RATE OF IN VITRO DIFFERENTIATION OF MINITUBERS IN THE "SANTÉ" POTATO VARIETY (SOLANUM TUBEROSUM L) DEPENDING ON TIME OF SAMPLING AND MEDIUM COMPOSITION

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Abstract

Taking into account the phenomenon of "ecological degradation" in potato varieties and its causes, the present experiment was initiated in order to establish a protocol for obtaining planting material (tubers) through in vitro cultures, for the Santé variety. The experiment was initiated in May (2014). The biological material was made up of minitubers of verious sizes, differentiated in vitro (those 5mmØ were sectioned, the ones 1-2mmØ left intact). Culture mediums were conceived to stimulate tuberation using a combination of a high dosage cytokine (2mg/l) and the same auxin in a low dosage (1mg/l), with the variants: ***Mt. = baseline*** *= MS + 100mg/l casein hydrolysate (H.C.);* ***V_1*** *= Mt + 2mg/l K + 1.0mg/l AIA;* ***V_2*** *= Mt + 2mg/l BA + 1.0mg/l AIA;* ***V_3*** *= Mt + 2mg/l 2iP + 1.0mg/l AIA. The reaction of the Santé variety was studied after 24, 30, 60 and 90 days, respectively. After less than a month, tuberation was between 4-10% on V_1, V_2, V_3, with differentiation of very small tubers, of circa 1mm Ø. 30, 60 and 90 days, respectively, after in vitro incubation of explants the regeneration capacity grew consistently reaching 100% after 90 days (on V_1 and V_2). After 90 days, we obtained tuberation of 80% on V_1, 50% on V_2 and 30% on V_3, with a number of 8-4-3 tubers/explant.*

Key words: tuberation rate, minitubers, Santé variety, rhizogenic callus, cytokines, auxins

INTRODUCTION

Obtaining potato planting material (tubers) through *in vitro* cultures has become important due to the phenomenon of "ecological degradation" signaled by Velican, 1959, which leads to the *filosity*[1] *of the variety*, a common disease in areas that are not favorable to potato crops. The causes of potato varieties degradation are environment conditions in the vegetative phase, especially when adaptation of the crop is attempted in a different climate (for example an excessively continental climate, with dry hot summers), the species' original climate being a temperate one (Roman, et all., 1912). Another phenomenon taking place in the classic potato crop is "degradation through ageing" , which manifests when using old planting

[1] Filosity = physiologic degradation of a plant due to lack of adaptation to a new climate

material (Velican, 1965, as quoted by Roman, 2012), resulting in plants with various degrees of degeneration and requires the rejuvenation of the entire clone (Morar, 1999). The potato is an annual plant that breeds vegetatively through tubers, the species being originary to the temperate zone (Draica, 1992). In the last decades, breeding through stem cuttings. In the practice of producing potato tubers for consumption, the requirements of local varieties and of acclimatized clones from other areas must be taken into account (Ianoși, 2002).

Studies that have targeted *in vitro* culture technlogies in order to perfect amelioration and multiplication techniques in potatoes were previously done by Agud, 2009 (on the micromultiplication of potatoes), Cachiță et al., 1997 (on production of planting material free of viral diseases, on rejuvenation of different varieties and old clones). Of particular interest are the applications to *in vitro* mutagenesis induction (Kulcarni et. al, 2007) and to obtaining batches of potatoes resistent to stress factors (Cachiță et al 2004). We also mention the success in stimulation of growth organs formation - bulbils – *in vitro* on species from the family Iridaceae (Zăpârțan, 1996), stimulation of bulbils' breeding through the use of bioreactors (Ziv, 1990), and the attainment of large quantities of bulbs in Liliaceae through *in vitro* cultures (Zăpârțan et al. 1999-2000; Zăpârțan et al. 2006).Studies done on varieties of the species *Solanum tuberosum* L. have followed various aspects, among them: the reaction of some otato genotypes to *in vitro* cultures (Baciu, 2008) with the establishment of some crop protocols; the implications of the photoperiod combined with high dosages of sugar in the culture medium in tuberation (Agud et al, 2009); the relationship between the prelevation time and the regeneration capacity of the explant in local varieties of potatoes, compared to foreign ones (Agud et all., 2013).

MATERIAL AND METHODS

The Santé poatato veriety we used in our experiment has certain biological and consumption particularities (Gravoueulle, 2011), which make it extremely interesting for amelioration studies. *The biological material* was made up of *in vitro* differentiated minitubers of various sizes (which were sectioned if larger than 0.5cmØ and left intact if between 1-2mmØ): the bulbils come from a previous experiment that also used the Santé variety (Agud and Laslo, 2014, publishing under way). *Culture mediums* were conceived to stimulate tuberation using a combination between a varying cytokine (the implications of using this group in the tuberation of potato varieties is known due to Butiuc-Keul et. al, 1996) and the same auxin, used on an improved baseline medium (Mt.). The following variants of culture

mediums emerged: **Mt.= baseline** = MS + 100mg/l casein hydrolysate (H.C.); **V_1 =** Mt + 2mg/l K + 1.0mg/l AIA; **V_2 =** Mt + 2mg/l BA + 1.0mg/l AIA; **V_3 =** Mt + 2mg/l 2iP + 1.0mg/l AIA. After inoculation, the explants were kept in the growing room, on a photoperiod of 16 hours light out of 24, a temperature of 26-27^{0}C and humidity of 80-85%.

RESULTS AND DISCUSSION

The first observations were made **after 24 days** from the implantation of the minitubers and the Santé variety segments *in vitro* (Table 1). After 3 weeks we noticed the following :

Tabel 1

Rate of minitubers differentiation in vitro, from apex tissue

Time (days)	Var.	% Regen.	% stagnation/ necrosis	% Callus growth	% Tuberation	Nr. tubers/ explant	Bonification/ observations
30 days (1 month)	Mt.	10	80	-	-	-	**X** 1sd. of 5.5cm
	V_1	50	10	10	20	2 small tub.	XX thickenings on nodes
	V_2	60	20	20	10	1 small tub.	XXX thickenings on nodes
	V_3	30	40	-	-	-	XX Ev. without tub., uneven
60 days (2 months)	Mt	28	72	-	-	-	XX 1 -2 sd/expl.
	V_1	70	-	20	48	4tub./expl. 1-2mmØ	XXXXX nodes' thickenings become minitubers
	V_2	70	-	45	25	2 tub./expl. 1-2mmØ	XXXX ½ of thickenings become minitubers
	V_3	55	35	20	-		XXXX rhizogenic callus
90 days (3 months)	Mt.	40	60	-	-	-	XXX weak ev.
	V_1	100	-	22	80	6 – 8 tub/ explant □ 0,5cmØ	XXXXXX very good and uniform ev.
	V_2	100	-	50	50	3 – 4 tub./ explant □ 0,5cmØ	XXXXXX 50% tub; 50% callus growth, rhizogenic callus, regenerative
	V_3	60	40	30	30	2 – 3 tub/ explant □ 0,3cmØ	XXXX even ev. 30% rhizogenic callus

- On Mt. the regeneration percentage is circa 20%, cca. 80% of explants stagnate, with no tuberation;
- On V_1 regeneration is at 40%, with the formation of 3-4 new seedlings on the explant and of a large number of roots, at the endings of which small tuberated formations of circa 1mm were formed (on circa 4% of them);

- On V_2 regeneration is at 50%, 1-2 new seedlings of circa 1 cm appear and tuberation starts with the formation of circa 2bulbils/explant of 3/4mm Ø (circa 10%);
- On V_3 regeneration is double compared to Mt. (40%), with the formation of a 1.5-1.6 cm plant with a number of respective roots (circa 20%), and 30% of explants form a fiable callus.

After 24 days we observed that : on the Mt sample the presence of just the aminoacid from MS is insufficient for good regeneration and stimulation of tuberation in the Santé variety; on the other hand, in the presence of kinetin and indole-3-acetic acid (V_1) tuberation is initiated to a small degree (4%) but thickenings of the tips of the root are observed; in the presence of BA and AIA (V_2) tuberartion is well developed (circa 10%) with differentiation of aprox. 2 minitubers of 1-2mm Ø/explant. A similar reaction takes place on medium containing 2iP and AIA (V_3). Here we could also observe the differentiation of friable callus, ideal for obtaining cell suspensions.

The explants, made up of minitubers and segments, were analized after 30, 60 and 90 days, from the standpoint of explants' capacity for regeneration (%), for callus growth (%), and for tuberation (%) and that of the average number of differentiated tubers/explant (Table 1).

The tissue's capacity for regeneration after 30 days on the baseline sample is at 10%, while on the other variants it is between 30-60%. The greatest capacity for regeneration was obtained on the medium containing BA. After 60 days, the variants containing cytokines regeneration increases significantly,exceeding 70% on V_1 (Mt. + 2mg/l K + 1.0mg/l AIA) and on **V_2** **(**Mt + 2mg/l BA + 1.0mg/l AIA) . After 90 days, the regeneration capacity on these mediums was 100% (Figure 1). The baseline sample only reaches 40% regeneration capacity (after 90 days) and stagnation, followed by necrosis of the tissue was observed in 60% of explants. The phenomenon of in vitro caulogenesis is present form the first observation (after 30 days) and reaches maximum value after 90 days (22-50% of explants). After the first 30 days, the callus tissue appears as thickenings (bulges) of the nodes on V1 and V2,and after 60 days the percentage of caulogenesis doubles on these variants, as the thickenings of the nodes (present on almost all nodes) become small minitubers of circa 2 mmØ, that after another 30 days (for a total of 90 days of in vitro culture) become tubers.

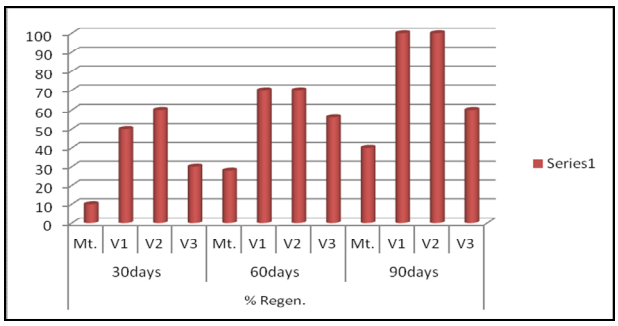

Fig. 1 Regeneration capacity of potato tissue after 30, 60 and 90 days

We remark that on the 2iP variant (V_3) differentiation of rhizogenic callus on circa 20-30% of explants regenerates 2-3 tubers/explant, showing an even evolution.

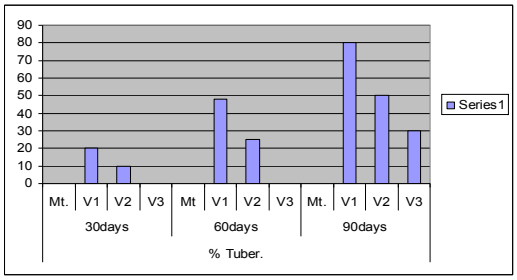

Fig. 2 Tuberation of potato explants (after 30, 60 and 90 days)

The rate of tuber formation after the 3 time periods is shown in Figure 2. Most significant are the top valuesof the parameter after 90 days on variants withK and BA (V_1 şi V_2), that reach a tuberation rate of 80%, and 50% respectively. On the i2P variant tuberation reaches circa 30% while the baseline sample is negative. It appears that adding only an aminoacid to the basic culture medium MS is insufficient to stimulate tuberation.

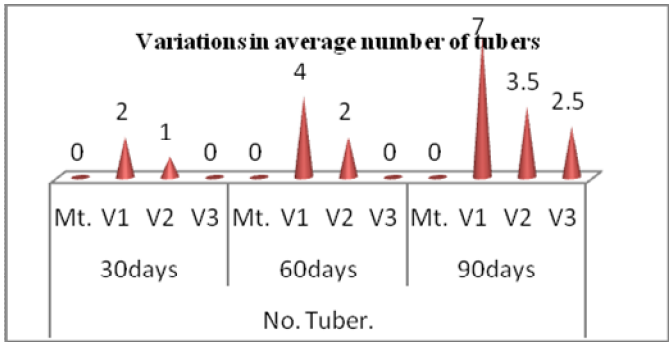

Fig. 3 Average number of tubers obtained *in vitro* (după 30, 60 respectiv 90 zile)

An average number of tubers/explant could only be assessed 60 days after *in vitro* culture inception, when we recorded an average of 4 tubers/explant on V_1 and 2 tubers/explant on V_2. After another 30 days these numbers doubled, and tuberation also appeared on the i2P medium (V_3).

CONCLUSIONS

After 24 days of *in vitro* culture on MS with only casein hydrolysate, tuberation in the Santé variety is not stimulated. In the presence of K and AIA (V_1) the tuberation process commences and thickenings appear on the roots. BA and AIA (V_2) determine tuberation in circa 10% of explants, through the differentiation of an average of 2 minitubers of 1-2mmØ. A similar reaction we found on la V_3 (2iP and AIA), where friable callus is also formed. The location of tuber differentiation is on the stem at the level of branching nodes or, in a medium, on the roots.

After 30 days *in vitro* regeneration reaches 50-60% on variants with K and BA (V_1 și V_2), and caulogenesis reaches 10 and 20%, respectively; concerning tuberation, thickenings (bulges) appear at the level of each node.

After 60 days regeneration on mediums containing cytokines is between 55-70%, caulogenesis is at 45% on V_2, and the node thickenings become minitubers of circa 1-2 mmØ. Tuberation after this time reaches 48% on V_1 and 25% on V_2: BA and K in the medium favors tuberation and differentiation of circa 2-4 tubers/explant of about 1-2 mmØ;
After 90 days *in vitro* regeneration reaches 100% on V_1 and V_2 (compared to 40% on Mt.). On V_3 ,40% of explants stagnate and show necrosis. tuberation on the medium with K and BA reaches 80% - 50% with differentiation of 4-6-8 even tubers (0,5cmØ).

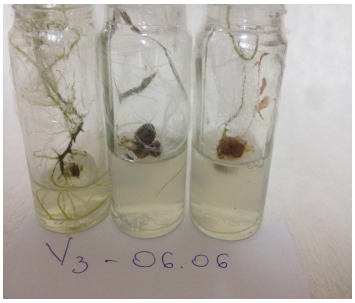

Foto 1

Foto 2

Tuberation on V 2 and V_3

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