Researches Concerning Improving Methods of *In Vitro* Microtubers Production

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Abstract

Five genotypes of potato (Solanum tuberosum L.) were induced to form microtubers under two *in vitro* culture conditions (continuos darkness and light). Cultures maintained under continuos darkness (with a temperatue of 17^{0}C) had a higher yield with a greater number of microtuber (1.394/plantlet) than those maintained under long days (16 h of light/day) combined with temperature of 20^{0}C. In the last case the microtubers were higher, with with the highest average weight of 0.602 g. Three phytohormones it was used: coumarine, benzylaminopurine and salicylic acid. Regarding the average number of microtubers obtained when it was used coumarine and salicylic acid, the highest average number of microtubers was recorded (1,135 microtubers/plantlet). The study had been performed also on two fractions of the of sugar quantity (80 and 140 g/l), but they had less influence compared to the varieties.

Keywords: *plantlets, microcuttings, phytohormones, varieties, microtuberization conditions, microtubers*

INTRODUCTION

Tissue culture represents the starting point for seed potato production.

Seed potato production technology is based on *in vitro* multiplication, plantlets regeneration or microtubers production. Microtuberisation is a highly complex process, which modified in various ways, increases the capacity of plantlets to produce microtubers.

Scheme for seed potato production involves: microtubers production with a high biological value corresponding from phytosanitary point of view and they are the first generation of potato seed, starting from *in vitro* plantlets; minitubers production by planting of plantlets and microtubers "in the protected spaces" and it is use a substrate consisting of a mixture of organic and inorganic material and under certain culture conditions; obtaining of first clonal links, by planting minitubers in clonal field.

Over time there have been performed extensive physiological researches which show that microtuberization is dependent on a number of factors, such as photoperiod, phytohormones combination, the composition of the nutrient medium, etc..

Influence of light

Wang and Hu (1982), Wattimena et al. (1983), Ortiz-Montiel and Lozoya-Saldana (1987), Forti et al. (1991) have issued the idea that on darkness microtuberization was rapidly.

Slimmon et al., 1989, Gopal et al., 1998 observed an increase in the microtubers weight on light conditions compared with induction of microtubers under darkness continuous (Gopal et al., 1997, 1998).

Influence of nutrient composition

Ewing and Struik (1992) notified that sugar - is the most effective factor in the formation of microtuber; Khuri and Moorby (1996) have

highlighted its role in inducing of microtubers, but at high concentrations; Radouani (1997) specified the effectiveness of coumarin in microtuberization; Gopal et al. (1998), on researches conducted over 22 potato genotypes, have reported that the cytokinin and benzylaminopurine influenced the number and size of microtubers formed. Handro et al (1997) have shown that salicylic acid promotes tuberization.

Clarke et al (2001) have revealed that the salicylic acid in small doses stimulate cell proliferation; General objective of the research is the improving method of microtuberization inducing the specific objectives are: identifying the optimal conditions for induction microtuberization and the best media of microtuberization; identification of varieties suitable to microtuberizare; promoting Romanian varieties that responded significantly at improved method of microtuberization

The research aim was: establishment of optimum values for the factors involved in potato microtuberization, such as rate of growth and multiplication of the different genotypes are not diminished by the recalcitrant reactions to the culture medium; introduction of microtubers in scheme of potato seed production (figure 1).

MATERIALS AND METHOD

Microcuttings resulting from uninodal segmentation were 15 inoculated, in recipients containing

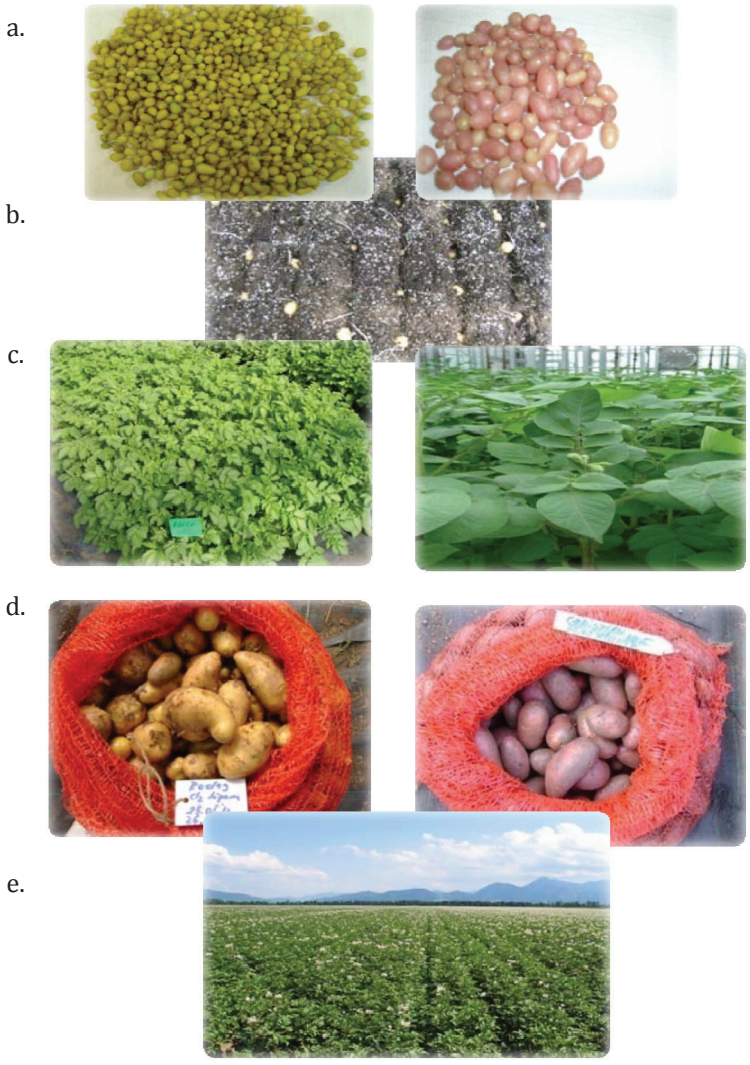

Fig. 1. Scheme of potato seed production.
Legend: a. microtubers; b. planting of microtubers in "protected spaces";
c. plants developed from microtubers; d. minitubers obtained from Romanian varieties; e. clonal field

Murashige & Skoog (1962) medium enriched with naphthyl acetic acid and 20 g / l sugar; the culture vessels were placed in the growth chamber, ensuring a light and temperature regime required for growth and development of the plantlets. After 4 weeks, in recipients with developed plantlets were applied microtuberization medium with sucrose, as an energy source (it was used in two quantities: 80 and 140 g / l) and phytohormones: coumarin (0.05 g/l), benzylaminopurine (0.006 g/l), salicylic acid (0.024 g/l); microtuberization conditions were continuous darkness (17^0C) and light (20^0C). Exposure of culture recipients at microtuberization conditions lasted a period of 3 months.

Calculation methods and interpretation of data from experience: the results were transformed by variation analysis; significance of differences was determined by the method of multiple comparisons (Duncan test).

Experimental variants:

Polifactorial experience, such as: 2x5x3x2, included the following factors, divided over 3 repetitions:

Experimental factor A- microtuberization conditions with 2 degrees:

a1-continuous darkness;

a2-light (photoperiod 16 h light / day).

Experimental factor B - variety, with five degrees:

b1-Roclas;

b2-Rustic;

b3-Gared;

b4-Kronstad;

b5-Nativ.

Experimental factor C - phytohormones, 3 degrees:

c1-coumarin;

c2-benzylaminopurine;

c3-salicylic acid

Experimental factor D - quantity of sugar (g/l) used on medium of microtuberization with 2 degrees:

d1-80 g / l;

d2-140 g / l;

RESULTS AND DISCUSSIONS

Microtuberization under continuous darkness proved to be optimal on microtubers formation in number (1.394) for Roclas variety, which differs significantly from the other variants (table 1) (figure 2, 3). Exposure to light favored varieties Gared (0.602 g) and Kronstad (0.471 g) in microtuberization, average weight of microtubers is significantly different from other variants. Conditions darkness provoked a negative reaction in microtuberization for Nativ variety, reflected by the minimum weight of microtubers (0.209 g).

Tab. 1 Influence of variety and conditions of microtuberization over the number of microtubers produced / plantlets and their average weight

Variety	Microtuberization conditions	Number of microtubers	Duncan test	The average weight of microtubers (g)	Duncan test
Roclas	Continuous darkness	1,394	A	0,297	CD
	Light	0,960	BC	0,247	D
Rustic	Continuous darkness	1,128	B	0,244	D
	Light	1,115	B	0,278	CD
Gared	Continuous darkness	1,016	B	0,316	CD
	Light	0,993	B	0,602	A
Kronstad	Continuous darkness	1,147	B	0,381	BC
	Light	0,758	C	0,471	AB
Nativ	Continuous darkness	1,155	B	0,209	D
	Light	1,155	B	0,459	B
		Lsd 5% = 0,2192		Lsd 5% = 0,1335	

Means followed by same letters are not significantly different according to Duncan test ($p \leq 0.05$)

Fig. 2. Microtuberization on darkness (in left) and light (in right)

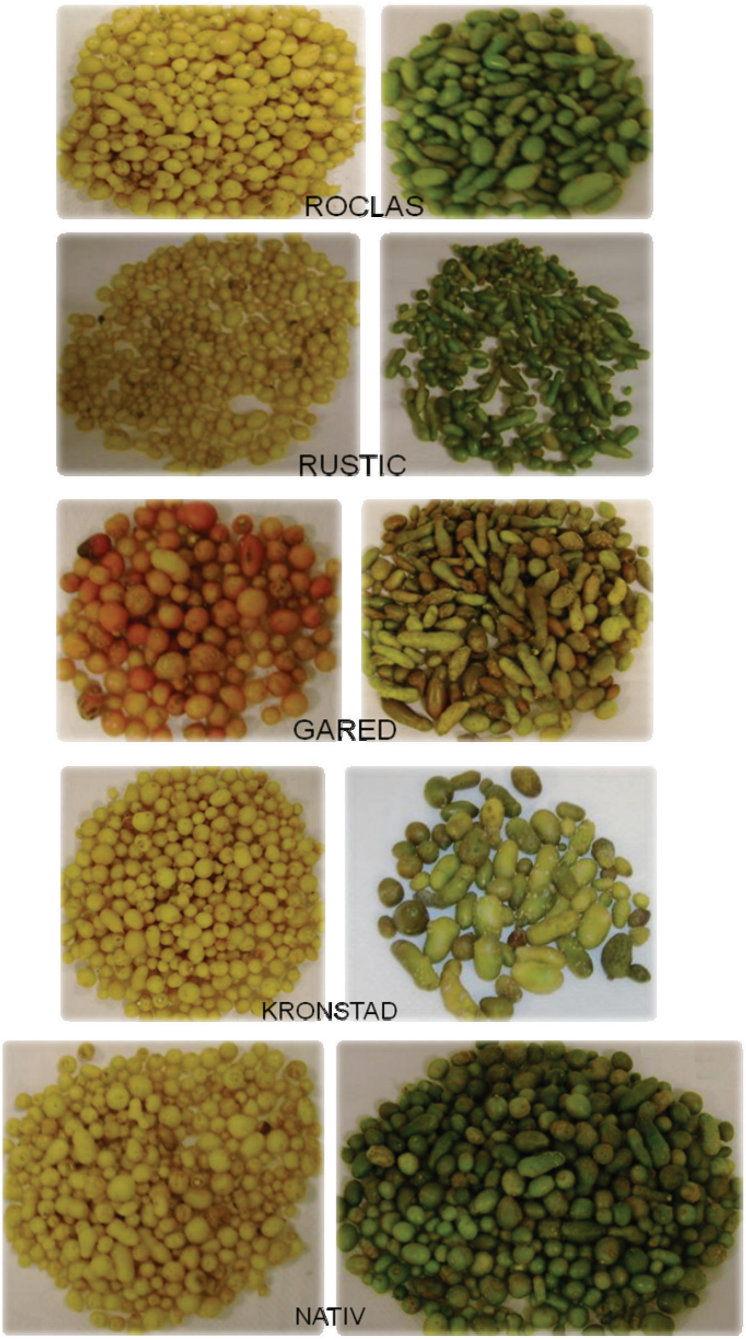

Fig. 3. Microtubers produced under continuous darkness (left) and light (right)

Average number of microtubers / plantlets, analyzed through the potential of variety shows the superiority of Roclas and Nativ varieties (1.177; 1.155); the produced microtubers differ significantly from Gared varieties and Kronstad (1.004; 0.953); instead, these varieties have produced microtubers with highest weight (0.459 g, 0.426 g), differing significantly from the other varieties (table 2).

Analyzing the results through the influence of phytohormones (figure 4), it shows that the effect of coumarin and salicylic acid is superior to benzylaminopurine, proved by the increased number of microtubers; the three phytohormones did not differ significantly in terms of the influence on the average weight of microtubers (table 3).

The combined influence of variety and phytohormones in microtuberization indicates achieving of differentiated results:

- salicylic acid prints for Roclas variety high capacity in microtuberization, by obtaining a large number of microtubers (1.330) and benzylaminopurine for Kronstad variety inhibits microtubers production (0.768);
- phytohormones coumarin and salicylic acid stimulates the production of microtubers for

Fig. 4. Influence of phyohormone in microtuberization
(depending by cumarine, benzylaminopurine, salicylic acid – from left to right)

Tab. 2 Influence of variety on number of microtubers produced / plantlets and their average weight

Variety	Microtubers number	Duncan Test	The average weight of microtubers (g)	Duncan test
Roclas	1,177	A	0,272	B
Rustic	1,122	AB	0,261	B
Gared	1,004	BC	0,459	A
Kronstad	0,953	C	0,426	A
Nativ	1,155	A	0,334	B
	Lsd 5% = 0,1302		Lsd 5% = 0,07934	

Means followed by same letters are not significantly different according to Duncan test ($p \leq 0.05$)

Tab. 3 Influence of phytohormones on the number of microtubers produced / plantlets and their average weight

Phytohormone	Microtubers number	Duncan Test	The average weight of microtubers (g)	Duncan test
Cumarine	1,135	A	0,316	A
Benzylaminopurine	0,977	B	0,388	A
Salicylic acid	1,135	A	0,348	A
	Lsd 5% = 0,1376		Lsd 5% = 0,08387	

Means followed by same letters are not significantly different according to Duncan test ($p \leq 0.05$)

Tab. 4 Influence of variety and phytohormones on the number of microtubers produced / plantlets and their average weight

Variety	Phytohormone	Microtubers number	Duncan Test	The average weight of microtubers (g)	Duncan Test
Roclas	Cumarine	1,167	ABCD	0,223	E
	Benzylaminopurine	1,034	BCDE	0,283	DE
	Salicylic acid	1,330	A	0,310	DE
Rustic	Cumarine	1,278	AB	0,260	DE
	Benzylaminopurine	0,916	EF	0,292	DE
	Salicylic acid	1,171	ABCD	0,232	E
Gared	Cumarine	0,943	DEF	0,348	BCDE
	Benzylaminopurine	1,063	BCDE	0,496	AB
	Salicylic acid	1,007	CDEF	0,533	A
Kronstad	Cumarine	1,060	BCDE	0,407	ABCD
	Benzylaminopurine	0,768	F	0,467	ABC
	Salicylic acid	1,031	CDE	0,402	ABCD
Nativ	Cumarine	1,227	ABC	0,340	CDE
	Benzylaminopurine	1,104	ABCDE	0,399	ABCD
	Salicylic acid	1,134	ABCDE	0,263	DE
		Lsd 5% = 0,2468		Lsd 5% = 0,150	

Means followed by same letters are not significantly different according to Duncan test ($p \leq 0.05$)

Roclas Rustic, Nativ varieties; for the latter one stimulative effect is given by the BAP, which is not significantly different from the others two phytohormones;

- salicylic acid and benzylaminopurine are beneficial to Gared variety by producing microtubers with increased weight (0.533 g, 0.496 g); in the same manner, benzylaminopurine promotes Kronstad variety (0.467 g).
- instead, not the same positive effect on the weight it had coumarin over microtubers of Roclas variety (0.223 g) (table 4).

Influence the sugar quantity used in the nutrient medium is smaller than the influence of variety in microtuberization:

- sugar in a quantity of 140 g / l, was established as a good support for the Roclas variety in microtuberization (which induces the formation of 1.262 microtubers / plant); fraction of 80 g / l sugar sustain Rustic, Gared, Kronstad varieties in producing microtubers (1.136, 1.159, 1.017) and for the variety Nativ the two sugar fractions did not differ significantly from viewpoint of obtaining microtubers.
- Sugar quantity of 80 g / l favors Gared variety in producing microtubers with highest weight (0.473) which is considered significant compared to Roclas and Rustic and insignificant to Kronstad and Nativ for both graduations of sugar (table 5).

CONLUSIONS:

Conduct of researches and interpretation of the results indicated that microtuberization depends of the interaction: microtuberization conditions - variety - type of phytohormone - the amount of sugar.

Weight of microtubers produced on light conditions, was significantly higher for Gared and Kronstad varieties compared to other varieties (0.602 g and 0.471 g) microtuberization induced in dark variety is beneficial for Roclas when the aim is to produce a large number of microtubers.

Addition of salicylic acid and coumarin in microtuberization medium, created the possibility

Tab. 5 Influence of variety and sugar quantity used in the nutrient medium on the number of microtubers produced / plantlets and their average weight

Variety	Sugar quantity (g/l)	Microtubers number	Duncan Test	The average weight of microtubers (g)	Duncan Test
Roclas	80	1,092	ABC	0,262	CD
	140	1,262	A	0,282	BCD
Rustic	80	1,136	AB	0,232	D
	140	1,108	ABC	0,291	BCD
Gared	80	1,159	AB	0,473	A
	140	0,849	D	0,445	AB
Kronstad	80	1,017	BCD	0,431	ABC
	140	0,889	CD	0,421	ABC
Nativ	80	1,156	AB	0,334	ABCD
	140	1,156	AB	0,334	ABCD
		Lsd 5% = 0,2192		Lsd = 0,1804	

Means followed by same letters are not significantly different according to Duncan test ($p \leq 0.05$)

of using these substances as growth regulator, in achieving effective results in microtuberization.

Although graduation of sugar factor has less influence compared to variety, beneficial effects presented:

- fraction of 140 g / l sugar in productivity of Roclas variety (1.262 / plant microtubers);
- fraction of 80 g / l sugar stimulated the formation of tubers with higher weight (0.473 g) for Gared variety.

Researches has led to a material with a high biological value:

- Roclas varieties and Nativ-which had the highest number of microtubers / plant (1.177, 1.155);
- Gared and Kronstad varieties, which recorded the highest value of the average weight of microtubers (0.459 g, 0.426 g).

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