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The 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. chaccoense*, 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

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induce 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 cells ($2n = 4x = 48$), 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 preocity: '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 week under a 16 h photoperiod (18000–20000 lux) at an approximately 80% relative humidity. Natural light was supplemented, from September to April, with artificial sodium lighting (400 Watts Sodiclaude) to maintain a photon flux density of 300–350 μE /m^{-2}/s^{-1} at the soil surface. Stresses such as pesticide treatment, water deficiency or temperature fluctuation were avoided during the plant growth.

Flower bud sampling and sterilization. Flower buds were collected when the microspores were at the uninucleate or early binucleate stages. This normally occurs when the buds are 4–6 mm long. The anthers were squashed in acetic carmine (5% carmine (w/v) in 45% (v/v) acetic acid boiled for 1 h and filtered) on a glass slide. Acetic carmine binds to DNA and delineates the location and the number of nuclei in the microspore. Flower buds were then sterilized in 70% ethanol for 5 min and rinsed in sterile distilled water for 5 min. The anthers were placed into Petri dishes 5 cm in diameter, 30 anthers per dish.

Anther pre-treatment. Thirty anthers collected from the same flower buds were incubated in a 5.5 cm diameter Petri dish in 10 ml of a medium containing mannitol (62 g l^{-1}) providing an osmotic pressure of 180 mosm l^{-1}. Anthers were pretreated at 4 °C in the dark for 4 days at a 80% relative humidity.

Anther culture. After pretreatment, anthers were transferred without rinsing on the medium [14] composed of macro-element salts including KNO_3 (1.9 g l^{-1}), KH_4NO_3 (0.166 g l^{-1}), KH_2PO_4 (0.170 g l^{-1}), $CaCl_2$ (0.020 g l^{-1}), $MgSO_4 \cdot 7H_2O$ (0.374 g l^{-1}) and micro-element salts including KI (0.830 mg l^{-1}), $MnSO_4 \cdot 4H_2O$ (22.3 mg l^{-1}), $Na_2MoO_4 \cdot 2H_2O$ (0.250 mg l^{-1}), H_3BO_3 (6.2 mg l^{-1}), $ZnSO_4 \cdot 4H_2O$ (8.6 mg l^{-1}), $CuSO_4 \cdot 5H_2O$ (2.500 mg l^{-1}), Fe-Na-EDTA (40 mg l^{-1}). This medium was supplemented with glutamine (752 mg l^{-1}), maltose (60 g l^{-1}) and mannitol (32 g l^{-1}). The pH was then adjusted at 5.6. Agarose (7 g l^{-1}), myoinositol (0.1 mg l^{-1}), thiamine-HCl (0.4 mg l^{-1}), as well as filter sterilized NAA (2 mg l^{-1}) and BAP (1 mg l^{-1}) were added.

Thirty anthers were plated per a Petri dish. The dishes were sealed with parafilm and maintained in the culture chamber at a constant temperature of 26 ± 2 °C in the dark for 2–4 weeks, monitoring the initiation of embryoid formation.

Plant regeneration. When microspore-derived embryoids measured approximately 1–2 mm, responding anthers were collected and transferred to a regeneration medium [14]. 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 and a 85% relative humidity with a 16 h photoperiod at 18000 lux.

When the green regenerants reached the length of approximately 5–7 cm with the coleoptiles, 1–2 cm roots and 1–2 green leaves, they were removed from the culture tubes using pincers and transferred into pots containing a sand / turf / soil mixture (1 / 1 / 1). The covered pots were kept in the climate chamber or in the greenhouse under controlled plant growth conditions (photoperiod 16 / 8 h, light intensity 18000–20000 lux, 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. Both the number of responding anthers and the formation of microspore-derived structures were genotype-dependent. The best res-

Table. **Formation of potato regenerants from embryoids in the anther culture of Lithuanian cultivars**

Cultivars	Origin	RA (%)	EM/RA	RP/RA	RP/EM
'Venta'	Tubers	7.7	8.7	0.0	0.0
	Minitubers	4.7	0.0	0.0	0.0
'Goda'	Tubers	5.0	106.7	53.3	50.0
	Minitubers	7.0	28.6	19.0	66.7
'Nida'	Tubers	24.0	11.1	15.3	137.5
	Minitubers	10.7	137.5	62.5	45.5
'Rasa'	Tubers	16.0	2.1	0.0	0.0
	Minitubers	10.0	3.3	0.0	0.0
'Aista'	Tubers	11.0	18.2	6.1	33.3
	Minitubers	15.7	204.3	74.5	36.5
$LSD_{0.01}$	3.98	2.48	9.14	5.71	

RA, responding anthers; EM/RA, embryoids per 100 responding anthers; RP/RA, regenerated plantlets per 100 responding anther; RP/EM, regenerants per 100 embryoids.

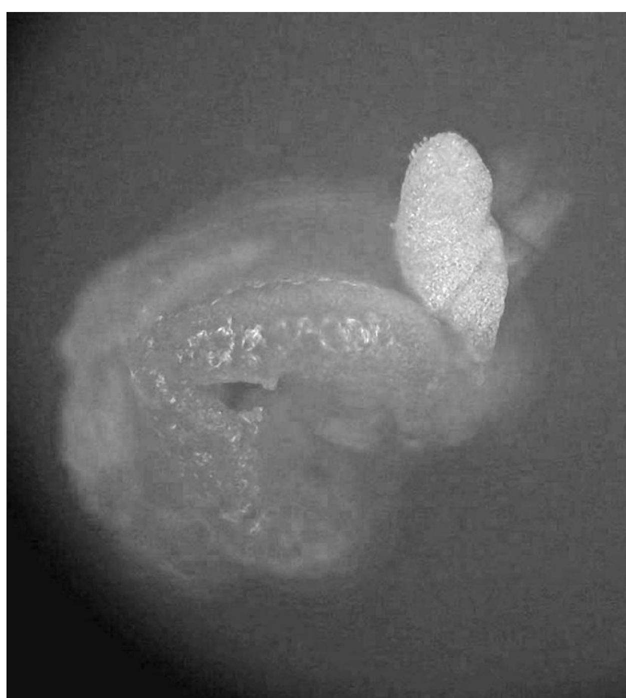

Fig. 1. *In vitro* culture of anthers of potato, showing direct embryoid formation

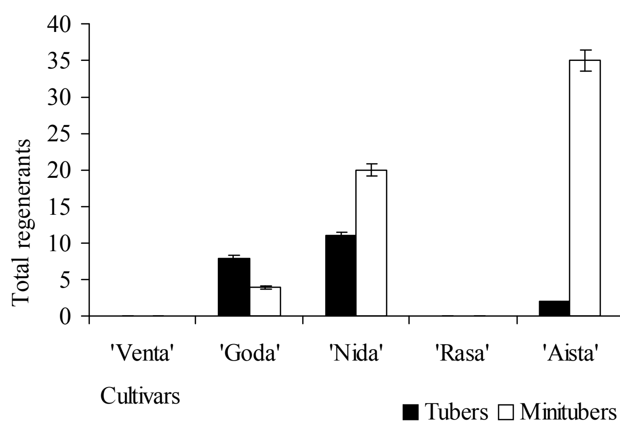

Fig. 2. The effect of donor material used for the formation of plants-regenerants

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 embryoids 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

are then used in $4x \times 2x$ maiting 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 sergregant analysis. If a gene is located, it could be transferred into agronomically 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.

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GENETINIAI ASPEKTAI LIETUVIŠKŲ BULVIŲ VEISLIŲ DULKINIŲ KULTŪROJE

Santrauka

Bulvių dvigubų haploidų (DH) gavimas dulkinių kultūros metodu atliktas 2006 m. Reimso augalų reprodukcijos ir streso apsaugos laboratorijoje (Prancūzija). Bandymo metu įvertintas bulvių veislių androgeninis potencialas dulkinių kultūroje pagal regenerantų išeigą ir kitus morfogenetinius potencialo rodiklius.

Tiriant lietuviškų bulvių veislių androgeninį potencialą nustatyta, kad daugiausia produktyvių dulkinių suformavo veislės 'Nida' (17,4%), o embrioidų – veislės 'Aista' (111,2 embrioidų, tenkančių 100-ui produktyvių dulkinių) augalai. Palyginus lietuviškas bulvių veisles pagal regenerantų išeigą nustatyta, kad daugiausia regenerantų gauta iš veislės 'Aista' dulkinių (40,3 regenerantų, tenkančių 100-ui produktyvių dulkinių). Augalai regenerantai buvo gauti iš veislių 'Goda', 'Nida' ir 'Aista' embrioidų.