post-effect of light conditions on dormancy of potato microtubers. *Acta Agronomica Hungarica* **48**, 127-132
Teisson C, Alvard D (1999) *In vitro* production of potato microtubers in liquid medium using temporary immersion. *Potato Research* **42**, 499-504
Thieme R (1988/89) An *in vitro* potato collection: microtuberization and storage of microtubers. FAO/IBPGR *Plant Genetic Resources Newsletter* 17-19
Tovar P, Estrada R, Schilde-Rentschler L, Dodds JH (1985) Induction and use of *in vitro* potato tubers. *CIP Circular* **13**, 1-5
Turnbull CGN, Hanke DE (1985) The control of bud dormancy in potato tubers. Evidence for the primary role of cytokinins and a seasonal pattern of changing sensitivity to cytokinin. *Planta* **165**, 359-365
Van den Berg JH, Ewing EE (1991) Jasmonates and their role in plant growth and development, with special reference to the control of potato tuberization: a review. *American Potato Journal* **68**, 781-794
Van den Berg JH, Vreugdenhil D, Ludford PM, Hillmann LL, Ewing EE (1991) Changes in starch, sugar, and abscisic acid contents associated with second growth in tubers of potato (*Solanum tuberosum* L.) one-leaf cuttings. *Journal of Plant Physiology* **139**, 86-89
Vanderhofstadt B (1999) Pilot units of potato seed production in Mali, using *in vitro* material: micro/minitubers. *Potato Research* **42**, 593-600
Van Ittersum MK (1992) Variation in the duration of tuber dormancy within a seed potato lot. *Potato Research* **35**, 261-269
Van Ittersum MK, Struik PC (1992) Relation between stolon and tuber characteristics and the duration of tuber dormancy. *Netherlands Journal of Agricultural Science* **40**, 159-172
Vecchio V, Benedettelli S, Andrenelli L, Palchetti E, Espen L (2000) Inductive and noninductive conditions on *in vitro* tuberisation and microtuber dor-

mancy in potato (*Solanum tuberosum* subspecies *tuberosum* and subspecies *andigena*). *Potato Research* **43**, 115-123

Veramendi J, Sota V, Fernández-San Millán A, Villafranca MJ, Martin-Closas L, Pelacho AM, Mingo-Castel AM (2000) An *in vitro* tuberization bioassay to assess maturity class of new potato clones. *Journal of Horticultural Science and Biotechnology* **75**, 733-738

Vreugdenhil D, Struik PC (1989) An integrated view of the hormonal regulation of tuber formation in potato (*Solanum tuberosum*). *Physiologia Plantarum* **75**, 525-531

Vreugdenhil D, Sergeeva LI (1999) Gibberellins and tuberization in potato. *Potato Research* **42**, 471-481

Vreugdenhil D, Bindels P, Reinhoud P, Klocek J, Hendriks T (1994) Use of growth retardant tetcyclacis for potato tuber formation in vitro. *Plant Growth Regulation* **14**, 257-265

Wang P, Hu C (1982) *In vitro* mass tuberization and virus-free seed-potato production in Taiwan. *American Potato Journal* **59**, 33-37

Wiersema SG, Cabello R, Tovar P, Dodds JH (1987) Rapid seed multiplication by planting into beds microtubers and *in vitro* plants. *Potato Research* **30**, 117-120

Wiltshire JJJ, Cobb AH (1996) A review of the physiology of potato tuber dormancy. *Annals of Applied Biology* **129**, 553-569

Wurr DCE, Fellows JR, Akehurst JM, Hambidge AJ, Lynn JR (2001) The effect of agricultural and environmental factors on potato seed tuber morphology and subsequent sprout and stem development. *Journal of Agricultural Science* **136**, 55-63

Xu X, vanLammeren AAM, Vermeer E, Vreugdenhil D (1998) The role of gibberellin, abscisic acid, and sucrose in the regulation of potato tuber formation *in vitro*. *Plant Physiology* **117**, 575-584

Zarrabeitia A, Lejarcegui X, Veramendi J, Mingo-Castel AM (1997) Influence of nitrogen supply on micropropagation and subsequent microtuberization of four potato cultivars. *American Potato Journal* **74**, 369-378

Zhang ZJ, Zhou WJ, Li HZ, Zhang GQ, Subrahmaniyan K, Yu JQ (2006) Effect of jasmonic acid on *in vitro* explant growth and microtuberization in potato. *Biologia Plantarum* **50**, 453-456