IN VITRO REACTION AND EX *VITRO ACCLIMATIZATION* OF SOME AUTOCHTHONOUS VARIETIES OF SOLANUM TUBEROSUM L. (MURESAN, SUPER, CATY)

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Abstract

*The experiment is a continuation of our idea of conserving in vitro the potato varieties and populations ameliorated within the country on economical grounds and based on the fact that autochthonous elements are much better adapted to our climate conditions, more resistant to unfavorable conditions becoming more common (drought, attack of diseases and pests). Three varieties were taken in study: Mureşan, Super and Caty ameliorated at The Potato Research Institute Braşov – Stupini, around the 1980s. The plant material consisted of apical meristem cultivated on Murashige-Skoog medium, 1962 improved with 825mg/l NH_4NO_3, with the following variants: C_o=MS1/2; C_1=MS+825mg/lNH_4NO_3+**5g/l coal**; C_2=MS+825mg/lNH_4NO_3+0,1mg/lANA+1,0mg/l BA; C_3=MS+825mg/lNH_4NO_3+0,1mg/lANA+2,0mg/lBA**;** C_4=MS+825mg/lNH_4NO_3+0,1mg/lANA+ **5g/**lEP; C_5=MS+825mg/lNH_4NO_3+0,1mg/lANA+80mg/l$AdSO_4$ (Table 1).*

*After three months from the incubation of the tissue on the mediums, observations were made concerning: the regeneration, multiplication and rooting percentage and observations concerning some parameters: the length of the plants, of the roots and layout changes of the varieties. The in vitro reaction of some varieties is dependent on the variety and on the composition of the culture mediums: the variety with the best reaction proved to be **Mureşan**, but this reaction is different on the five mediums: on **C_3** it regenerates and multiply in vitro in a percentage of over 80%, rooting about 52%, generating a rich radicular system (10-12 roots with aerial roots on which there are placed tubers with a Ø of 0,5-0,6cm, proving to be the best medium. This variety has a reaction on all variants, but it is inferior to C_3 medium. On C4 variant with 5g/l EP, Mureşan variety reaches good values (we believe that due to the content of natural zeatin that can be found in the maize germ extract, and citokinin strongly involved in the regeneration of the vegetal tissue in vitro): the variety also presents a good reaction on the medium with cu $AdSO_4$ (C_5), but it is inferior to the other mediums. The other varieties have an inferior or a loose reaction depending on the medium: on Super and Caty they have the best reaction C_3 and on C_4 also (but inferior to Mureşan variety). On the control medium C_o the parameters are small and regeneration is situated between 10-8% and without rooting: on C_1 in the presence of the vegetal coal and of NH_4NO_3 regeneration is much more better, but rooting does not take place in this case also (see Table 2). The method can ensure vegetal material for amelioration, can conserve in vitro the autochthonous populations and varieties, for the maintenance of a Romanian potato collection of interest. The potato varieties ameliorated at Braşov-Stupini have a different reaction, manifested in vitro in a longer time and inferior to the potato populations from Harghita county (experimented by us), but they are varieties which are resistant to unfavorable environmental conditions, with a certain genetic value for the Romanian ameliorators. For the success of the technique there shall be tested other medium formulas which may prove more effective.*

Key words: apical meristem, Murashige Skoog medium (MS)+ NH_4N0_3, BA, ANA, maize germ extract (EP), adenine sulfate, *Solanum tuberosum L*, autochthonous varieties: *Mureşan, Super, Caty* (ameliorated at The Potato Research Institute from Stupini-Braşov, 1985).

INTRODUCTION

Vegetal biotechnologies have revolutionized the culture techniques and the amelioration of many species of plants with an economical value (Raicu, Badea, 1990), determining the appearance of a new science related to the nonconventional amelioration of the culture plants (Savatti et al. 2003), the field knowing a large-scale in this century and it intensifies in the future (Cachiță, Ardeleanu, 2009). If conventional methods are based on Genome manipulation as a whole, *in vitro* techniques contribute to supplementing classical methods of amelioration (Chiru, 1991). In applying conventional methods gene sources of interest determine useful characters only to related species, sexually compatible with the specie to be ameliorated (Botez et al., 1995). Because of this, it was intensified the interest for the promotion of the nonconventional methods of potato multiplication and conservation by *in vitro* cultures (Maior et al., 2005).

Biotechnologies can ensure the manufacture of biotypes, lines, varieties with advanced characteristics, can ensure a healthy multiplication vegetal material, can maintain the biological biodiversity of the specie and its conservation (Bajaj, 1987). The method guarantees the manufacture of new productive varieties for the culture mediums (Ardeleanu et al., 1995), and also the involvement in the conservation of the genetic vegetal resources (Halmádyi, Butiuc-Keul 2007), or the multiplication of a genetically tested biological and phytosanitary material (Chiru et al., 1993).

The potato has a well known economic value occupying the second place in human nutrition, after the corn (Brown, 1994), and *in vitro* culture has brought many practical applications not only to the potato, but also in the amelioration of many plant species of economic importance (Savatti et al. 2004), the method putting initially in value the totipotency of the plant cells (Cachiță et al., 2004). To the potato, the method allowed the discovery of the tissues` capacity (apex, meristem explant from the leaf, steam, bud, etc.) of generating new plants (Agud, 2008a; Agud et al., 2009a) or of multiplying in an unlimited number (Pătru, Cachiță, 2005).

The interest for the promotion and conservation of the autochthonous potato varieties was born due to the fact that the specie is of economic interest for Romania, and on the other hand, we know that the autochthonous varieties from any other specie have a greater capacity to adapt to the conditions of temperate continental climate, with an undeniable resistance to diseases, pests, drought, etc. Our research noted the importance of the autochthonous potato populations from Ciuc Hollow and their behavior at the *in vitro* culture (Agud et al., 1013). A much studied field and of a great interest for the in vitro culture of the potato, was also the manufacture of the multiplication material (tubers), in this sense being used

certain hormonal balances, with a different photoperiod and a certain quantity of sucrose (Butiuc-Keul et al., 1997-1988; Agud et al., 2009b). Then we recall the experiments for the stimulation of the in vitro differentiation of tubers by replacing sucrose with honey or fructose (Pătru, Cachița, 2005), or the manufacture of economically advantageous techniques for the stimulation of the in vitro rooting (Agud, 2011), and even the testing of a balanced hormonal balance also in order to obtain the much more economical *in vitro* potato plant material (Agud et al., 2008).

MATERIAL AND METHODS

The studied potato varieties, Mureşan, Super and Caty are ameliorated at the Institute for Potato Amelioration from Brasov, around 1980s and they present some valuable characteristics as they come from a part of the country with a favorable climate for the culture of the potato: a certain dynamic in the root differentiation and the formation manifested depending on the variety, hence a pronounced heterogeneity, the vegetation periods unfolding between 70 and 80 days, with a high index of exfoliation, good resistance to drought, diseases and pests. The recalled potato varieties have been researched in vitro since the beginning of 1990s under the aspect of testing their in vitro regeneration capacity on mediums with a content of substances still untested on the potato, as some retardants are (Zăpârțan et al., 1991), or the manufacture of in vitro tubers at these varieties by using an original technique in a double layer, solid – liquid, with remarkable results (Cachiță, Zăpârțan, 1991).

The plant material consisted of apical meristem detached from the tubers of the three Mureșan, Super and Caty varieties, after forcing it in April, this type of tissue was also experimented to other potato varieties (foreign or Romanian) with good regenerative results (Agud et al., 2011), and in other cases was tested a balanced balance of phitohormones and a surplus of sucrose (Agud, 2012). The time of tissue sampling for the in vitro culture plays an essential role in the success of the technique, our research has proved that the proper time of year for good results is March – April (Agud, 2013). After the sterilization the material was inoculated on the basal medium according to Murashige-Skoog -1962 (MS), to which initially was added 825mg/l NH_4NO_3, hence it was intervened for the first time in a basal recipe of a medium formula (hence an improved MS) to which there were added the hormonal components specified in Table 1.

Table 1

The medium variants for the in vitro culture of the autochthonous varieties of Solanum tuberosum L(Mureşan, Super, Caty)

Var.	The basal medium	Vegetal coal	Auxin ANA (mg/l)	Citokinin BA (mg/l)	Maize	Adenine sulfate
C_o	MS1/2	-	-	-	-	-
C_1	MS+ 825mg/l NH_4N0_3	5 g/l	-	-	-	-
C_2	MS+ 825mg/l NH_4N0_3	-	0,1	1,0	-	-
C_3	MS+ 825mg/l NH_4N0_3	-	0,1	2,0	-	-
C_4	MS+ 825mg/l NH_4N0_3	-	0,1	-	5 g/l	-
C_5	MS+ 825mg/l NH_4N0_3	-	0,1	-	-	80mg/l

RESULTS AND DISCUSSION

After two months of in vitro culture, the proliferation capacity of the three potato varieties was law, a unprecedented reaction to the varieties experimented by us, this is why the observations were made only after three months of in vitro culture of the apical meristematic tissue detached from the three varieties. The observations contained data related to the regeneration, multiplication percentage and also to the implication of the hormonal balance (composition of the medium) for the *in vitro* rooting of the potato varieties, assessments being made of the evolution of some parameters (the aspect of the stem, of the radicular system, of the foliar system, etc.).

The percentage values are listed in Table 2, for all three varieties: Mureşan, Super, Caty. Following the *in vitro* regenerative and rooting capacity of the potato varieties, we can state that the best percentages are obtained at Mureşan variety, followed by Super and Caty, at these latter varieties the values are much more lower. So, Mureşan variety reaches a regeneration percentage of 80%, 52% rooting on medium C_3 (MS+825mg/lNH_4NO_3+0,1mg/lANA+**2,0mg/lBA),** with the maximum dose of BA**,** resulting 10-12 long roots with a multitude of aerial roots on which mini tubers of 0,5-0,6cm have differentiated. This variety has a good in vitro reaction on the medium with 5mg/lEP (C_4), obtaining 75% regeneration and 50% rooting, with 2-3 long roots with tubers of 4-5mm. On the other variants the percentage values are much smaller between 20-45% regeneration and 11-25 % rooting. On the witness sample C_o and C_1 with vegetal coal, rooting did not take place (see Fig. 1 and Fig. 2).

Table 2

The values of the analyzed parameters of the in vitro culture of the autochthonous potato varieties: Mureşan, Super, Caty (after three months)

Var.	Variety	Regener. %	Multiplic. %	Root. %	Evalut.	Observations
C_o	Mureşan	10	5	-	xx	2-3 roots
	Super	8	-	-	x	1 root
	Caty	8	-	-	x	Without roots
C_1	Mureşan	20	18	-	xx	1-2 root+ aerial roots
	Super	12	10	-	xx	1-2 root+ aerial roots
	Caty	8	5	-	xx	1-2 root+ aerial roots
C_2	Mureşan	45	45	25	xxxx	5-6 root+ aerial roots with tubers (2mm)
	Super	32	32	15	xxx	5 roots+aerial roots with tubers (1mm)
	Caty	25	25	2	xxx	3 roots + aerial roots, small tubers
C_3	**Mureşan**	**80**	**80**	**52**	**xxxxx xx**	**10-12 roots+aerial roots with tubers of 0,5-0,6 cm**
	Super	55	50	20	xxxxx	8 roots + aerial roots with small tubers on the roots
	Caty	45	28	12	xxxx	5 roots + aerial roots with small tubers on the roots
C_4	**Mureşan**	**75**	**75**	**50**	**xxxxx xx**	**2-3 roots+long aerial roots: tubers of 4-5mm**
	Super	50	50	35	xxxxx	2-3 roots + aerial roots: small tubers (2mm)
	Caty	40	40	21	xxxx	2-3 roots + aerial roots with small tubers (1mm)
C_5	Mureşan	45	45	11	xxxx	1-2 roots + aerial roots with 1-2 tubers
	Super	38	30	10	xxxx	1-2 roots + aerial roots with 1-2 tubers
	Caty	22	20	2	xx	1-2 roots + aerial roots

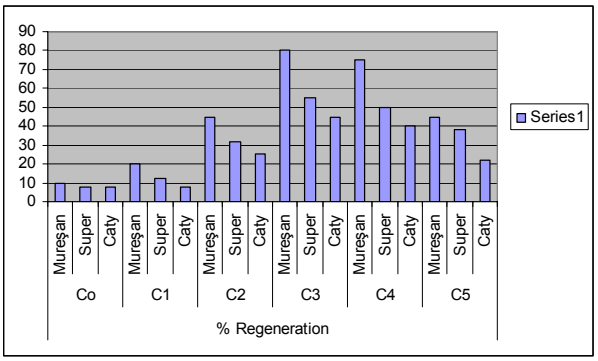

Fig. 1. In vitro regeneration percentage of the autochthonous potato varieties Mureşan, Super and Caty (after three months)

Super and Caty varieties have an inferior in vitro reaction but to these varieties the mediums with doses of 2 mg/l BA (C_3) and with added maize germs 5g/l (C_4) have given the best results (see Fig. 1 and 2). The regeneration percentage is of 55-50% at Super and of 45-40% at Caty, and rooting reaches values of 35-20% at Super variety and of 21-12% at Caty, with the formation of 2-3 roots with small tubers of 1-2mm (see Table 2).

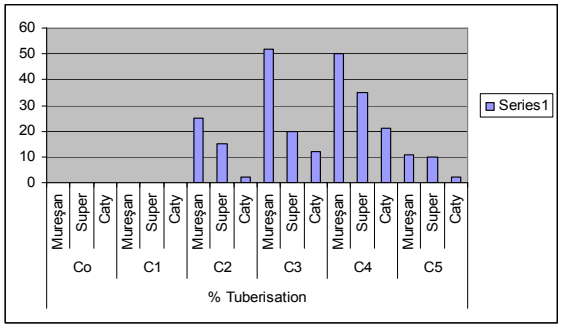

Fig. 2. In vitro rooting percentage of the autochthonous potato varieties Mureşan, Super and Caty (after three months)

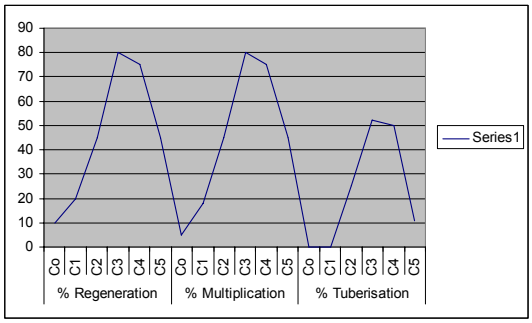

Fig. 3. The evolution of Mureşan potato variety cultivated in vitro (after three months)

Hence, the best in vitro reaction was manifested by Mureşan variety which regenerates, multiplies and is rooting depending on the variant (see Fig. 3). Rooting is signaled at a rate of 52-11% depending on the composition of the culture medium, with the differentiation of a basal and aerial radicular system on which mini tubers are developing (depending on the composition of the medium), from 1 up to 0,5cm (see Table 2).

CONCLUSIONS

1. Autochthonous potato varieties: Mureşan, Super and Caty cultivated in vitro from apical meristematic tissue have a reaction manifested in a longer time than to other varieties (in three months), reaction dependent on the variety and on the components of the culture medium;
2. Mureşan variety proved to have the best in vitro reaction, regenerating 80% and rooting 52% on the medium with a large dose of BA (variant C3), the mini tubers reaching about 0.5 – 0.6 cm. On the witness sample it is not rooting and it is regenerating only 10%;
3. The reaction of Super and Caty varieties is inferior to Mureşan variety, regenerating in a percentage of 50-22% depending on the medium, and is rooting in a percentage of 20-35% at Super variety and of 11-12% at Caty;

4. On the witness sample C_o the regeneration percentage is small, of 8-10%, rooting does not take place, and on the sample with vegetal coal C_1 regeneration is something bigger, of 12-20%;
5. Potato varieties ameliorated at Braşov-Stupini have a very different in vitro reaction, manifested depending on the variety and in a longer time;
6. The evolution of Mureşan, Super and Caty varieties is inferior to the populations from Harghita county (experimented by us), but they are varieties with a genetic value, very important for the amelioration works and with superior qualities concerning their resistance to unfavorable environmental conditions.

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