Genetic aspect in anther culture of Lithuanian potato (Solanum Tuberosum L.) cultivars

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The anther culture method was used for the production of doubled haploids (DH) in Lithuanian potato cultivars. Two types of donor material, (i) tubers produced from sectoring and (ii) minitubers produced from tissue culture were applied to determine the androgenic potential according to the regenerant yield and other morphogenetic factors.

‘Nida’ was found to be superior by the rate of responding anthers (17.4%). The highest rate of embryoid formation was identified for the ‘Aista’ potato cultivars (111.2 embryoids per 100 responding anthers). The regeneration potential of Lithuanian potato cultivars by direct microspore embryogenesis in anther culture was evaluated in this experiment. Regenerants were obtained in three cultivars (‘Goda’, ‘Nida’ and ‘Aista’) out of the five studied. In the ‘Aista’ cultivar, up to 40.3 regenerants per 100 responding anthers developed from embryos.

Key words: potato, anther culture, responding anthers, embryoids, regenerants

INTRODUCTION

Ploidy manipulation has been used in potato (Solanum tuberosum L.) breeding since the 1980s. The main ploidy manipulation procedures were described by Chase [1], Mendiburu and Peloquin [2], Iwanaga [3], and Ortiz [4].

The genetic basis of anther culture responsiveness has been studied in potato. Wenzel and Uhrig [5] as well as Sonnino et al. [6] proposed more than one recessive allele to control anther culture response. Singst and Veilleux [7] suggested that one dominant allele controls anther culture competence, using a diploid potato species. Using anther culture and leaf disc culture in S. chacoense, Veronneau et al. [8] concluded that two genes control the induction of embryo formation from microspore and two additional genes control embryo regeneration. Taylor and Veilleux [9], working with S. phurea to determine the inheritance system for leaf disc regeneration, anther culture response and protoplast culture, proposed the action of one codominant gene with an additive effect for anther culture response.

The regeneration capacity of microspores is dependent on the genotype and can be transferred via sexual recombination [5]. Studying crosses between S. chacoense and S. tuberosum, Cappadocia et al. [10] concluded that the regenerative capacity can be transferred via breeding and recover highly responsive genotypes in order to obtain clones more efficient in the development of embryoids. Jacobsen and Sopory [11] also showed the possibility of sexual transfer for the ability to form embryos from microspores. Therefore, incorporating the genes controlling high responsiveness to anther culture into non- or low-responsive genotypes may enhance the yield of microspore embryogenesis in potato [7].

One of the limitations of the 2n gametes approach has been the poor tuber characters of the 4x progenies inherited from the male or female diploid parents [12], although some dihaploid 2x clones with a different genetic background and first division restitution (FDR) 2n gametes have been obtained [13]. Since the male parent exerts a strong effect on the performance of 4x progenies, it is valuable to improve the tuber characteristics of 2x male parent characters. First, wild and closely related diploid potatoes with first division restitution (FDR) 2n gametes were crossed with cultivated tetraploid potato breeding lines or 2n egg diploid hybrids. Then, dihaploids were induced from the tetraploid hybrid populations (2n = 4x = 48) by means of anther culture and microspore embryogenesis.

In potato, there are a number of factors that influence the triggering of microspore embryogenesis. Considering the improvement of culture techniques, it is now possible to induce microspore-derived embryoids in a large number of plant species. These factors can be genetical, physiological, physical or chemical, which
indene the microspores to enter a new developmental pathway. In this work, we aimed at obtaining microspore-derived embryos from Lithuanian potato cultivars known to respond poorly to microspore embryogenesis.

MATERIALS AND METHODS

The potato (Solanum tuberosum L.) has 48 chromosomes per somatic cell (2n = 4x = 48), so that the number of chromosomes in the egg cell and in haploid somatic cells is 24.

Plant material. The research was carried out using the anther culture protocols previously optimised in other species such as barley [14]. A study of potato microspores during pretreatment of the uninucleate stage to the early culture stage was conducted utilizing five genotypes differing in their precocity: ‘Venta’ and ‘Goda’ (early), ‘Nida’ and ‘Rasa’ (maincrop) and ‘Aista’ (late).

The donor material used was (i) tubers produced from sectoring [15] and (ii) minitubers produced from tissue culture [16].

Seeds were germinated on humidified filter paper in a Petri dish for four days at room temperature and ambient light. Seedlings were planted in 20 cm diameter pots containing a mixture of peat moss and soil (1:1). Plants were grown in the greenhouse at 25 °C for a 16 h photoperiod at 18000–20000 lux. The differences between the regeneration and the culture medium consisted in the replacement of maltose (60 g l⁻¹) by sucrose (30 g l⁻¹), of agarose (7 g l⁻¹) by agar washed (6 g l⁻¹) and in lower concentrations of plant growth regulators (0.4 mg l⁻¹ NAA and BAP). The Petri dishes were maintained in the culture chamber at 26 ± 2 °C with a 85% relative humidity with temperature 14–16 ± 2 °C.

Data statistics. At least 300 anthers from different donor plants were used for each test. Data were processed using statistical analysis for quantitative and qualitative parameters and the set of statistical data analysis software “SELEKCIJA” (author P. Tarakanovas).

RESULTS

The process of microspore embryogenesis in potato can be divided into three stages: (i) induction – the usual development of gametophyte is blocked, and an alternative sporophyte programme is induced; (ii) cultivation – the microspores produce embryoids structures; (iii) regeneration – haploid plants are regenerated from androgenic embryoids.

The regeneration potential of five Lithuanian potato cultivars by direct microspore embryogenesis in the anther culture was evaluated in this experiment using the anther culture method. Embryoids were formed in the anther culture of all the five potato cultivars (Table).

The data of our experiment show that the conditions for the growth of the donor plant affect the efficiency of microspore embryogenesis. The best res-
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ponding anthers were obtained from ‘Nida’ (24.0%) when the plants were used from tuber.

The lowest rate of responding anthers was obtained in ‘Goda’ (5.0%), despite the rather high number of microspore-derived structures per 100 responding anthers (106.0). The highest rate of embryoid (Fig. 1) formation was identified for ‘Aista’ and ‘Nida’, reaching respectively 204.3 and 137.5 embryoids per 100 responding anthers (Table).

Using the anther culture, plants-regenerants from microspore-derived structures developed only in three cultivars, ‘Goda’, ‘Nida’ and ‘Aista’ (Fig. 2), suggesting that anther culture response is predetermined by the genotype.

**DISCUSSION**

Lithuanian cultivars show a high variation in terms of anther culture response and some of them respond quite interestingly. According to the results presented here, the ‘Aista’ cultivar has the highest androgenic potential among the Lithuanian potato cultivars tested. Our results confirm that the induction response in anther culture, embryoid formation, regeneration potential and the ratio of regenerants are controlled genetically as was discussed in the literature [4, 7, 10].

The frequency of haploids obtained from anther culture of potatoes is also highly dependent on the genotype. In many cases, haploids are difficult to recognize from anther-derived plants with an unreduced chromosome composition [1]. Therefore, determination of the ploidy level in the regenerated plantlets using nuclear DNA content analysis is suggested.

The study on five cultivars by this method has shown that regenerants can be obtained from embryos of cvs. ‘Goda’, ‘Nida’ and ‘Aista’. In these specific cultivars, which commonly regenerate into plants with unreduced ploidy, the first regenerated plants are mainly tetraploids. Thus, dihaploids seem to have a slower regeneration rate compared to tetraploids [17].

In potato breeding programs, dihaploids are used to cross them with diploid species. Diploid hybrid species

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Origin</th>
<th>RA (%)</th>
<th>EM/RA</th>
<th>RP/RA</th>
<th>RP/EM</th>
</tr>
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<tbody>
<tr>
<td>‘Venta’</td>
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<td>7.7</td>
<td>8.7</td>
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<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Minitubers</td>
<td>4.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<td>‘Goda’</td>
<td>Tubers</td>
<td>5.0</td>
<td>106.7</td>
<td>53.3</td>
<td>50.0</td>
</tr>
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<td>28.6</td>
<td>19.0</td>
<td>66.7</td>
</tr>
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<td>15.3</td>
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</tr>
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<td>137.5</td>
<td>62.5</td>
<td>45.5</td>
</tr>
<tr>
<td>‘Rasa’</td>
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<td>3.3</td>
<td>0.0</td>
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</tr>
<tr>
<td>‘Aista’</td>
<td>Tubers</td>
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<td>18.2</td>
<td>6.1</td>
<td>33.3</td>
</tr>
<tr>
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<td>74.5</td>
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<td>LSD 0.01</td>
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<td>3.98</td>
<td>2.48</td>
<td>9.14</td>
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are then used in $4x \times 2x$ mating after screening the diploids ($2n = 2x = 24$) for ability to produce $2n$ gametes. These plants are used in crosses with tetraploid $S. tuberosum$ cultivars ($2n = 4x = 48$) to produce $4x$ progenies. The genetic control of $2n$ gamete formation appears to be due to a few major recessive genes. This fact makes the $2n$ trait a good target for gene identification by methods such as bulked segregant analysis. If a gene is located, it could be transferred into agriculturally desirable diploids for crossing with tetraploids.

We have shown that the ability to produce microspore-derived plants is dependent on the genotypes and suggest that deeper genetic studies should be undertaken to characterize this parameter.

Received 8 December 2006  
Accepted 22 January 2007

References