Plant Cell Reports (1998) 17: 787–790

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Effect of physiological age of mother tuber and number of subcultures on in vitro tuberisation of potato (*Solanum tuberosum* L.)

Received: 8 July 1996 / Revision received: 28 March 1998 / Accepted: 28 March 1998

Abstract The physiological age of mother tubers (*Solanum tuberosum* L. cv. Kennebec) used as a source of material influenced kinetin-induced in vitro tuberisation. Tuberisation significantly increased with physiological age. Kinetin- or ancymidol-induced tuberisation, plantlet and microtuber dry weight decreased with increasing number of subcultures. Single-node segments obtained from tubers stored for more than 9.5 months at 4 °C showed increased kinetin-induced tuberisation rates and earlier tuberisation than those obtained from younger tubers. For any physiological age, material may be safely multiplied using node propagation until the third subculture and bioassayed for tuberisation without variation in the response.

Key words Physiological age · Potato · Tuberisation

Abbreviations *PA* Physiological age

Introduction

Potato tuberisation is a complex developmental process known to be influenced by genetic, environmental and physiological variables. Available evidence indicates that the physiological age (PA) of the mother tuber, photoperiod, temperature, irradiance and nitrogen fertilisation act on tuberisation, either directly or indirectly mediating changes in hormone concentrations (Van der Zaag and Van Loon 1987; Vreugdenhil and Struik 1989; Burton 1989; Ewing 1990; Ewing and Struik 1992).

Communicated by N. Amrhein

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The influence of PA of mother tubers (Van der Zaag and Van Loon 1987; Ewing and Struik 1992) on tuberisation of whole plants was demonstrated by Madec and Perennec (1959). They showed that tuberisation was induced by both the top of the plant and by the mother tuber and that the effect of both parts was additive. There is also evidence for the importance of sprout PA on tuberisation (Okazawa 1959). Tuber PA depends on chronological age and storage conditions (Burton 1989; Ewing and Struik 1992).

While there are studies on the effect of the above-mentioned factors on in vitro tuberisation (Ewing and Struik 1992), knowledge on the influence of PA is lacking. Etiolated single-node segments derived from old tubers spontaneously formed in vitro tubers in the absence of plant hormones, the rate of tuberisation increasing with tuber age (Koda and Okazawa 1983), which is in agreement with the fact that, under field conditions, older tubers tuberise earlier (Van der Zaag and Van Loon 1987).

The tuberisation bioassay using in vitro stolons in the dark is excellent in terms of specificity and sensitivity but, unfortunately, it greatly depends on the availability of tubers of a defined PA. Preliminary experiments in our laboratory (unpublished results) indicated that tubers of various PAs may produce misleading results. It would therefore be desirable to develop a tuberisation system less dependent on the PA of mother tubers. One possibility would be to use plant material previously multiplied in vitro under long days. The question then arises as to the extent, if any, of a lingering effect of PA on tuberisation throughout repetitive in vitro subculturing.

To meaningfully compare tuberisation results obtained in different laboratories using tubers of different PAs or unevenly subcultured in-vitro-grown material, we need to examine the effect of the PA of the mother tuber as well as the effect of the number of subcultures performed before the induction of tuberisation. We undertook this task using two different tuberisation inducers: kinetin and ancymidol.

Materials and methods

Plant material

Freshly harvested potato tubers (*Solanum tuberosum* L. cv. Kennebec) were stored at 4 °C in the dark for at least 5 months (August–December). We labelled the tubers used after 5, 8, 9.5 and 11 months as of PAs I, II, III and IV, respectively.

The protocol for tuber sprouting and culture initiation was performed as previously described (Mingo-Castel et al. 1991) with the following modifications: potatoes were surface-sterilized with a 1% (vol/vol) NaClO, 0.1% (vol/vol) Tween-20 solution for 5 min, and rinsed with sterile water.

Tissue plugs, 2 cm in diameter and 2.5 cm thick, bearing a single sprouting bud, were excised by means of a cork borer and placed in a plastic tray containing moistened vermiculite and sprayed with 27.7 mM $(NO_3)_2Ca \cdot 4H_2O$. Trays were kept at 20 °C in the dark and watered with the $(NO_3)_2Ca \cdot 4H_2O$ solution as needed. After 4–5 weeks, etiolated shoots were obtained.

Single-node sprout sections were aseptically cultured in Murashige and Skoog basal salts and vitamins supplemented with 2% (wt/vol) sucrose and 0.8% (wt/vol) Difco Bacto-agar. The pH was adjusted to 5.7. Cultures were maintained at 20 °C under 16 h photoperiod and a 70 $\mu E \cdot m^{-2} \cdot s^{-1}$ photon fluence rate for 3–5 weeks to allow plantlet growth.

Micropropagation

Single-node sections from plantlets were excised, removing the leaf to ensure uniform growth of developing shoots. Sections were aseptically cultured as described above, and allowed to form new plantlets (first subculture). A total of five subcultures were performed. Single-node sections from first, third and fifth subcultures were used for tuberisation assays.

Tuberisation

Intact single-node segments were cultured in Murashige and Skoog basal salts and vitamins, 6% (wt/vol) sucrose, 0.8% (wt/vol) Difco Bacto-agar, with either 11.6 μM kinetin or 20 μM ancymidol as tuberisation inducers. A control medium without tuber-inducing growth regulator was also prepared. Thus, the effect of the PA of the mother tuber on three different tuberising systems was investigated. The medium was adjusted to pH 5.7. Treatment cultures were kept at 20 °C under 8 h photoperiod for 4 weeks. At the end of the 4th week, the rate of tuberisation was calculated as (number of tuberised plantlets/total number of emerged plantlets)×100. Tuberisation was scored whenever an incipient tuber appeared either directly on an axillary bud or on the side or apex of a stolon or plantlet. Fresh and dry weights were also measured. The number of biological units used to compute the different means are indicated under each figure.

Analysis of variance (ANOVA) and the Fisher LSD test, P=0.05, were applied to the data to look for significant differences between means. Error bars on figures are mean standard errors of the biological units used in each case.

Results

Effect of mother tuber PA on tuberisation

The general trend was increased tuberisation (tuberisation percentage after 4 weeks in culture) with ageing. A maximum tuberisation rate of 80% was achieved with kinetin for the oldest PA. IV (11 months of storage at 4 °C) (Fig. 1).

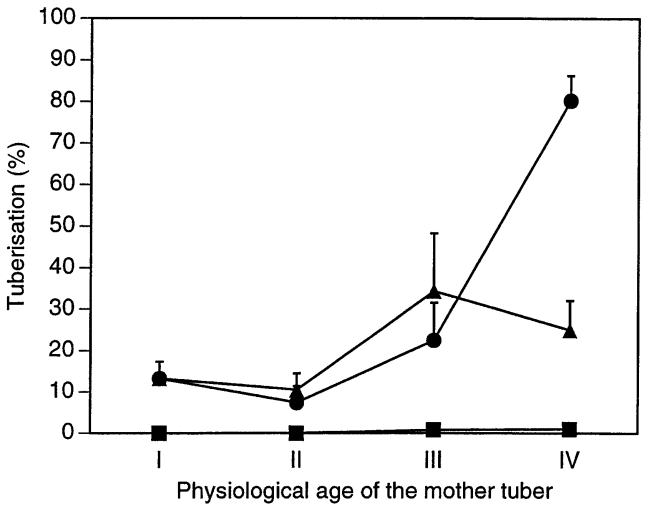

Fig. 1 In vitro tuberisation for four different PAs of mother tubers [control (■), kinetin (●), ancymidol (▲)]. Tuberisation values are means (±SE) obtained from three sets of explants produced after one, three and five subcultures. Each set had 24 explants and there were two replicated sets

Significant differences were found between PA IV and the others (5, 8 and 9.5 months of storage at 4 °C). Despite the slight increase in tuberisation with ageing, no significant differences were observed using ancymidol as an inducer.

Effect of number of subcultures on tuberisation

Tuberisation rates (mean values of results obtained for all PAs) showed a tendency to decrease when the material was obtained after a greater number of subcultures (Fig. 2). Significant differences were found using kinetin as an inducer, between tuberisation in subcultures 1 and 5 for PA I and between 1 and 3, and 1 and 5 for PA III (Fig. 2). A decreasing tuberisation trend was also found with ancymidol. Significant differences were observed between tuberisation in subcultures 3 and 5 for PA II, between 1 and 3, and 1 and 5 for PA III, and between 1, 3 and 5 for PA IV.

Plantlet and microtuber dry weight

Plantlets cultured in the presence of kinetin were always heavier than those in ancymidol (Fig. 3). A continuous decrease in plantlet dry weight was observed in material obtained after a greater number of subcultures, both with kinetin and ancymidol (Fig. 3). Significant differences were found between plantlets of subcultures 1 and 3, 1 and 5, and 3 and 5 in both treatments.

Microtubers developed in the presence of kinetin or ancymidol had similar dry weights (Fig. 4). Tuber weight decreased with increasing number of subcultures, both with kinetin and ancymidol. Significant differences were obtained between tubers of subcultures 1, 3 and 5 for kinetin, and subcultures 1 and 3 and 1 and 5 for ancymidol.

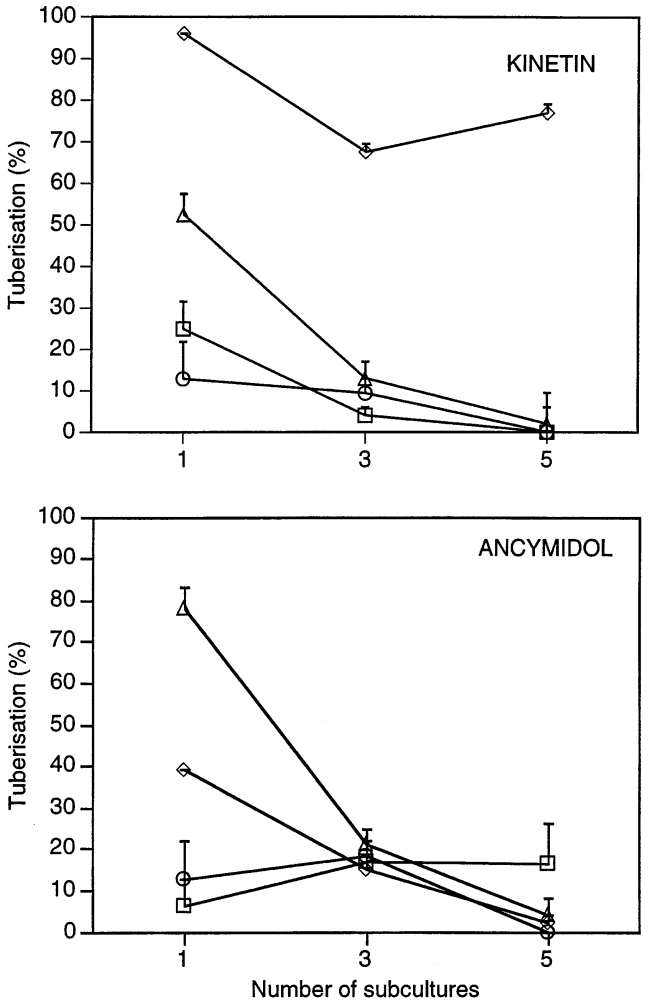

Fig. 2 Effect of number of subcultures on kinetin- or ancymidol-induced in vitro tuberisation for four different PAs of mother tubers: I (□), II (○), III (△) and IV (◇). Tuberisation values are means of two sets of 24 explants

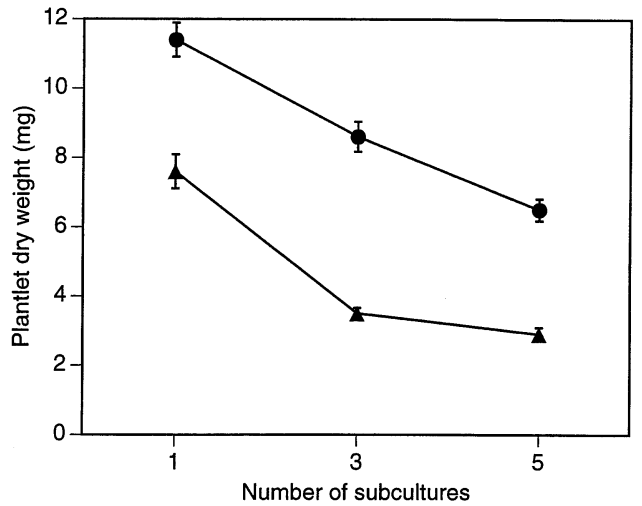

Fig. 3 Effect of number of subcultures on in vitro plantlet dry weight (● medium with kinetin, ▲ medium with ancymidol). Weight values are means (±SE) obtained from four sets of different PAs of 20 plantlets each, with two replications

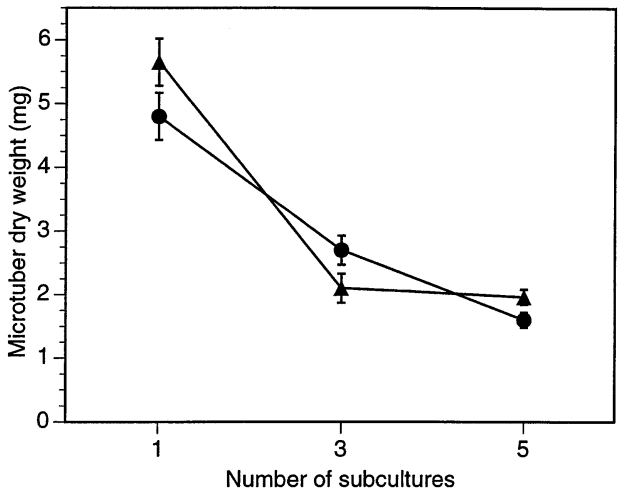

Fig. 4 Effect of number of subcultures on kinetin- (●) or ancymidol-induced (▲) in vitro microtuber dry weight. Weight values are means obtained from four sets of different PAs. The number of kinetin-induced microtubers weighed after 1, 2 and 3 subcultures was 62, 47 amd 35, respectively. The number of ancymidol-induced microtubers weighted after 1, 2 and 3 subcultures was 46, 37 and 11, respectively

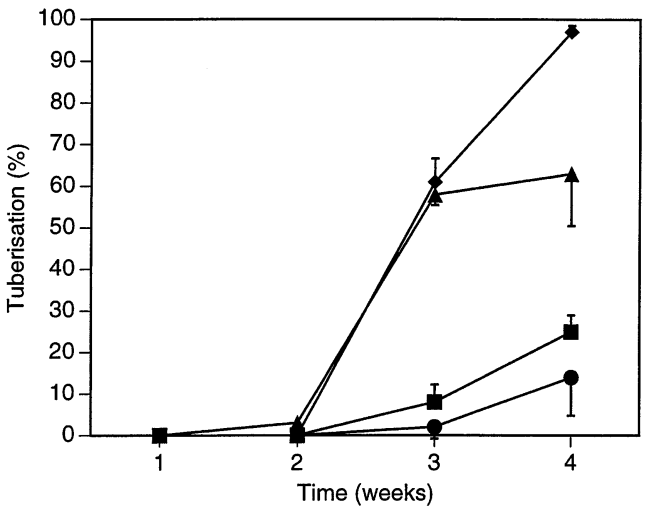

Fig. 5 Time course experiment of kinetin-induced in vitro tuberisation for four PAs of mother tubers: I (■), II (●), III (▲) and IV (◆). Values are averages of two replications of 24 plants each (±SE), from the first subculture

Earliness in tuberisation

Earliness of tuberisation was studied using material obtained after the first subculture and kinetin as inducer. PA influenced not only the final tuberisation but also the timing of the process. There were no differences on kinetin-induced tuberisation rate for the four ages analysed during the first 2 weeks of culture (Fig. 5). However, at the 3rd week, tuberisation increased to 60% for PA III and IV, while remaining very low for PA I and II. That is, tuberisation in older tubers was 1 week earlier than in younger tubers.

Discussion

The two growth regulators assayed, kinetin and ancymidol, showed an inducing effect on in vitro tuberisation. Gibberellin inhibitors such as paclobutrazol, CCC and ancymidol have also been used to accelerate in vitro tuberisation (Harvey et al. 1991; Nasiruddin and Blake 1991; Simko 1994).

In our in vitro experiments, tuberisation was promoted by increasing PA of the mother tubers. Single-node segments obtained from tubers stored for more than 9.5 months at 4 °C showed increased tuberisation rates and earlier tuberisation than those obtained from younger tubers. This behaviour of cv. Kennebec is in agreement with the experiments of Koda and Okazawa (1988) who found that the rate of tuberisation of single-node segments increased with the age of cv. Irish Cobbler tubers. In field experiments, early tuberisation with advancing age was reported by Madec and Perennec (1959). Physiologically old tubers contained higher levels of reducing sugars from sprouting onwards (Caldiz et al. 1986).

Van Ittersum et al. (1990) classified cv. Kennebec as a low-rate variety in terms of physiological ageing. An even greater effect of PA on in vitro tuberisation may be expected for cultivars with a higher rate of physiological ageing (e.g. cv. Jaerla).

Our results show that the number of subcultures had an adverse effect on plantlet vigour. Plantlet dry matter in tuberisation medium continuously decreased with increasing number of subcultures. The tuberisation rate also diminished in material obtained after more subcultures, regardless of the PA of mother tubers. Moreover, microtuber dry weight was lower with an increasing number of subcultures. It may be argued that the lower tuberisation with more subcultures is due to vigour loss as a consequence of shoot culture in a suboptimal medium. This does not seem to be the case, however, because the growth rate through successive subcultures steadly decreased in the presence of tuberisation. Alternatively, the tuberising stimulus originally present in the mother tuber (Bodlaender and Marinus 1969; Claver 1971) is progressively being diluted with ongoing subcultures.

From our results it follows that, for any PA, we can safely multiply the material using single-node propagation until the third subculture and use the cultures at this stage in tuberisation assays without expecting any changes in the results.

Thus, for reliable data in tuberisation experiments, it is necessary to take into account both the PA of mother tubers and the number of subcultures performed with the plant material after in vitro establishment.

Acknowledgements We are indebted to CICYT (Spain) for supporting this research through grant AGF92-0453 and EU (BIO2 CT93 0400).

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Note added in proof. For any physiological age kinetin-induced tuberisation is stable for 3 or more subcultures.