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Inductive and noninductive conditions on in vitro tuberisation and microtuber dormancy in potato (*Solanum tuberosum* subspecies *tuberosum* and subspecies *andigena*)

V. VECCHIO[1], S. BENEDETTELLI[1], LUISA ANDRENELLI[1], E. PALCHETTI[1] and L. ESPEN[2]

[1]Dip. di Scienze Agronomiche e Gestione del Territorio Agroforestale, Piazzale delle Cascine 18, 50144 Firenze, Italy
[2]Dip. di Fisiologia delle Piante Coltivate e Chimica Agraria, Via Celoria 2, 20132 Milano, Italy

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Summary

The effect of growth conditions (medium and photoperiod) on in vitro tuberisation and microtuber dormancy of the cv. Désirée (*Solanum tuberosum* subspecies *tuberosum*) and Imilla Negra (*Solanum tuberosum* subspecies *andigena*) was evaluated.
The short photoperiod and presence of CCC in the medium reduced the dry matter of plants as well as the size of microtubers. The two cultivars showed a difference, in favour of Désirée, in the percentage of plants having more than one tuber and in the average time of sprouting after storage. The average time of sprouting was more rapidly obtained under long days and in a medium without CCC. The CCC and short days caused the same dormancy conditions and the same K^+ quantity in the microtubers. The microtubers mineral composition is involved in the dormancy status.

Introduction

Potato plants are readily propagated in vitro (Goodwin et al., 1980; Espinoza et al., 1986; Ahloowalia, 1988) and when culture is carried out under suitable growth conditions of medium and photoperiod, potato plants produce microtubers (diameter range from 2–10 mm). These represent a suitable material both to be introduced into the tuber seed production system and also for germplasm exchange (van der Zaag, 1990; Estrada et al., 1986; Vecchio et al., 1991; Haverkort et al., 1991; Desiré et al., 1995).

Microtuber induction was promoted by the addition of BAP and CCC in combination with 8% sucrose (Tovar et al., 1985; Estrada et al., 1986) in MS medium (Murashige & Skoog, 1962). Sucrose is an important source of carbon and this concentration represents a favourable osmolarity for microtuber development (Khuri & Moorby, 1995).

CCC concentrations of 500–1000 mg l^{-1} in the medium promoted tuberisation both in short and long photoperiodic conditions; in these conditions, the effect of BAP in tuberisation promotion was reinforced (Hussey & Stacey, 1984). The addition of gibberellins to the medium inhibited tuberisation (Okazawa, 1960; Tizio, 1971; Koda & Okazawa, 1983), while the effect of CCC as an antigibberellic factor, compared

with other growth regulators, is limited (Cheng & Zhang, 1990). In noninductive photoperiodic conditions, CCC anticipated and increased in vitro tuberisation (Vecchio et al., 1994; Andrenelli, 1996), whereas the use of CCC in unrecalcitrant cultivars inhibited tuberisation (Harvey et al., 1991; Andrenelli, 1996). Other results showed that the absence of growth regulators, medium enrichment with 8% sucrose and plantlet transfer in short photoperiods are conditions that promote in vitro tuberisation (Garner & Blake, 1989).

The use of CCC on field crops led to increased (Radwan et al., 1971) or unincreased yield (Sekhon & Singh, 1985), and to a decrease in average tuber weight (Rex, 1992). Observed discrepancies in in vitro tuberisation carried out with or without growth regulators resulted from different genotype behaviour (Vecchio et al., 1994, 1997) and from different growing conditions including medium amount, container shape, number of nodal cuttings per container and light intensity.

Short photoperiod is considered inductive for potato tuberisation (Gregory, 1956), but results of Hussey & Stacey (1981) showed positive effects of long photoperiod on tuberisation. Increase in light hours, both in the presence and absence of kinetin, reduced the percentage of tuberisation (Pelacho & Mingo-Castel, 1991).

Solanum tuberosum subspecies *andigena* has a critical photoperiod of tuberisation that is shorter than *Solanum tuberosum* subspecies *tuberosum*, and has also shown low tuberisation in noninductive conditions of photoperiod as well as in the presence of tuberisation promoters (Pelacho et al., 1993).

Dormancy break in microtubers is under the control of hormonal mechanisms as well as enzymatic activity and studies carried out on this subject have often led to contrasting results. Nevertheless, there is a threshold of endogenous ABA that, in relation to the length and storage modality of microtubers, can reduce the dormancy period (Suttle, 1995).

The research on two cultivars, Désirée and Imilla Negra reported here studied the effect of photoperiod and medium conditions on growth of in vitro plants, tuberisation, chemical composition of the produced microtubers and their level of dormancy after storage.

Materials and methods

Nodal cuttings of cvs Désirée (*Solanum tuberosum* subspecies *tuberosum*) and Imilla Negra (*Solanum tuberosum* subspecies *andigena*) from plants obtained by meristematic culture, were used to evaluate the effect of two culture media, modified MS (Murashige & Skoog, 1962) (MS whole strength + 8% Sucrose + 2 g l^{-1} Phytagel – SIGMA P-8169) and MSC (MS whole strength + 500 mg l^{-1} CCC [(2-chloroethyl)trimethylammonium chloride] + 8% Sucrose + 2 g l^{-1} Phytagel) and also the effect of two photoperiods, short days of 8 light hours (SD) and long days of 16 light hours (LD).

The experimental unit was a 250 ml Magenta GA-7 vessel (SIGMA V 8505) containing 25 ml of medium and six nodal cuttings. Ten replications for each treatment were made. The experiment lasted 85 days and was performed in a

controlled environment cabinet with light intensity of 70 μmol m^{-2} sec^{-1} and with a temperature of 18 and 24 °C, respectively during the dark and light periods. Plant length, number of nodes per plant, percentage of plants with more than one tuber, number and size of tubers (diameter and average weight) and dry weight of plants after 85 days were scored.

The microtubers produced were stored for 20 and 60 days at 4 °C and then placed in sprouting conditions (26 °C, 90% RH, 70 μmol m^{-2} sec^{-1} of light intensity) to evaluate dormancy both as a percentage and the average time of sprouting. Microtubers were considered sprouted when their sprouts were 5 mm long.

Chemical analysis of K^+, Ca^{2+}, Mg^{2+} and Na^+ content was determined on stored microtubers, following the methods of Cocucci & Morgutti (1986).

Analysis of variance was carried out on the factorial experiment for the in vitro culture data with three factors: cultivar, medium and photoperiod, each with two levels. Storage time factor was added to first three factors in the analysis of variance for data referring to the sprouting trial. To verify the null hypothesis the fixed model was adopted.

Results

The analysis of variance (Table 1) showed that the factors studied significantly influenced some growth parameters of plants and tubers.

Plant growth parameters. Plantlet length was not significantly different between cultivars, while the long photoperiod and presence of CCC in the culture medium caused a decrease in plantlet size (Table 1). The interaction photoperiod per medium ($P<0.05$) showed that decrease in plant length was more marked under LD in combination with CCC, while the interaction photoperiod per cultivar ($P<0.01$) showed an increase only in cv. Imilla Negra. The number of nodes per plant increased in Imilla Negra and also in the presence of CCC in the medium (Table 1). Second order interactions of this parameter were significant (Table 1); CCC caused an increase in node number in Imilla Negra, while cv. Désirée showed a decrease under SD; the combination of SD and CCC led to an increase in node number. Dry weight of plants, which expressed the response to the growth conditions better than other parameters, showed a reduction in the presence of CCC in the medium: this effect was more evident in LD than in SD (Fig. 1A). Désirée showed a greater reduction in dry weight of plants in the presence of CCC than Imilla Negra (Fig. 1B).

Microtuber production. Tuber number per plant was not significantly affected by the different treatments. But plants with tubers were about 65% of the total plants, with 5 microtubers per vessel; also the two cultivars were different in the percentage of plants producing more than one tuber (Table 1). Short days and MS medium increased the percentage of plants with more than one tuber; by contrast, the CCC in the culture medium decreased this parameter (Table 1). The weight of microtubers was lower in the medium with CCC (Table 1).

Table 1. Effect of cultivar, photoperiod and culture medium on plant and microtuber parameters.

Source of variation	Plant length (cm)	Nodes per plant (no.)	Plants with more than 1 tuber (%)	Tuber weight (mg)	Tuber diameter (mm)
Cultivar (C)					
Désirée	5.2	11	15.9	150	4.2
Imilla Negra	5.6	13	8.5	187	5.2
Significance level	ns	**	**	ns	**
Photoperiod (P)					
Long (16 h)	4.6	12	10.3	190	4.8
Short (8 h)	6.2	12	14.2	150	4.5
Significance level	**	ns	ns	ns	ns
Medium (M)					
MS	6.1	11	16.0	202	5.0
MSC	4.7	13	8.4	134	4.5
Significance level	**	**	**	*	*
Interaction:					
C×P	**	**	ns	ns	ns
C×M	ns	**	ns	*	ns
P×M	*	**	*	ns	ns

ns: not significant differences; *, **: significant difference at $P<0.05$ and $P<0.01$ levels respectively.

Mineral composition of microtubers. Analysis of K^+ and Ca^{2+} contents (Table 2) on microtubers showed that the K^+ content was higher in Imilla Negra than in Désirée in both photoperiodic conditions and also in MS medium; addition of CCC to the medium had a greater effect on Désirée, in which the K^+ content increased twofold as compared with microtubers from MS medium. The Ca^{2+} content of Imilla Negra tubers was also higher than in Désirée. Growth regulators increased the Ca^{2+} content in both cultivars. Also LD caused an increase in Ca^{2+} with both cultivars. The Na^+ and Mg^{2+} contents showed similar responses respectively to those of K^+ and Ca^{2+} with regard to cultivar and CCC in the medium. Photoperiod did not affect Mg^{2+}.

Dormancy of microtubers. The average time of sprouting was significantly influenced by genotype, photoperiodic conditions and culture medium. Also the interaction between photoperiod and medium was significant. Microtubers produced under LD showed a sprouting percentage higher than 70% (Fig. 2). Furthermore, microtubers produced in MS medium and under LD showed rapid sprouting, whereas under SD sprouting was delayed and was similar to that in presence of CCC (Fig. 3A). Désirée showed quicker formation of sprouts on microtubers (Fig. 3B). Storage period did not affect sprouting.

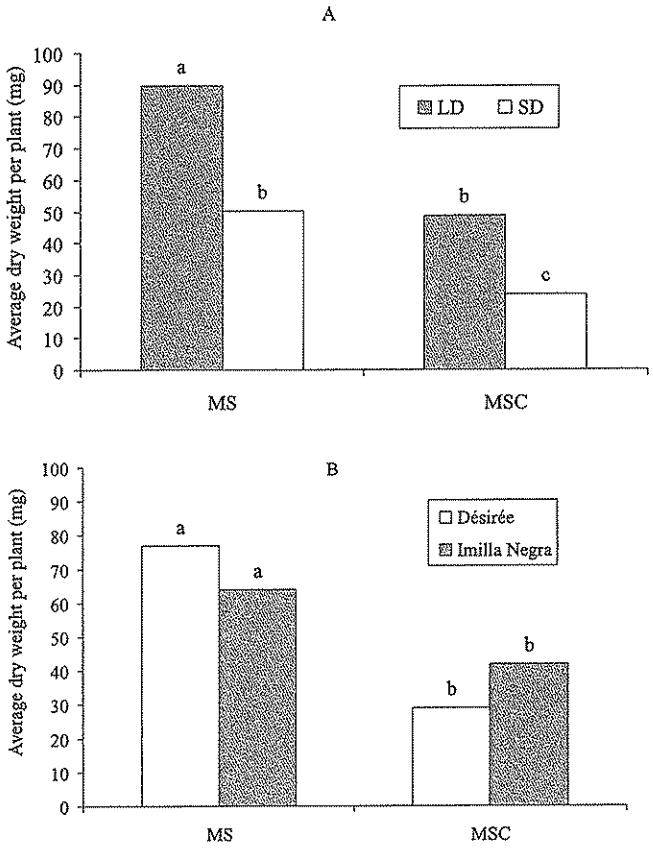

Fig. 1. Interaction of long days and short days (LD, SD) and medium (MS, MSC) (A) and interaction of cultivar (Désirée, Imilla Negra) and medium (MS, MSC) (B) on the dry weight of plants. Different letters show significant differences tested by the Bonferroni method.

Table 2. Chemical analysis of microtubers (no. mol/g of fresh weight).

Treatments	Imilla Negra				Désirée			
	K	Ca	Na	Mg	K	Ca	Na	Mg
Long days								
MS	124	6.6	4.2	5.5	65	4.2	5.8	2.6
MSC	139	10.6	33.9	7.1	142	9.8	14.8	7.3
Short days								
MS	154	2.1	10.5	5.5	64	1.3	5.8	4.0
MSC	110	2.8	7.0	5.0	124	2.4	21.8	7.5

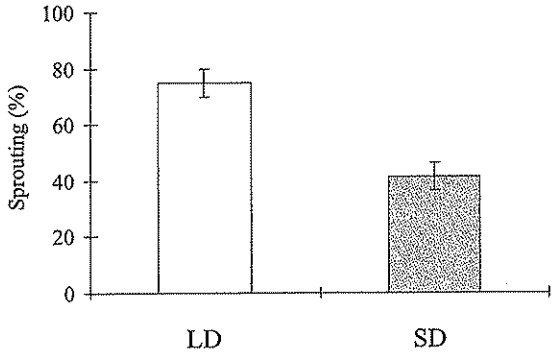

Fig. 2. Average effect of long days and short days (LD, SD) on percentage sprouting. Bars show standard errors.

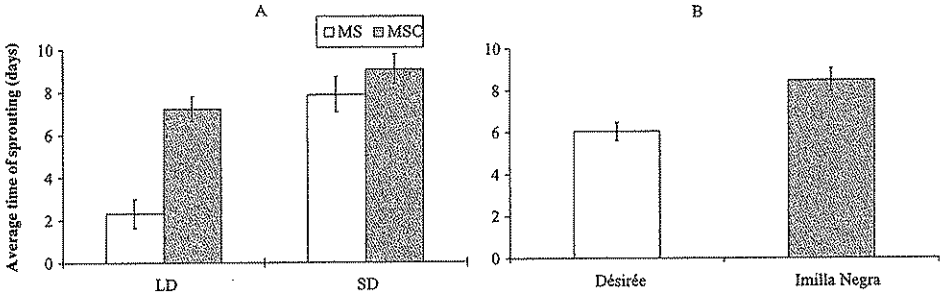

Fig. 3. Interaction of long days and short days (LD, SD) and medium (MS, MSC) (A) and effect of cultivar (Désirée, Imilla Negra) (B) on the average time of sprouting. Bars show standard errors.

Discussion

Results of this research confirmed that growth parameters and some tuberisation variables are controlled by both genotype and growth conditions and their interaction.

CCC in the medium reduced the internode length as found by Hussey & Stacey (1984). In both cultivars this effect contributed to dry matter reduction under short and long photoperiod in combination with CCC. This demonstrated that the negative effect of short photoperiod had been reinforced by CCC added to the medium. CCC affected microtuber size more than number, and it is probable that CCC, as an antigibberellic complex, could have caused early senescence of plantlets, so decreasing potential microtubers enlargement. This has been already observed both in vitro (Hussey & Stacey, 1984; Vecchio et al., 1997) and in vivo (Rex, 1992), while Harvey et al. (1992) reported that paclobutazol, an inhibitor of gibberellin synthesis,

incorporated in the tuberisation medium did not reduce microtuber fresh weight.

The colour of microtubers produced in this experiment was green or clear red in Désirée and nearly black in Imilla Negra; microtubers were long in Désirée and round in Imilla Negra.

The large differences in the K^+ and Ca^{2+} content of tubers of the two cultivars, and the large effects of our experimental conditions on these parameters, suggested that the control of in vitro tuberisation and dormancy breaking may be linked to mineral nutrition. For example microtubers with high Ca^{2+} content (Table 2), produced in a long photoperiod and in both media, showed the highest sprouting percentage and the quickest average time of sprouting. The high amount of K^+ found in Désirée in the presence of CCC both in long and short photoperiods is linked with a reduced size of microtubers and extended dormancy.

The lack of a positive effect of short photoperiod on tuberisation induction found in both cultivars in this trial may be explained by the high Ca^{2+} content of microtubers produced under long days. This hypothesis is in agreement with the stimulus effect of Ca^{2+} on tuberisation as previously reported (Balamani et al., 1986; MacIntosh et al., 1996).

The highest percentage of microtubers sprouting in long photoperiodic conditions not only confirmed earlier reports (Tovar et al., 1985; Estrada et al., 1986; Gopal et al., 1997), but also highlighted some effects linked with the chemical composition of microtubers. CCC in the medium created the same dormancy conditions and the same amount of K^+ in the microtubers as produced in a short photoperiod. These conditions highlighted greater differences between cultivars with regard to microtuber dormancy than the formation of microtubers. This suggested that different physiological and biochemical mechanisms are responsible for the in vitro tuberisation and dormancy breaking. Therefore, a different relationship between the gibberellic and antigibberellic complexes may be responsible for these two phenomena (Krauss & Marschner, 1982; Suttle, 1995).

Our results are in partial agreement with indications given by Estrada et al. (1986), who noted a lengthened dormancy period when microtubers are produced in the dark, and a reduced time of sprouting with long photoperiodic conditions during the inductive phase.

These results have practical implications (Gopal et al., 1997), and especially with regard to the production of microtubers with different levels of dormancy, their use and the subsequent crop performance.

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