IN VITRO TUBERIZATION IN SOME POTATO CULTIVARS (CHRISTIAN, ROCLAS, OSTARA AND DÉSIRÉE)

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Abstract.

Four potato cultivars Christian, Roclas, Ostara and Désirée , were studied for in vitro tuberization. Cultivar Christian was the most receptive to in vitro tuber induction. The best results were obtained on culture media to which kinetin(0.5 mg/l) or benzyladenine (1mg/l and 0.5mg/l), plus α-naphtylacetic acide (0.5 mg/l) were added. The December/February period was found to be the most favorable time for in vitro tuberization. Of the four cultivars studied: Christian, Roclas, Ostara and Désirée, cultivar Christian was the most receptive to charcoal and phytohormones added to the culture media (C_2, C_3 and C_5).

Key words: tuberization, cultivars, *in vitro*, regeneration, tuber induction, Christian, Roclas, Ostara, Désirée, phytohormones, acclimatization, multiplication, micropropagation

INTRODUCTION

In vitro tuberization aims at obtaining seed tubers that can be used e.g. for in vitro multiplication, micropropagation, selection and conservation of valuable cultivars (Agud, E., et al., 2008; Agud, E., et al., 2009 et al.; and Agud, E., 2009). This process is influenced by several factors: photoperiod, temperature, season, endogenous hormones and last but not least, the nature and concentration of sugar, mineral salts and phytohormones in the culture medium. In vitro tuberization also depends on the nature of cultivars (Cachiţa-Cosma, D., and M. Zăpârţan, 1991; Butiuc, Keul, A., et al. 1997-1998; Baciu, A., 2008; Pătru, D. M., D. Cachiţă, 2005). Wang and Hu reported on in vitro mass tuberization and virus-free potato production in Taiwan. They established that the in vitro mass tuber production was optimal when the incubation was carried aut at 20^{o}C and the culture medium contained sucrose (8%) and BAP (10mg/l). Over a period of 4 months, about 36,000 dormand minitubers were harvested from the 20-m^2 area of an aseptically incubated container. (Wang, P.J. Hu, C.Y. 1982).

Kodo et al. found that, under in vitro conditions, salicylic acid in 10^{-5}M concentration exhibited a potato tuber-inducing effect (Kodo, Y., et al., 1992). Ahloowalia presented an integrated system of potato micropropagation for minituber seed production (Ahloowalia, B. S., 1990). Minicultures were obtained from stable cultivars cloned on the revised medium of Murashige and Skoog and micropropagated serially (Murashige, T., and Skoog, A., 1962). There were no significant differences between the

productivity performances of minitubers and standard tubers or between the numbers of tubers. The zeatin, coumarin and chlorocholine chloride (CCC) showed that the in vitro tuberization mainly depended on the nature of cultivars, while the effects of coumarin and CCC were season-dependent (Zăpârțan, M., 1992; Zăpârțan, M., et al., 1989).

MATERIALS AND METHODS

For the experiments, minicuttings formed of 1-2 nodes were used. They were cultured in vitro on a basal medium consisting of macroelements, trace elements, mineral salts and FeEDTA as used in the revised medium of Murashige and Skoog, vitamins (thiamine HCl, pyridoxine HCl and nicotinic acid, 1 mg/l each), and meso-inositol, 100mg/l, sucrose-30g/l, agar- 6g/l, pH- 5,7. The basal medium (BM) were used for preparing 5 media by addition of charcoal or pfytohormones: benzyladenine(BA), α-naphtylacetic acide(NAA), kinetin (K), and gibberellic acid (GA_3). The media dad the following composition:

M = MS1/2 (MS in which the concentration of mineral salts was reduced to half)

C_1 = BM1/2 + charcoal – 5mg/l

C_2 = BM + BA-1mg/l + NAA – 0.5 mg/l + GA_3 – 0.1mg/l

C_3 = BM + BA -0.5mg/l + NAA – 0.5 mg/l

C_4 = BM + K/ 0.5mg/l + NAA – 0.5 mg/l

The minicutting cultured on these media were examined periodically for assessing the evolution of culture and determining the number of minitubers/flask and the number and length of the shoot and root neoformations. Four potato cultivars (Christian, Roclas, Ostara and Désirée) were cultured in vitro in a growth chamber (temperature: 25^{o}C; illumination intensity; 2000lx for 8 hours/day).

RESULTS AND DISCUSSION

Details the minitubers formed *in vitro* in the four potato cultivars studied and the tuberization are prezented in Table 1. One can see from this table that, of the cultivars studied, cultivar Christian was most receptive to the charcoal and hormonal mixtures added to the culture media: it had the highest regenerative capacity and exhibited a nearly steady in vitro evolution. On media C_2 – C_4, cultivar *Christian* produced 18-20 shoots (9-12 cm long) and 10-15 roots (4.5 – 8.0 cm long). In this cultivar, 5-8 minitubers/flask appeared on media C_1 – C_3, whereas the minitubers were most numerous on medium with K + NAA (C_4). It should be emphasized that evolution of this cultivar was good even on medium with MS1/2 + charcoal (Fig. 1).

Table 1

In vitro tuberization in four potato cultivars

Cultivar	Me - dium	No. length	Length (cm)	No. root	Length Root(cm)	No. tubers	Notes
CHRISTIAN	M	10	10.0	3	2.0	-	Thin roots and neopl.
	C_1	12	8.0	5	2.0	5	Good evolution
	C_2	20	12.0	15	8.0	5	Very good evolution
	C_3	20	10.0	10	6.5	8	Very good evolution
	C_4	18	9.0	15	4.5	12	Best evolution
ROCLAS	M	2	5.0	3	2.0	-	Poor evolution
	C_1	5	6.0	3	2.5	-	Slightly better evol.
	C_2	15	8.5	9	5.2	-	multiplication
	C_3	15	12.0	8	1.8	2	Slightly better evol.
	C_4	13	15.0	7	1.2	-	Slightly better evol.
OSTARA	M	3	4.5	2	1.5	-	Poor evolution
	C_1	4	8.0	5	3.2	2	Thin shoots
	C_2	25	10.0	15	5.5	3	0.8-1.0 cm ø tubers
	C_3	12	5.5	17	8.0	4	Good evolution
	C_4	15	6.2	19	12.0	5	Good steady evolution
DÉSIRÉE	M	2	4.0	3	1.4	-	Poor evolution
	C_1	4	2.5	2	1.3	2	Good evolution
	C_2	17	12.5	6	5.0	2	1 cm ø tubers
	C_3	13	6.9	19	7.4	5	Very steady evolution
	C_4	11	8.5	30	10.6	5	1 cm ø tubers

The in vitro tuberization in cv. Roclas was quite poor. Regeneration of neoplantlets on media M and C_1 was weak. Two minitubers/l\flask appeared on media C_1 and C_3. Medium C_2 favored the multiplication and rooting with no tuberization, but with the generation of a whitish-farinaceous callus mass on the contact area of explants with the medium. After six months, this zone generated small-diameter minitubers in an impressive number.

Cultivar Ostara produced 3 minitubers of 0.8-1 cm in diameter on medium C_2 with BM + BA + NAA + GA_3. the best results were, however obtained on media C_3 and C_4. The behaviour cultivar Désirée was similae to that of cultivar Ostara (Fig. 2).

The results prove that for in vitro tuberization the nature of cultivars and the hormonal balance in the culture medium play a key role. Our other results over a period of about 6 years indicated that the season is also essential for the in vitro tuberization, the most favorable period being between December and February.

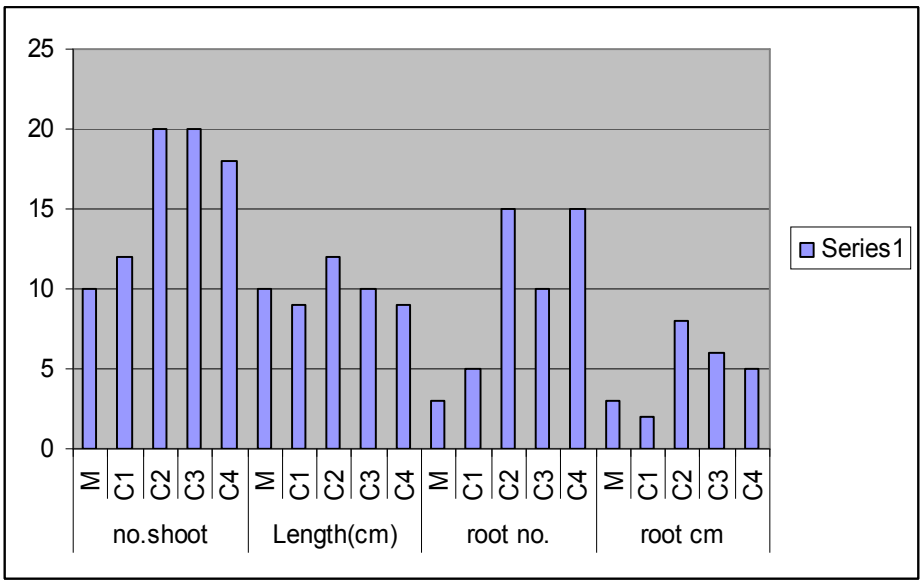

Fig. 1 *In vitro* evolution of *Christian* cultivar

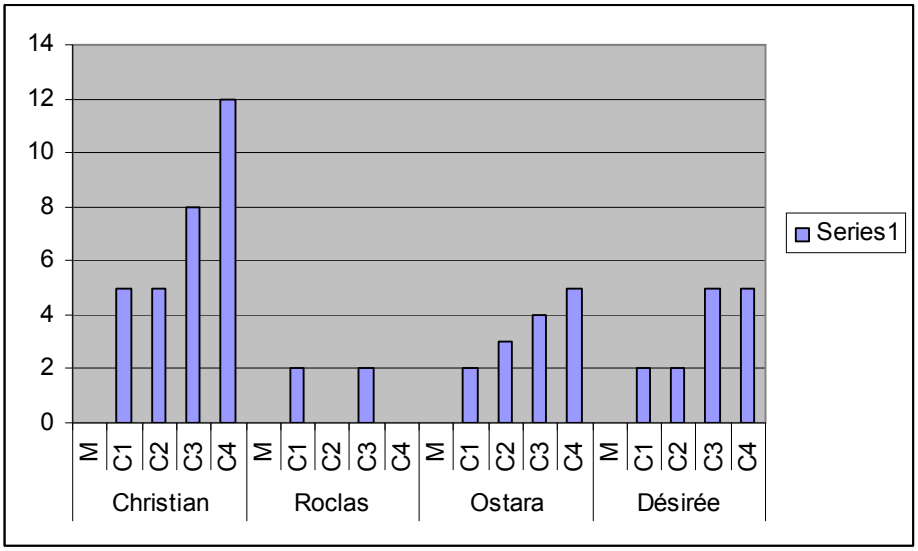

Fig. 2 Number of tubers regeneration *in vitro*

CONCLUSIONS

1. Of the four potato cultivars: Christian, Roclas, Ostara and Désirée, cultivar Christian was the most receptive to charcoal and hormonal mixtures added to the culture media.
2. The in vitro tuberization of cultivars Christian, Ostara and Désirée was strongest on media C_3 and C_4, containing benzyladenune + α-naphtylacetic acid, and kinetin + α–naphtylacetic acid, respectively.
3. The minitubers appeared on the nodes along the shoots and on the area in contact with the culture medium.
4. The most favorable period for in vitro tuberization was found to be between December and February

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