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: Mureșan, Super and Caty ameliorated at The Potato Research Institute Brașov – Stupini, around the 1980s. The plant material consisted of apical meristem cultivated on Murashige-Skoog medium, 1962 improved with 825mg/l NH₄NO₃, with the following variants: C₁=MS1/2; C₂=MS+825mg/lNH₄NO₃+5g/l coal; C₃=MS+825mg/lNH₄NO₃+0,1mg/lANA+1,0mg/l BA; C₄=MS+825mg/lNH₄NO₃+0,1mg/lANA+2,0mg/lBA; C₅=MS+825mg/lNH₄NO₃+0,1mg/lANA+5g/lEP; C₆=MS+825mg/lNH₄NO₃+0,1mg/lANA+80mg/lAdSO₄ (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 Mureșan, but this reaction is different on the five mediums: on C₃ 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 C₅ medium. On C₄ variant with 5g/l EP, Mureșan variety reaches good values (we believe that due to the content of natural zeatin that can be found in the maize germ extract, and cytokinin strongly involved in the regeneration of the vegetal tissue in vitro): the variety also presents a good reaction on the medium with cu AdSO₄ (C₅), 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 C₃ and on C₄ also (but inferior to Mureșan variety). On the control medium C₀ the parameters are small and regeneration is situated between 10-8% and without rooting: on C₁ in the presence of the vegetal coal and of NH₄NO₃ 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 Brașov-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)+ NH₄N0₃, BA, ANA, maize germ extract (EP), adenine sulfate, Solanum tuberosum L, autochthonous varieties: Mureșan, Super, Caty (ameliorated at The Potato Research Institute from Stupini-Brașov, 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, Cachiţa, 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 (Zăpărtan 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 (Cachiţă, Zăpărtan, 1991).

The plant material consisted of apical meristem detached from the tubers of the three Mureşan, 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 NH₄NO₃, 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

<table>
<thead>
<tr>
<th>Var.</th>
<th>The basal medium</th>
<th>Vegetal coal</th>
<th>Auxin ANA (mg/l)</th>
<th>Cytokinin BA (mg/l)</th>
<th>Maize</th>
<th>Adenine sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀</td>
<td>MS/2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C₁</td>
<td>MS⁺ 825 mg/l NH₄NO₃</td>
<td>5 g/l</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C₂</td>
<td>MS⁺ 825 mg/l NH₄NO₃</td>
<td>-</td>
<td>0,1</td>
<td>1,0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C₃</td>
<td>MS⁺ 825 mg/l NH₄NO₃</td>
<td>-</td>
<td>0,1</td>
<td>2,0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C₄</td>
<td>MS⁺ 825 mg/l NH₄NO₃</td>
<td>-</td>
<td>0,1</td>
<td>-</td>
<td>5 g/l</td>
<td>-</td>
</tr>
<tr>
<td>C₅</td>
<td>MS⁺ 825 mg/l NH₄NO₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>80 mg/l</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

After two months of in vitro culture, the proliferation capacity of the three potato varieties was low, an 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: Mureșan, Super, Caty. Following the in vitro regenerative and rooting capacity of the potato varieties, we can state that the best percentages are obtained at Mureșan variety, followed by Super and Caty, at these latter varieties the values are much more lower. So, Mureșan variety reaches a regeneration percentage of 80%, 52% rooting on medium C₃ (MS⁺ 825 mg/l NH₄NO₃ + 0,1 mg/l ANA + 2,0 mg/l BA), with the maximum dose of BA, resulting 10-12 long roots with a multitude of aerial roots on which mini tubers of 0,5-0,6 cm have differentiated. This variety has a good in vitro reaction on the medium with 5 mg/l IEP (C₄), obtaining 75% regeneration and 50% rooting, with 2-3 long roots with tubers of 4-5 mm. On the other variants the percentage values are much smaller between 20-45% regeneration and 11-25% rooting. On the witness sample C₀ and C₁ with vegetal coal, rooting did not take place (see Fig. 1 and Fig. 2).
Table 2

<table>
<thead>
<tr>
<th>Var.</th>
<th>Variety</th>
<th>Regener. %</th>
<th>Multiplic. %</th>
<th>Root. %</th>
<th>Evalut.</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀</td>
<td>Mureșan</td>
<td>10</td>
<td>5</td>
<td>-</td>
<td>xx</td>
<td>2-3 roots</td>
</tr>
<tr>
<td></td>
<td>Super</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>x</td>
<td>1 root</td>
</tr>
<tr>
<td></td>
<td>Caty</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>x</td>
<td>Without roots</td>
</tr>
<tr>
<td>C₁</td>
<td>Mureșan</td>
<td>20</td>
<td>18</td>
<td>-</td>
<td>xx</td>
<td>1-2 root+ aerial roots</td>
</tr>
<tr>
<td></td>
<td>Super</td>
<td>12</td>
<td>10</td>
<td>-</td>
<td>xx</td>
<td>1-2 root+ aerial roots</td>
</tr>
<tr>
<td></td>
<td>Caty</td>
<td>8</td>
<td>5</td>
<td>-</td>
<td>xx</td>
<td>1-2 root+ aerial roots</td>
</tr>
<tr>
<td>C₂</td>
<td>Mureșan</td>
<td>45</td>
<td>45</td>
<td>25</td>
<td>xxxx</td>
<td>5-6 root+ aerial roots with tubers (2mm)</td>
</tr>
<tr>
<td></td>
<td>Super</td>
<td>32</td>
<td>32</td>
<td>15</td>
<td>xxx</td>
<td>5 roots+aerial roots with tubers (1mm)</td>
</tr>
<tr>
<td></td>
<td>Caty</td>
<td>25</td>
<td>25</td>
<td>2</td>
<td>xxx</td>
<td>3 roots + aerial roots, small tubers</td>
</tr>
<tr>
<td>C₃</td>
<td>Mureșan</td>
<td>80</td>
<td>80</td>
<td>52</td>
<td>xxxx</td>
<td>10-12 roots+aerial roots with tubers of 0.5-0.6 cm</td>
</tr>
<tr>
<td></td>
<td>Super</td>
<td>55</td>
<td>50</td>
<td>20</td>
<td>xxxx</td>
<td>8 roots + aerial roots with small tubers on the roots</td>
</tr>
<tr>
<td></td>
<td>Caty</td>
<td>45</td>
<td>28</td>
<td>12</td>
<td>xxxx</td>
<td>5 roots + aerial roots with small tubers on the roots</td>
</tr>
<tr>
<td>C₄</td>
<td>Mureșan</td>
<td>75</td>
<td>75</td>
<td>50</td>
<td>xxxx</td>
<td>2-3 roots+aerial roots: tubers of 4-5mm</td>
</tr>
<tr>
<td></td>
<td>Super</td>
<td>50</td>
<td>50</td>
<td>35</td>
<td>xxxx</td>
<td>2-3 roots + aerial roots: small tubers (2mm)</td>
</tr>
<tr>
<td></td>
<td>Caty</td>
<td>40</td>
<td>40</td>
<td>21</td>
<td>xxxx</td>
<td>2-3 roots + aerial roots with small tubers (1mm)</td>
</tr>
<tr>
<td>C₅</td>
<td>Mureșan</td>
<td>45</td>
<td>45</td>
<td>11</td>
<td>xxxx</td>
<td>1-2 roots + aerial roots with 1-2 tubers</td>
</tr>
<tr>
<td></td>
<td>Super</td>
<td>38</td>
<td>30</td>
<td>10</td>
<td>xxxx</td>
<td>1-2 roots + aerial roots with 1-2 tubers</td>
</tr>
<tr>
<td></td>
<td>Caty</td>
<td>22</td>
<td>20</td>
<td>2</td>
<td>xx</td>
<td>1-2 roots + aerial roots</td>
</tr>
</tbody>
</table>

Fig. 1. In vitro regeneration percentage of the autochthonous potato varieties Mureșan, 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 (C₃) and with added maize germs 5g/l (C₄) 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).
Hence, the best in vitro reaction was manifested by Mureșan 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: Mureșan, 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. Mureșan 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 Mureșan 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 C₀ the regeneration percentage is small, of 8-10%, rooting does not take place, and on the sample with vegetal coal C₁ regeneration is something bigger, of 12-20%;
5. Potato varieties ameliorated at Brașov-Stupini have a very different in vitro reaction, manifested depending on the variety and in a longer time;
6. The evolution of Mureșan, Super and Caty varieties is inferior to the populations from Harghita county (experienced 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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