

Potato Microtubers as Research Tools: A Review¹

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ABSTRACT

Although *in vitro* production of potato tubers or microtuberization was achieved more than 40 years ago, the application of microtubers in reliable model research systems has been slow to develop. Several factors such as the use of growth regulators in microtuber induction and growth media, the mixotropic nature of the *in vitro* system, and cultivar-specific responses have led to interpretive difficulties. A cautionary note is also necessary in view of apparent growth and development differences, metabolic alterations, and somaclonal variation encountered in microtubers that may not be found in field-grown tubers. Evidence for strong and consistent analogies between microtubers and field-grown tubers for their induction, growth and development, and metabolism often is lacking. However, several components such as the rapid and near-synchronous induction and growth, which can be modified by a range of exogenous compounds or conditions, make the microtuber a valuable model system. Complex problems such as dormancy also appear to be particularly amenable to examination by the microtuber system. In addition, the use of microtubers as experimental research tools has potential in the areas of plant metabolism, germplasm selection and evaluation, genetic transformation, somatic hybridization, and molecular farming.

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RESUMEN

Aunque la producción *in vitro* de tubérculos de papa, o microtuberización, data de hace 40 años, la aplicación de microtubérculos en modelos confiables de sistemas de investigación ha tenido un lento desarrollo. Diversos factores, como el uso de reguladores y medios de crecimiento en la inducción de microtubérculos, la naturaleza mixotrófica del sistema *in vitro* y las respuestas de cultivares específicos han conducido a dificultades de interpretación. Una anotación preventiva es también necesaria en vista de las aparentes diferencias en el crecimiento y desarrollo, alteraciones metabólicas y variaciones somaclonales encontradas en los microtubérculos, que no se encuentran en los tubérculos que crecen en campo. La evidencia de fuertes y consistentes analogías para inducción, crecimiento y desarrollo y metabolismo entre microtubérculos y tubérculos que crecen en campo con frecuencia es defectuosa. Sin embargo, diversos componentes, como la rápida y cercana inducción y el crecimiento sincrónico, que pueden ser modificados por un rango de combinaciones y condiciones exógenas, hace de la microtuberización un modelo valioso para el sistema. Problemas complejos como la dormancia también aparentan estar particularmente sujetos a examen por el sistema de microtubérculos. Adicionalmente, el uso de microtubérculos como herramientas de investigación experimental tiene potencial en las áreas del metabolismo de plantas, evaluación y selección de germoplasma, transformación genética, hibridación somática y cultivo molecular.

INTRODUCTION

Potato (*Solanum tuberosum* L.) tuber production *in vitro* was first described as an experimental tool for examining tuberization and problems in potato pathology (Barker 1953; Mes and Menge 1954). Although *in vitro* tubers are occasionally referred to as "mini-tubers" produced *in vitro* (e.g., Abbott and Belcher 1986; Lewis *et al.* 1998) or "vitrotubers" (Mandolino *et al.* 1996), the accepted terminology supports the use of "microtubers" (Wattimena 1983), and this term will be used in the present review. While microtubers have become integrated into numerous potato research programs, physiochemical conditions *in vitro* that maximize microtuber production may not be appropriate for evaluating tuber induction or physiological events such as regulation of carbohydrate metabolism during tuber bulking, response to low temperatures, or the physiology of dormancy. Most studies have used complex media containing plant growth regulators that affect microtuber induction and dormancy. Consequently, inconsistent results have led to uncertainty regarding the validity of microtubers as model systems for tuber physiology research. The purpose of this review is to summarize the available information comparing microtubers with greenhouse-grown minitubers or field-grown tubers in order to examine the potential usefulness of microtubers as experimental tools. A

subsequent review (in preparation) will examine microtuber production and performance.

TUBER DORMANCY

Microtuber dormancy can be regarded as extending from tuber initiation or induction *in vitro* to the resumption of active and continuous tuber bud growth as noted by Burton (1957), Goodwin (1967), and Wiltshire and Cobb (1996) for field-grown tubers. However, it is unknown whether the mechanism of dormancy in microtubers is similar to field-grown tubers. Although microtuber dormancy duration has been positively and significantly correlated with dormancy duration in field-grown tubers for a limited number of cultivars (Leclerc *et al.* 1995), microtubers may exhibit a wide range of dormancy responses. Microtubers may be nondormant, or exhibit dormancy durations of up to 7 months depending on cultivar, and *in vitro* induction and growth environment (Hussey and Stacey 1984; Tovar *et al.* 1985; Estrada *et al.* 1986; Joyce and McCown 1987; Ortis-Montiel and Lozoya-Saldana 1987; Rosell *et al.* 1987; Harvey *et al.* 1991; Choi *et al.* 1994; Ranalli *et al.* 1994; Vecchio *et al.* 1994; Désiré *et al.* 1995a,b; Leclerc *et al.* 1995; Hoque *et al.* 1996). For example, when microtubers were induced in continuous darkness and then stored at 4 C, the average dormancy duration was 210 days. However, when microtubers were induced under an 8-h photoperiod, the average dormancy was 60 days for the same cultivar (Tovar *et al.* 1985; Estrada *et al.* 1986). Whether dormancy duration was measured from 50% tuber initiation or from the start of storage is unknown. The need for a consistent frame of reference for descriptions of tuber dormancy is evident, both in field-grown tubers (Cho *et al.* 1983) and microtubers. As noted previously, dormancy duration appears to extend from tuber initiation to sprouting while use of a harvest date is highly subjective and may have little or no physiological significance. On the other hand, the date of top pull or top kill may be very important in the regulation of tuber dormancy (Coleman and King 1984). This field event (i.e., top kill) has yet to be modelled and studied using microtubers.

Since variability in tuber dormancy duration due to growing conditions *in vitro* also has not been studied systematically, there are no well-defined protocols for consistent production of microtubers of known dormancy status for use in seed certification programs or as propagules for basic research into dormancy mechanisms. However, highly variable dormancies reported within one cultivar (e.g., Estrada *et al.* 1986) due to defined exogenous "triggers" available to the experimentalist suggest that the microtuber system may prove to be effective in unravelling hormonally based dormancy mechanisms.

Abscisic Acid

Initially considered a naturally occurring inhibitor of seed and bud dormancy (Lang 1996), endogenous ABA is found primarily in periderm tissues of potato tubers (Korableva et al. 1980). Abscisic acid concentrations were significantly correlated with dormancy duration in small (< 250 mg FW) and large (> 250 mg FW) microtubers (Leclerc et al. 1995). Small microtubers exhibited significantly longer dormancy duration than large microtubers. These differences in dormancy duration possibly were due to differences in microtuber age or surface-to-volume ratios (Leclerc et al. 1995). When exogenous fluridone (a carotenoid biosynthesis inhibitor that also inhibits ABA synthesis) or ABA were added to induction media, subsequent observations on the onset and maintenance of microtuber dormancy suggested that they required endogenous ABA (Suttle and Hultstrand 1994). While ABA may play a critical role in dormancy induction and maintenance, a direct and obvious link between dormancy loss and endogenous ABA levels has not been forthcoming in potato. In seed dormancy, there is evidence that dormancy release is related to the gradual depletion of ABA (Taiz and Zeiger 1998). In field-grown tubers, however, there is no clear relationship between tuber sprouting and endogenous levels of free ABA. For example, there was no evidence for a rapid decline in endogenous ABA below a threshold level for loss of dormancy at a storage temperature of 4 C (Coleman and King 1984) and similar observations were evident for greenhouse-grown microtubers stored at 3 C (Suttle 1995). However, other work has supported the concept of a depletion of endogenous ABA preceding dormancy release at 4 C (Cvikrová et al. 1994) or 20 C storage temperatures (Suttle 1995). Certainly all published studies support the decline of ABA levels after the resumption of rapid and continuous sprout growth. A subsequent study indicated that a rapid and reversible decline in ABA content caused by controlled atmospheres composed of altered nitrogen, oxygen and carbon dioxide levels preceded dormancy release in field-grown tubers and greenhouse-produced microtubers (Coleman 1998).

Cytokinins

Cytokinins can enhance microtuberization (Wang and Hu 1985) as well as modify tuber dormancy duration depending on cultivar (Wattimena 1983). High levels of IAA (56 µM) reduced microtuber dormancy duration in five cultivars when dormancy was measured from tuber initiation to sprouting (Wattimena 1983). However, when measured from harvest to sprouting, dormancy duration was only reduced in one (Russet Burbank) of the five cultivars. In field-grown tubers, exogenous cytokinins can break dormancy (Hemberg 1970) with greatest efficacy when applied near the end of the dormancy period (Turnbull and Hanke 1985a) as the concentration of endogenous cytokinins begins to increase (Turnbull and Hanke 1985b; Sukhova et al. 1993). Whether similar changes in apparent sensitivity to exogenous cytokinins is present in microtubers is currently unknown.

Gibberellins

Numerous studies indicate that exogenous GA enhances stolon formation, but is an effective inhibitor of microtuber induction (Palmer and Smith 1970; Garcia-Torres and Gomez-Campo 1973; Stallknecht and Farnsworth 1982a,b; Xu et al. 1998). Similar inhibition of tuber induction as well as reductions in tuber dry matter and altered tuber size distribution (i.e., smaller grades) occurred in field-grown tubers that had been treated at different times after planting with GA₃ (Struik et al. 1989). The use of ¹⁴C-GA₃ in a microtuberization medium indicated limited degradation of GA₃ after 3 wk of storage (Couillerot 1993) suggesting that GA₃ was relatively long lived in microtubers.

Treatments with promise for reducing microtuber dormancy duration have included cutting with or without a subsequent brief dip (5 min) in GA₃ (5 mg/L) solution (Ewing *et al.* 1987). The GA₃ dip was of variable effectiveness, possibly due to lack of effect on dormant tubers (Turnbull and Hanke 1985a) or the dosage used. Soaking whole microtubers for 1 h in 1 g/L GA₃ was suggested as being effective for breaking dormancy (Choi *et al.* 1994). In field-grown tubers, endogenous GAs increase during sprouting (Smith and Rappaport 1961). Exogenous GAs can promote tuber sprouting (van Istersum 1992) but usually only after dormancy is complete (Turnbull and Hanke 1985a; Couillerot 1993). GA₁ may be the active GA form in potatoes although its role in field-grown tuber or microtuber dormancy is unknown (Suttle, 1996). These discrepancies indicate that further work is necessary to characterize GA effects on dormancy duration compared to sprout growth. In addition, possible carry-over effects from GA₃ treatment of microtubers on the subsequent plant development (e.g., modified leaf morphology, aerial stolons, reduced yield) will have to be identified for individual cultivars.

Ethylene

Endogenous ethylene may be an important component of early dormancy development in microtubers (Suttle 1998). Whether endogenous ethylene plays a role in dormancy release of microtubers remains to be demonstrated. In field tubers and microtubers, ethylene production increased during dormancy inception and then decreased to low levels until tuber sprouting (Cvikrová *et al.* 1994; Suttle 1998). Previous work with field-grown tubers has shown repeatedly that exogenous ethylene can reduce tuber dormancy duration (Rosa 1925; Rylski *et al.* 1974; Alam *et al.* 1994). However, treatment of dormant field tubers with 2% (v/v) Ethrel (ethephon), an ethylene-release compound, led to prolonged dormancy (Sukhova *et al.* 1993) while treatment of microtubers with the ethylene antagonists, silver nitrate and 2,5-norbornadiene, led to precocious sprouting (Suttle 1998). Since ethylene is known to inhibit sprout growth (Rylski *et al.* 1974), "any evaluation of dormancy release mechanisms must include a study of sprout

elongation since we define the former developmental event in terms of the latter growth feature" (Coleman 1998). Microtubers may allow us to examine dormancy and sprout growth regulation as well as to study possible interactions between such plant hormones as ABA and ethylene (Suttle 1998).

Dormancy-Release Agents

A number of exogenous chemicals can remove dormancy from field-grown tubers (Coleman 1987; Wiltshire and Cobb 1996), but similar evaluations with microtubers have been limited. Rindite (7 parts ethylene chlorohydrin:3 parts dichloroethane: 1 part carbon tetrachloride) or carbon disulfide were effective dormancy-release agents for microtubers and field tubers (Wattimena 1983; Kim *et al.* 1996, 1997; Nasiruddin and Blake 1997). However, their mutagenicity, carcinogenicity, and high toxicity make their commercial use unacceptable. The use of BE vapor in conjunction with a carbon dioxide, oxygen and ethylene treatment was highly effective in dormancy release in microtubers and may provide a more environmentally-acceptable alternative for microtubers, greenhouse- and field-grown tubers (Coleman *et al.* 1992; Coleman 1998; Coleman and Coleman 2000).

The probable occurrence of multiple plant growth regulator controls in tuber dormancy make any simple interpretation unlikely. Any experimental system used to address this regulator problem will have to possess superior handling and manipulation characteristics for the examination of hormonal interactions (e.g., ABA/KIN ratio effects), timing of application or concentration effects. Since microtubers can be induced rapidly in a relatively synchronous manner under defined conditions in a short period of time and in large numbers, they should provide a suitable experimental system for closely examining the problem of tuber dormancy. The value of using a microtuber induction system for exploring the roles of dormancy release agents as well as endogenous and exogenous plant growth regulators has been demonstrated (Suttle and Hulstrand 1994; Suttle 1995, 1996, 1998; Xu *et al.* 1998) and should allow additional insight into the complexities of potato tuber dormancy.

MICROTUBER METABOLISM

Researchers have been quick to develop research systems that use microtubers for the examination of plant metabolism, ranging from pigment biosynthesis in tubers (Lewis *et al.* 1998) to tuber protein gene expression (Bourque *et al.* 1987; Musín and Troffits 1988; Ulloa *et al.* 1997). The use of microtubers to investigate carbohydrate metabolism associated with tuber formation and growth is an excellent example of this application.

Carbohydrates

Studies of carbohydrate metabolism in tubers have benefited from the use of microtuber systems since sucrose was demonstrated to be an obligatory external carbon substrate for microtuber induction and development (Mes and Menge 1954; Leclerc *et al.* 1994). Also, developing microtubers have a capacity to rapidly accumulate sucrose and reducing sugars when stored at 2 C (Classen *et al.* 1992). Such results suggest that a microtuber experimental system could prove useful to explore metabolic events surrounding low-temperature induction of reducing sugars, a phenomenon with significant ramifications for potato processors. Similarly, high sucrose concentrations in conjunction with fluridone promote rooting of nodal stem segments cultured on microtuber induction media (Harvey *et al.* 1994). This observation may be the basis of another promising experimental system that should be examined in our attempts to unravel sugar/ ABA interactions on organogenesis.

The apparent regulation of starch synthesis by sucrose during tuberization, possibly through the control of orthophosphate or triose phosphate concentrations (Taiz and Zeiger 1998), may be another research area where micro-tubers are a superior experimental tool compared with field tubers. For example, starch appears in the induced axillary buds within 72 h of tuber induction on a sucrose-containing medium (Pevalek-Kozlina and Berljak 1997). In field-grown tubers, sucrose induced the expression of tuber-specific genes, and this induction was suppressed by GA3 (Park 1990). In addition, application of G~ to field-grown tubers increased reducing sugar levels, possibly by reducing starch synthesis through a reduction in ADP-glucose pyrophosphorylase activity (Mares *et al.* 1981). When sucrose was applied to cut slices of growing field tubers, most of the carbon was incorporated into starch during the initial 24-h period (Geiger *et al.* 1998). The authors concluded that sucrose stimulated the expression of ADP-glucose pyrophosphorylase. Whether a similar role for sucrose in the promotion of starch production is evident in conjunction with GA modulation remains to be demonstrated in microtubers. However, we know that sucrose can serve as an osmotic agent (Khuri and Moorby 1995) as well as a regulator of endogenous GA levels (possibly through increased GA conjugation) in developing tubers from cuttings (Simko 1994) and microtubers (Xu *et al.* 1998).

In a microtuber system, Kim *et al.* (1997) concluded that the parallels between sucrose and starch contents and sucrose synthase activity supported the importance of this enzyme in starch biosynthesis. Similarly, high sucrose levels in the culture medium appeared to increase sucrose synthase in potato tissue cultures (Sowokinos and Varns 1992). Whether microtuber size can be altered by a modified sucrose metabolism due to the expression of cytosolic or apoplasmic invertase as found in tubers remains to be determined (Sonnewald *et al.* 1997). The apparent interactions between GAs and sucrose during tuber initiation and growth as well as starch synthesis suggest that the use of the microtuber system

should assist in the further characterization of carbohydrate metabolism in the context of plant growth regulators and their effects on microtuber growth.

As a cautionary note, there is conflicting evidence for possible differences in carbohydrate metabolism between field-grown tubers and microtubers. A report by Veramendi *et al.* (1999) concluded that microtubers were "an adequate model system for studying primary carbohydrate metabolism. In addition, a study of temperature effects on starch synthesis and composition in microtubers suggested that these features are similar to field-grown tubers (Debon *et al.* 1998). However, in the latter study, the modal diameter of starch granules from microtubers was considerably smaller (8-9 μm) than those found in field-produced tubers (21 μm). Although starch granule size may be a function of tuber size (Christensen and Madsen 1996), starch granules from microtubers increased in size with incubation temperature. This result was contrary to temperature effects on granule size in tubers produced from potted plants under controlled environmental conditions. The authors (Debon *et al.* 1998) hypothesized that this discrepancy was caused by "the artificial nature of the microtuber system, although it is difficult to conceive how this could be so." Similarly, in an earlier study it was found that the activity of a primer-independent phosphorylase could be modulated by exogenous sucrose and had greater activity in microtubers than field-grown tubers (Moreno and Tandecarz 1996). Since starch deposition is related to photosynthetic patterns, the mixotrophic nature of the cultures may play a key, although as yet undefined, role. These studies indicate that any future metabolic studies should include, during the initial stage of the study, a comparative evaluation with field-grown tubers in order to confirm the suitability of the microtuber analogy. Intrinsic physiological responses may be evaluated best in growth-regulator-free culture media (Garner and Blake 1989) since agronomic events may be assessed best when *in vitro* environments mimic the major components (light, temperature, moisture, inorganic nutrients) of field conditions (Gopal and Minocha 1997, 1998; Gopal *et al.* 1997).

GENETIC TRANSFORMATIONS AND MOLECULAR FARMING

In addition to contributing to germplasm selection and evaluation (Gopal and Minocha, 1997, 1998), potato microtubers have provided a valuable addition to the collection of tools for studies of genetic transformation (e.g., Choi *et al.* 1997; Sandhu *et al.* 1998; Tu *et al.* 1998; Choi *et al.* 1999; Inui *et al.* 1999). The successful development of transgenic potato plants has been achieved primarily by the use of disabled strains of *Agrobacterium tumefaciens*. This transformation was followed by regeneration of plants from *in-vitro*-grown microtubers (Ishida *et al.* 1989). In addition to providing the widely accepted advantages of sterility and ease of storage and transport (Kumar *et al.* 1995), the regenerated plants exhibited lower levels of somaclonal variation compared with regenerated plants derived

from protoplasts (Snyder and Belknap 1993). Snyder and Belknap (1993) used this approach to increase resistance to blackspot bruise in the cultivars Lemhi and Russet Burbank, by the introduction of a tyrosine- rich protein gene that appeared to reduce the levels of free tyrosine.

The recent extension of this approach from *Solanum tuberosum* to five wild *Solanum* species (Kumar *et al.* 1995) should allow access to unique genes and eventual development of effective somatic hybridization systems. The successful use of *Agrobacterium* and regeneration from microtuber slices for transgenic potato plant production may find broader applications as genetic transformation methods become more intensively used for agricultural research. Recent research areas include the characterization and control of tuberization (Perl *et al.* 1991; Visser *et al.* 1994; Galis *et al.* 1995; Ivana *et al.* 1997), starch granule synthesis (Smith *et al.* 1996), enhanced protein quality (Davies 1996) and export and storage of sugars (Stitt and Sonnewald 1995).

Transgenic potato plants have also served as experimental vehicles for the demonstration of the synthesis of foreign carbohydrates. For example, by introducing microbial fructosyltransferase genes, microtubers were produced that accumulated the foreign carbohydrate fructan (Meer *et al.* 1994). Similarly, after introducing trehalose synthesis enzymes from *Escherichia coli* and inhibiting trehalase activity with validamycin A, considerable trehalose accumulated in the microtubers of the transformed plants (Goddijn *et al.* 1997).

In addition to microtubers serving as a source for transgenic plants, the highly synchronous nature of tuberization *in vitro* has served as the basis for visualizing differential gene expression during microtuber development using an AFLP technique for RNA fingerprinting (Bachem *et al.* 1996). The study isolated two tuber-specific, transcript-derived fragments coding for the lipoxygenase enzyme and suggested that developmentally regulated genes could be identified by this technique. These studies indicate that microtuber systems may provide considerable flexibility for exploring the genetic, physiological and biochemical components of microtuber initiation and growth as well as the production of unique or foreign biochemicals.

However, unintended growth and development changes, metabolic alterations, or somaclonal variation during or after culturing *in vitro* can lead to complications in interpretation. For example, Romanov *et al.* (1998) failed to obtain a clear correlation between tuber formation and yield in field-grown tubers and microtubers. Similarly, high (8%) sucrose levels can activate class I B33 patatin promoter in tissues where it normally is not expressed (Rocha-Sosa *et al.* 1989; Perl *et al.* 1991). Mandolino *et al.* (1996) discovered different variants in "vitrotubers" (microtubers) derived from MS-induction medium containing Ia N when they used a RAPD fingerprinting approach. The authors concluded that the observed changes were caused by culturing *in vitro* in the presence of plant-gro

regulators. This somaclonal variability has been observed repeatedly from the morphological to the DNA level (Larkin and Scowcroft 1981; Ball 1987; Rietveld *et al.* 1991, 1993). Consequently, the potential impact of culturing *in vitro* should be considered before any conclusions are drawn from the microtuber system.

Perhaps the most challenging future role for microtubers will focus on their potential use for the large-scale production of proteins for industrial, pharmaceutical, and agricultural use. "Molecular farming" has become a reality in a range of plant species such as tobacco, canola, and maize. The potato plant has entered this high-value area of biotechnology with the development of edible vaccines for such diseases as diabetes and hepatitis (e.g., Mason and Arntzen 1995; Featherstone 1996; Ma *et al.* 1997; Tacket *et al.* 1998; Arakawa *et al.* 1999). The ease of use of microtubers for producing transgenic potatoes and as mass-produced, optimally aged, and soluble protein sources make potato potentially one of the most suitable, cost-effective molecular farming hosts.

CONCLUSIONS

Microtubers grown in an aseptic manner on defined nutrient media in a controlled environment are an attractive model for biochemical and physiological studies of conventional and transformed potatoes. Their synchronous and rapid induction in response to a range of exogenous "triggers," their small size, structure comparable to field tubers, protein, and starch composition are important characteristics of the model system. Studies of microtuber dormancy once appeared to consist of a confusing plethora of culture-specific observations. With the use of microtuber systems, the confusion can now be resolved into a well-defined set of experimental questions. At a practical level, dormancy issues that restricted the use of microtubers for field planting in nuclear seed stock programs are being overcome (Jones 1988; Coleman and Coleman 2000). However, a cautionary note is necessary in view of apparent growth and development changes, metabolic alterations and somaclonal variation mentioned earlier. Often evidence for a strong and consistent analogy for induction, growth and metabolism between microtubers and field-grown tubers is lacking. The lack of a close analogy has led to artifacts that preclude generalized conclusions being applied to all potato tubers, regardless of source. Similar concerns in other *in vitro* systems have been expressed, e.g., the control of antioxidative mechanisms in *Nicotiana tabacum* L. (Yun *et al.* 1998). These studies support the need for caution in interpreting results that may reflect system-dependent artifacts. Nevertheless, during the 21st century, microtubers may emerge as the propagule of choice in the nuclear production phase of the seed potato industry and as an essential research tool in a wide range of tuber-related studies.

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LITERATURE CITED

- Abbott, A.J. and A.R. Belcher. 1986. Potato tuber formation *in vitro*. *In*: Withers, L.A, and P.G. Alderson (eds), Plant Tissue Culture and Agricultural Applications. Butterworths, London. pp. 113-122.
- Alam, S.M.M., D.P. Murr, and L. Kristof. 1994. The effect of ethylene and of inhibitors of protein and nucleic acid syntheses on dormancy break and subsequent sprout growth. *Potato Res* 37:25-33.
- Arakawa, T., J. Yu, and W.H. Langridge. 1999. Food plant-delivered cholera toxin B subunit for vaccination and immunotolerization. *Adv Exp Med Biol* 464:161-178.
- Bachem, C. W.B., R.S. van der Hoeven, S.M. de Bruijn, D. Vreugdenhil, M. Zabeau, R.G.F. Visser, R.S. Van der Hoeven, and S.M. De Bruijn. 1996. Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: analysis of gene expression during potato tuber development. *Plant J* 9:745- 753.
- Ball, S.G. 1987. Molecular genetics of somaclonal variations. *In*: Bajaj, Y.P.S. (ed), *Biotechnology in Agriculture and in Forestry*. Springer-Verlag, Berlin. pp. 165-169.
- Barker, W.G. 1953. A method for the *in vitro* culturing of potato tubers. *Science* 118:384-385.
- Bourque, J.E., J.C. Miller, and W.D. Park. 1987. Use of an *in vitro* tuberization system to study tuber protein gene expression. *In Vitro Cell Develop Biol* 23:381-386.
- Burton, W.G. 1957. The dormancy and sprouting of potatoes. *Food Sci Abstr* 29:1-12.
- Cho, J.L., W.M. Iritalú, and W.M. Martjn. 1983. Comparison of methods for measuring dormancy of potatoes. *Am Potato J* 60:169-177.

- Choi, Y.W., J.L. Cho, and L.S. Kim. 1994. Studies on rapid *in vitro* multiplication of potato (*Solanum tuberosum* L.) microtubers and their practical use. m. Dormancy of microtubers. J Kor Soc Hort Sci 35:213-219.
- Choi, K.H., J.H. Jeon, H.S. Kim, Y.H. Joung, H. Joung, and Y.P. Lim. 1997. Genetic transformation of intact potato microtuber by particle bombardment. Korean J Plant Tissue Culture 24:87-91.
- Choi, K.H., J.H. Jeon, H.S. Kim, Y.H. Joung, H. Joung, K.H. Choi, J.H. Lee, H.S. Kim, Y.H. Joung, and H. Joung. 1999. Stability of transgenic potato plants and their progenies expressing herbicide resistant gene. J Korean Soc Hort Sci 40:31-34.
- Christensen, D.H. and M.H. Madsen. 1996. Changes in potato starch quality during growth. Potato Res 39:43-50.
- Classen, P.A.M., M.H. van Ca1ker, and J. Marinus. 1992. Accumulation of sugars in microtubers of potato node cuttings (cv. Kennebec) during cold storage. Potato Res 35:191-194.
- Coleman, W.K. 1987. Dormancy release in potato tubers: a review. Am Potato J 64:57-68.
- Coleman, W.K. 1998. Carbon dioxide, oxygen and ethylene effects on potato tuber dormancy release and sprout growth. Ann Bot 82:21-27.
- Coleman, W.K. and S.E. Coleman. 2000. Modification of potato microtuber dormancy during induction and growth *in vitro* or *ex vitro*. Am J Potato Res 77:103-110.
- Coleman, W.K., G. Hawkins, J. McInemey, and M. Goddard. 1992. Development of a dormancy release technology: a review. Am Potato J 69:437-445.
- Coleman, W.K. and R.R. King. 1984. Changes in endogenous abscisic acid, soluble sugars and proline levels during tuber dormancy in *Solanum tuberosum* L. Am Potato J 61:437-449.
- Couillerot, J.P. 1993. Effects and distribution of 14 C-gibberellic acid after application to stored potato microtubers. Phyton 54:67-73.
- Čoičková, M., L.S. Sukhova, J. Eder, and N.P. Korobleva. 1994. Possible involvement of abscisic acid, ethylene and phenolic acids in potato tuber dormancy. Plant Physiol Biochem 32:685-691.
- Davies, H. V. 1996. Recent developments in our knowledge of potato transgenic biology. Potato Res 39:411-427.

- Debon, S.J.J., R.F. Tester, S. Millam, and H.V. Davies. 1998. Effect of temperature on the synthesis, composition and physical properties of potato microtuber starch. *J Sci Food Agric* 76:599-607.
- Désiré, S., J-P. Couillerot, and J. Vasseur. 1995a. Dormance et germination des microtubercules de pomme de terre (*Solanum tuberosum* L.) produits *in vitro*: effets de la concentration en saccharose du milieu de tuberisation, de la durée de conservation à 4°C et d'un traitement avec de l'acide gibberellique. *Acta Bot Gallica* 142:371-378.
- Désiré, S., J-P. Couillerot, and J. Vasseur. 1995b. Germination en serre des microtubercules de pomme de terre (*Solanum tuberosum* L.) produits *in vitro*: influence du diamètre, de la densité de plantation et de l'âge des microtubercules sur le rendement. *Acta Bot Gallica* 142:379-387.
- Estrada, R., P. Tovar, J.H. Dodds. 1986. Induction of *in vitro* tuber in a broad range of potato genotypes. *Plant Cell Tiss Org Cult* 7:3-10.
- Ewing, L.L., S.E. McMurry, and E.E. Ewing. 1987. Cutting as a method of breaking dormancy in microtubers produced *in vitro*. *Am Potato J* 64:329-332.
- Featherstone, C. 1996. Vaccines by agriculture. *Mol Med Today* 2:278-281.
- Galis, I., J. Macas, J. Vlasak, M. Ondrej, and H.A. Van Onckelen. 1995. The effect of an elevated cytokinin level using the *ipt* gene and N⁶-benzyladenine on single node and intact potato plant tuberization *in vitro*. *J Plant Growth Regul* 14:143-150.
- García-Torres, L. and C. Gómez-Campo. 1973. *In vitro* tuberization of potato sprouts as affected by ethrel and gibberellic acid. *Potato Res* 16:73-79.
- Garner, N. and J. Blake. 1989. The induction and growth of potato microtuber *in vitro* on media free of growth regulating substances. *Australian Bot* 63:663-674.
- Geiger, M., M. Stitt, and P. Geigenberger. 1998. Metabolism in slices from growing potato tubers responds differently to addition of sucrose and glucose. *Planta* 206:234-244.
- Goddijn, O.J.M., T.C. Verwoerd, E. Voogd, R.W.H.H. Krutwagen, P.T.H.M. de Graaf, J. Poels, K van Dun, A.S. Ponstein, B. Dammann, and J. Pen. 1997. Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. *Plant Physiol* 113:181-190.
- Goodwin, P.B. 1967. The control of branch growth on potato tubers. I. Anatomy of buds in relation to dormancy and correlative inhibition. *J Exp Bot* 18:78-99.

- Gopal, J. and J.L. Minocha. 1997. Effectiveness of selection at microtuber crop level in potato. *Plant Breeding* 116:293-295.
- Gopal, J. and J.L. Minocha, 1998. Effectiveness of *in vitro* selection for agronomic character in potato. *Euphytica* 103:67-74.
- Gopal, J., J.L. Minocha, and J.S. Sidhu. 1997. Comparative performance of potato crops raised from microtuber15 induced in the dark versus microtuber15 induced in light. *Potato Res* 40:407-412.
- Harvey, B.M.R., S.H. Crothel15, N.E. Evans, and C. Selby. 1991. The use of growth retardants to improve microtuber formation by potato (*Solanum tuberosum*). *Plant Cell Tiss Org Cult* 27:59-64.
- Harvey, B.M.R., G. Boeden, C. Reavey, and C. Selby. 1994. Stimulation of *in vitro* root and shoot growth of potato by increasing sucrose concentration in the presence of fluridone, an inhibitor of abscisic acid synthesis. *Plant Cell Tiss Org Cult* 37:271-276.
- Hemberg, T. 1970. The action of some cytokinins on the rest-period and the content of acid growth-inhibiting substances in potato. *Physiol Plant* 23:850-858.
- Hoque, M.I., N.B. Mila, Md.S. Khan, and R.H. Sarker. 1996. Shoot regeneration and *in vitro* microtuber formation in potato (*Solanum tuberosum* L.) Bangladesh J Bot 25:87-93.
- Hussey, G. and N.J. Stacey. 1984. Factors affecting the formation of *in vitro* tubel15 of potato (*Solanum tuberosum* L.). *Aru Bot* 53:565- 578.
- Inui, H., Y. Ueyama, N. Shiota, Y. Ohkawa, and H. Ohkawa. 1999. Herbicide metabolism and cross-tolerance in transgenic potato plants expressing human CYP1A1. *Pesticide Biochem and Physiol* 64:33-46.
- Ishida, B.K, G.W. Snyder, and W.R. Belknap. 1989. The use of *in vitro*- grown microtuber disks in *Agrobacterium-mediated* transformation of Russet Burbank and Lemhi Russet potatoes. *Plant Cell Rep* 8:325-328.
- Ivana, M., S. Lidiya, O. Milos, Z. Oksana, K. Tatyana, E. Josef, O. Jaroslava, G. Svetlana, R. Yuri, and A. Nina. 1997. Growth pattern, tuber formation and hormonal balance *in vitro* potato plants carrying *ipt* gene. *J Plant Growth Regul* 21:27-36.
- Jones, E.D. 1988. A current assessment of *in vitro* culture and other rapid multiplication methods in North America and Europe. *Am Potato J* 65:209-220.

- Joyce, P.J. and B.H. McCown. 1987. A system for producing uniform microtubers. *Am Potato J* 64:445 (Abst).
- Khuri, S. and J. Moorby. 1995. Investigations into the role of sucrose in potato cv. Estima microtuber production *in vitro*. *Ann Bot* 75:295-303.
- Kim, H.S., J.H. Jeon, K.H. Choi, Y.H. Joung, B. Y. Lee, H. Joung, H.S. Kim, J.H. Jeon, K.H. Choi, Y.H. Joung, B.Y. Lee, and H. Joung. 1997. Changes of starch and sugar contents and activity of sucrose synthase during microtuberization. *J Kor Soc Hort Sci* 38:211-215.
- Kim, S.Y., J.C. Jeong, J.K Kim, and M.S. Lim. 1996. Effect of chemical treatments for the dormancy breaking of *in vitro* microtubers of *Solanum tuberosum* L. cv. Dejima. *J Kor Soc Hort Sci* 37:19-23.
- Korablev~ N.P., R.A. Karavaeva, and I.V. Metlitskii. 1980. Changes of abscisic acid content in potato tuber tissues in the period of deep dormancy and during germination. *Fiziol Rast* 27: 585-591.
- Kumar, A., M. Miller, P. Whitty, J. Lyon, and P. Davie. 1995. *Agrobacterium* mediated transformation of five wild *Solanum* species using *in vitro* microtubers. *Plant Cell Rep* 14:324-328
- Lang, G.A. (ed.). 1996. Plant dormancy: Physiology, biochemistry and molecular biology. CAB International, Wallingford, Oxon, UK 386 pp.
- Larkin, P.J. and W.R. Scowcroft. 1981. Somaclonal variations .a novel source of variability from cell cultures for plant improvement. *Theor Appl Genet* 60:197-214.
- Leclerc, Y., D. Donnelly, and J.E.A. Seabrook. 1994. Microtuberization .of layered shoots and nodal cuttings of potato: The influence of growth regulators and incubation periods: *Plant Cell Tiss Org Cult* 37:113-120.
- Leclerc, Y., D.J. Donnelly, W.K. Coleman, and R.R. King. 1995. Microtuber dormancy in three potato cultivars. *Am Potato J* 72:215-223.
- Lewis, C.E., J.R.L. Walker, J.E. Lancaster, and A.J. Conner. 1998. Light regulation of anthocyanin, flavonoid and phenolic acid biosynthesis in potato microtubers *in vitro*. *Aust J Plant Physiol* 25:915- 922.
- Ma, S.W., D.L. Zhao, Z.Q. Yin, R. Mukherjee, B. Singh, H.Y. Qin, C.R. Stiller, and A.M. Jevnikar. 1997. Transgenic plants expressing autoantigens fed to mice to induce oral immune tolerance. *Nat Med* 3:793-796.
- Mandolino, G., S. de Marco, V. Faeti, M. Bagatta, A. Carboni, and P.

- Ranalli. 1996. Stability of fingerprints of *Solanum tuberosum* plants derived from conventional tubers and microtubers. *Breeding* 115:439-444.
- Mares, D.J., H. Marschner, and A. Krauss. 1981. Effect of gibberellic acid 31\nd carbohydrate metabolism of developing tubers of potato (*Solanum tuberosum*). *Physiol Plant* 52:267- 274.
- Mason, H.S. and C.J.. 1995. Transgenic plants as vaccine production systems. *Trends Biotechnol*13:388-392.
- Meer, I.M. van der, M.J.M. Ebskan1p, R.G.F. Visser, P.J. Weibeek, and S.C.M. Smeekens. 1994. Fructan as a new carbohydrate sink in transgenic potato plants. *The Plant Cell* 6:561-570.
- Mes, M.G. 1954. Potato shoot and tuber cultures *in vitro*. *Physiol Plant* 7:637- 649.
- Moreno, S. and J.S. Tanderarz. 1996. Analysis of primer independent phosphorylase activity in potato plants: high levels of activity in sink organs and sucrose-dependent activity in cultured stem explants. *Cell Mol Biol* 42:637-643.
- Musin, S.M. and L.N. Trofimets. 1988. Use of microtuber proteins in identifying genotypes (of varieties, hybrids 311d lines) of potatoes *in vitro*. *Doklady Vsesoyuznoi Akadelnii* 7:23- 26.
- Nasiruddin, KM. and J. Blake. 1997. Effect of Rindite on storage behavior, dormancy break 311d sprout growth of potato microtubers (cv. Désirée). *A111 PotatD J* 74:325-330.
- Ortis-Montiel, G. 311d H. Lozoya-Sald311a. 1987. Potato minitubers: technology validation in Mexico. *And Potato J* 64:535-544.
- Palrner, C.E. and O.E. Snilitll. 1970. Effect of kinetin on tuber formation on isolated stolons of *Solanum tubelusum* L. cultured *in vitro*. *Plant Cell Physiol* 11:303-314.
- Park, W.D. 1990. Molecular approaches to tuberization in potato. *In: Vayda, M.E. 311d W.D. Park (eds), The Molecular 311d Celular Biology ofthe Potato. C.AB. Intemational, Wallingford, CT. pp. 43-56.*
- Perl, A, D. Aviv, L. Willnlitzer, and E. Galun. 1991. In vitro tuberization in tr311sgenic potatoes harboring B-glucuronidase linked to a patatin promoter: Effect of sucrose levels 311d photoperiods. *Plant Sci* 73:87-95.

- Pevalek-Kozlina, B. and J. Berljak. 1997. St31.ch accumulation as a marker for microtuberization in potato (*Solanum tuberosum*). *Biologia-Bratislava* 52:553-559.
- Ranalli, P., M. Bizarri, L. Borghi, 311d M. Mari. 1994. Genotypic influence on *in vitro* induction, dormancy length, advancing age and agronomical performance of potato microtubers (*Solanum tuberosum* L.). *Ann Appl Biol* 125:161-172.
- Rietveld, R.C., R.A. Bressan, and P.M. Hasegawa. 1991. Somaclonal variations tuber derived populations of potato. I. Evidence of genetic stability across tuber generations and diverse locations. *Theor Appl Genet* 82:430-440.
- Rietveld, R.C., R.A. Bressan, and P.M. Hasegawa. 1993. Somaclonal variations in tuber derived populations of potato. II. Differential effect of genotype. *Theor Appl Genet* 87:305-313.
- Rocha-Sosa, M., U. Sunnewald, W. Frollmeyer, M. Stratnlarul, J. Schell, and L. Willmitzer. 1989. Both developmental and metabolic signals activate the expression of a class 1 patatin gene. *EMBO J* 8:23-29.
- Romanov, G.A., T.N. Konstantinova, L.I. Sergeeva, S.A. Golyanovskaya, J. Kossmann, L. Willmitzer, T. Schmlulling, and N.P. Aksenova. 1998. Morphology and tuber formation of *in-vitro-grown* potato plants harboring the yeast invertase gene and/or the rol gene. *Plant Cell Rep* 18:315-324.
- Rosa, J. T. 1925. Shortening the rest period of potatoes with ethylene gas. *Potato Assoc America. Potato News Bull* 2:363-365.
- Rosell, G., F.G. de Bertoldi, and R. Tizio. 1987. *In vitro* mass tuberization as a contribution to potato micropropagation. *Potato Res* ;m: 111- 116.
- Rylski, I., L. Rappaport, 311d H.K. Pratl 1974. Dual effects of ethylene on potato tuber dormancy and sprout growth. *PI311t Physiol*53:G58-6G2.
- Sandhu, J.S., C.I. Webster, and J.C. Gray. 1998. Alr1 sequences act as quantitative enhancers of gene expression in transgenic tobacco and potato plants. *Plant Mol Biol*37:885-896.
- Simko, I. 1994. Sucrose application causes hormonal changes associated with potato tuber induction. *J. Plant Growth Regul* 13:73-77.
- Smith, O.E. and L. Rappaport. 1961. Endogenous gibberellins in resting and sprouting potato tubers. *Adv in Chem Ser* 28:42-48.

- Smith, A.M., K Denyer, and C. Martin. 1996. The synthesis of the starch granule. *Annu Rev Plant Physiol Plant Mol Biol* 48:67-87.
- Snyder, G. W. and W.R. Belknap. 1993. A modified method for routine *Agrobacterium-mediated* transformation of *in vitro* grown potato microtubers. *Plant Cell Rep* 12:324- 327.
- Sonnewald, U., M.R. Hajirezaei, J. Kossman, A. Heyer, R.N. and L. Willmitzer. 1997. Increased potato tuber size resulting from apoplastic expression of a yeast invertase. *Nature Biotech* 15:794-797.
- Sowokinos, J.R. and J.L Varns. 1992. Induction of sucrose synthase in potato tissue culture: Effect of carbon source and metabolic regulators on sink strength. *J. Plant Physiol* 139:672-679.
- Stallknecht, G.F. and S. Farnsworth. 1982a. General characteristics of coumarin-induced tuberization of axillary shoots of *Solanum tuberosum* L cultured *in vitro*. *Am Potato J* 59: 17 -32
- Stallknecht, G.F. and S. Farnsworth. 1982b. The effect of the inhibitors of protein and nucleic acid synthesis on the coumarin-induced tuberization and growth of excised axillary shoots of potato sprouts (*Solanum tuberosum* L) cultured *in vitro*. *Am Potato J* 59:69-75.
- Stitt, M. and U. Sonnewald. 1995. Regulation of metabolism in transgenic plants. *Annu Rev Plant Physiol Plant Mol Biol* 46:341-368.
- Struik, P.C., G. Kramer, and N.P. Smit. 1989. Effects of soil applications of gibberellic acid on the yield and quality of tubers of *Solanum tuberosum* L. cv. Bintje. *Potato Res* 32:203- 208.
- Sukhova, L.S., I. Machackova, J. Eder, N.D. Bibik, and N.P. Kovaleva. 1993. Changes in levels of free IAA and cytokinins in potato tubers during dormancy and sprouting. *Biol Plant* 35:387-391.
- Suttle, J.C. 1995. Postharvest changes in endogenous ABA levels and ABA metabolism in relation to dormancy in potato tubers. *Physiol Plant* 95:233-240.
- Suttle, J.C. 1996. Dormancy in tuberous organs: Problems and perspectives. pp. 133-143. *In*: Lang, G.A.(ed), *Plant Dormancy: Physiology, Biochemistry and Molecular Biology*. CAB International London.
- Suttle, J.C. 1998. Involvement of ethylene in potato microtuber dormancy. *Plant Physiol* 118:843-848.

- Suttle, J.C. and J.F. Hultstrand. 1994. Role of endogenous abscisic acid in potato microtuber dormancy. *Plant Physiology* 105:891-896.
- Tackett, C.O., H.S. Mason, G. Losonsky, J.D. Clements, M.M. Levine, and C.J. Amtzen. 1998. Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. *Nat Med* 4:607-609.
- Taiz, L. and E. Zeiger. 1998. *Plant physiology*. 2nd edn. Sinauer Associates, Sunderland, MA.
- Tovar, P., R. Estrada, L. Schilde-Rentschler, and J.H. Dodds. 1985. Induction of in vitro potato tubers. CIP Circular 13: 1-4, International Potato Centre, Lima, Peru.
- Tu, H.M., L.W. Godfrey, and S.S.M. Sun. 1998. Expression of the Brazil nut methionine-rich protein and mutants with increased methionine in transgenic potato. *Plant Mol Biol* 37:829-838.
- Tumbull, C.G.N. and D.E. Hanke. 1985a. 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.
- Tumbull, C.G.N. and D.E. Hanke. 1985b. The control of bud dormancy in potato tubers: Measurement of the seasonal pattern of changing concentrations of zeatin-cytokinins. *Planta* 165:366-376.
- Ulloa, R.M., G. C. McIntosh, M. Melchiorre, A.N. Mentaberry, P. Dallari, D.N. Moriconi, and M. T. Tellez-Inon. 1997. Protein kinase activity in different stages of potato (*Solanum tuberosum* L.) microtuberization. *Plant Cell Rep* 16:426-430.
- Van Ittersum, M.K. 1992. Dormancy and growth vigour of seed potatoes. Ph.D. thesis, Wageningen Agricultural University. 187 pp.
- Vecchio, V., L. Andrenelli, M.T. Pagano, and S. Benedetti. 1994. Influence of photoperiod and media culture on potato microtuber production and dormancy. *Potato Res* 37:440.
- Veramendi, J., L. Willnitzer, and R.N. Trethewey. 1999. *In vitro* grown potato microtubers are a suitable system for the study of primary carbohydrate metabolism. *Plant Physiol Biochem* 37:693-697.
- Visser, R.G.F., D. Vreugdenhil, T. Hendriks, and E. Jacobsen. 1994. Gene expression and carbohydrate content during stolon to tuber transition in potatoes. *Physiol Plant* 90:285-292.

Wang, P. and C. Hu. 1985. Potato tissue culture and its applications in agriculture. pp 503-577. *In*: P.H.U, ed. Potato physiology. New York: Academic Press.

Wattimena, G.A. 1983. Micropropagation as an alternative technology for potato production in Indonesia. Ph.D. thesis. Univ Wisconsin-Madison. 202 pp.