


Zal 1. Poster


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Trials on androgenesis in tomato *Solanum lycopersicum* L. with the use of microspore cultures



Agnieszka Kielkowska, Adela Adamus, Viyaleta Piasetskaya, Katarzyna Rolka

University of Agriculture, Al. Mickiewicza 21, 31-120 Krakow, Poland, email: a.kielkowska@urk.edu.pl



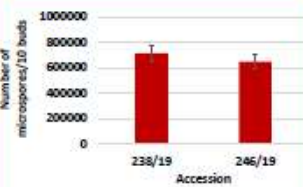
BACKGROUND

Tomato (*Solanum lycopersicum* L.) encounters as one of the world's most important vegetable crops. Doubled Haploids (DH) have a particular significance for tomato breeding, however tomato is strongly recalcitrant species in terms of haploidization (Segui-Simarro 2007). Although researches have tried to induce haploids of tomato, the results of using anther or microspore culture have remained unsatisfactory (Yuan et al. 1999; Segui-Simarro 2007).

METHODS


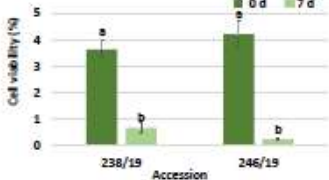
This study focused on the trials of induction of androgenesis tomato in microspore cultures. The research was conducted for two breeding lines (238/19 and 246/19). The stage of bud development was evaluated with 4,6-diamidino-2-phenylindole (DAPI). The optimal buds used for culture establishment were these, in which >50% of cells were microspores. Selected buds were surface sterilized, and microspores were released by crushing anthers in a petri dish with induction medium (190/2 and TMC1). The suspension was purified by filtering and centrifugation and the culture was established. The culture density was 50 000 microspores in 1 ml of medium. The analysis of cell viability was conducted with fluorescein diacetate (FDA). Temperature stress (4°C and 32°C) was applied to the cultured microspores for 1-3 days, controls were not treated.

RESULTS – effectiveness of isolation



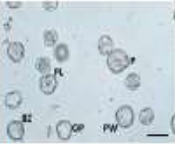
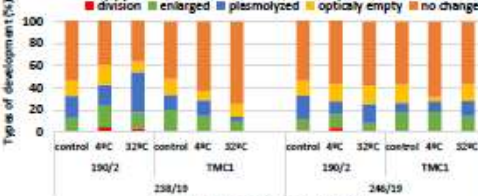
Efficiency of the isolation of microspores in two accessions (238/19 and 246/19) of tomatoes, based on 10 flower buds

RESULTS – cell viability



Viability of tomato microspores in the control culture depending on the duration of the culture and the accession. Bars marked with the same letter do not differ statistically significantly (p = 0.05, HSD).

RESULTS – development after 21 days of culture

Development in the culture of tomato microspores depending on the accession (238/19 and 246/19), the duration of the culture (7 and 21 days) and the medium (190/2 and TMC1).

RESULTS – callus development

First mitotic divisions of tomato microspores
Microspore-derived callus clump of tomato developed after cold treatment

CONCLUSIONS

- ◆ The analysis of cell viability conducted with fluorescein diacetate (FDA) showed surprisingly low viability cultured microspores
- ◆ Frequency of microspores undergoing divisions was very low (< 2%) and was noted mostly in the cultures subjected to thermal shocks (32°C and 4°C for 2 days).
- ◆ Undisturbed cell divisions resulting in the development of callus tissue was observed in one (238/19) out of two tested lines and was observed on the medium supplemented with 2,4-D, NAA and BAP (TMC1 medium) after application of cold stress (4°C).

ACKNOWLEDGMENTS


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Załącznik 2. Poster

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Trials on gynogenesis in tomato (*Solanum lycopersicum* L.) in ovary and isolated ovule cultures

Kielkowska Agnieszka*, Adamus Adela, Chachłowska Dorota
University of Agriculture in Kraków, Al. Mickiewicza 21, 31-120 Kraków, Poland,
*Corresponding author: a.kielkowska@urk.edu.pl, tel.: 12 662 51 90



Introduction


Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crop in the world in terms of both production and harvest area. However, despite its economic importance all over the world, doubled-haploid (DH) technology is not applied in tomato breeding programs, due to the lack of effective and repeatable method of haploid induction in this species. The study aimed to the induction of the development of haploid tissues by means of gynogenesis.

Methodology

The research was conducted on two breeding lines (238/19 and 246/19). Selected flower buds were taken from the donor plants and sterilized (10% chloramine T for 15 min.). Gynogenesis was induced in unpollinated ovary and ovule cultures. Whole ovaries were isolated from tomato flower buds and the style was removed. In the second experiment ovaries were dissected from the ovaries. During isolation ovaries were detached, as much as possible from the placenta. Both ovaries and ovules were cultured on two solid media: G1 (B5+BAP+2,4-D) and G4 (B5+BAP+2,4-D+NAA). Ploidy of obtained calluses was analyzed with flow cytometer.


Results

Donor plants



Plants of tomato were cultivated in the greenhouse and subjected to flowering.

Analyses of the flower development prior to selection of optimal stage of explants for culturing




Flower development in tomato. Observations of early (a), middle (b) and late (c) phase of stamen and pistil development. Scale bar 1 mm.

The optimal phase for culturing were buds in the middle stage, where the pistil was completely formed and pollen was not released from the anthers.

Ovary culture

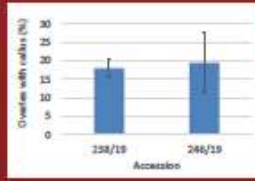
Accession	Media	No. of plated ovaries
238/19	G1	200
	G4	196
246/19	G1	198
	G4	200
Total		794

On both agar media near 800 ovaries were plated.



The edevelopment in isolated ovary culture of tomato. Scale bar 1 cm.


The ovaries, after being placed on the nutrient media usually enlarged in size (blue arrow), callused (red arrow) or died (green arrow). Callusing was observed mainly from outer layers of ovaries.



The analysis of the effectiveness of development of isolated ovaries showed no differences in the ability to callusing between the tested accessions ($p = 0.58$) nor the media ($p=0.72$). Callusing was in approx. 18 to 19.5% of the cultured ovaries.

Conclusion Ovary breaking and ovule growth was not observed, suggesting that the technique is ineffective.

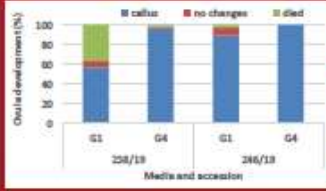
Ovule culture




Cross-section through the ovary of a tomato. The arrows mark the ovules on the placenta. Scale bar 1 mm.

Accession	Media	No. of ovaries used for isolation of ovules
238/19	G1	115
	G4	115
246/19	G1	125
	G4	125
Total		480

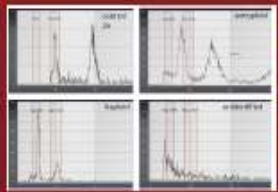
In total ovules from 480 ovaries were isolated. One ovary contains on average 100-150 ovules.



Development of isolated tomato ovules depending on accession and medium. In total, 3-37% of the cultured ovules get browned and died up to 4th week of culture, which may be the result of high stress and tissue damage during isolation. In the majority of survived ovules (56-100%) callus development was observed.




Tomato ovules on 7th day after isolation (a); callusing (red arrow) and dying ovules (blue arrow) after 14 days (b); callus obtained from ovules after 4 weeks of culture (c). Scale bar 1 cm.




The callus obtained from the cultured ovules in some cases was so abundant that it was possible to perform ploidy analysis. Most of the analyzed callus clumps was identified as diploid (64%), however haploid tissues (5%) were also detected. The remaining calluses (30%) were aneuploids. In 1% of samples the ploidy level was unidentified.

Conclusion Some portion of the callus obtained from the ovules was haploid, suggesting that the technique of isolated ovule cultures is promising.




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Załącznik 3. Poster

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Trials on the haploidization in *Vicia faba* L. and *Lactuca sativa* L. after distant pollination

Kielkowska Agnieszka¹, Adamus Adela¹, Skrzyżkowski Wiktor¹, Kiszczak Waldemar², Podwyszyńska Małgorzata², Marasek-Ciołakowska Agnieszka², Góraj-Koniarska Justyna², Burian Maria²

¹ University of Agriculture in Kraków, Al. Mickiewicza 21, 31-120 Kraków, Poland, a.kielkowska@urh.edu.pl, tel.: 12 662 51 90

² Department of Applied Biology, The National Institute of Horticultural Research, Konstytucji 3 Maja 1/3 Str., 96-100 Skierniewice, Poland, waldemar.kiszczak@inhort.pl



INTRODUCTION


A haploid is an organism that has a chromosome set of a reduced gamete in the sporophyte. Doubling of the genome of a haploid plant leads to the development of doubled haploid (DH) plant. The application of DH in breeding is based on their potential for producing true homozygous lines in one generation, which shortens the production of new varieties in plants. Both *Vicia faba* L. and *Lactuca sativa* L. are popular vegetables consumed worldwide; however, effective and repeatable protocols for their haploidization have not been developed.

METHODOLOGY


The aim of the study was to stimulate the development of haploid cells of the female gametophyte after distant pollination. *V. faba* was hand pollinated with *Phaseolus vulgaris* pollen, while *L. sativa* was hand pollinated with the pollen of *Helianthus annuus*. In both crossings, pollination was preceded by emasculation. The germination of foreign pollen grains on stigmas was analyzed under a fluorescence microscope with aniline blue. Approximately 3-7 days after pollination (DAP) pistils of *V. faba*, ovaries of *L. sativa* and unpollinated controls were cultured *in vitro*. The development of explants during *in vitro* culture was monitored.

RESULTS


Donor plants and pollinators




Plants of *V. faba* (a; mother plant) and *P. vulgaris* (b; pollen donor) cultivated in the greenhouse.




Flowers of mother and pollinator.



Tagged *V. faba* flowers pollinated with *P. vulgaris* pollen (a); aborted pollinated bud (b).




Plants of *L. sativa* cultivated in the greenhouse.




Flowering of *L. sativa* (a,b; mother plant) and *H. annuus* (c; pollen donor).

Analyses of flower development prior to selection of optimal phase for foreign pollination

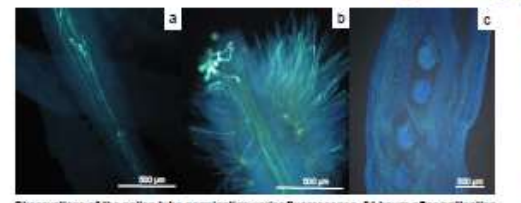


Flower development in *V. faba*. Observations of early (I), medium (II) and late (III) phase of stamen and pistil development. Hairy stigma marked with arrow.

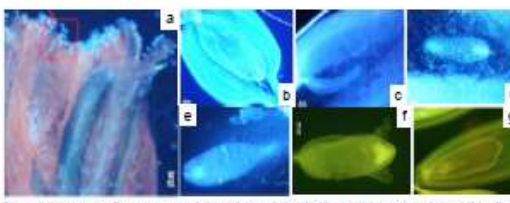


Flower development in *L. sativa*. Observations of early (I), medium (II) and late (III) phase of stamen and pistil development. 1 - stigma, 2 - anthers.

Analyses of foreign pollination with anilin blue

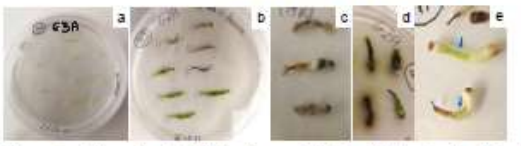


Observations of the pollen tube germination under fluorescence, 24 hours after pollination. In control (a); self-pollination of *V. faba*; and after pollination of *V. faba* with pollen of *P. vulgaris* (b,c). a) multiple pollen tubes in control pollination; b) germinating pollen tubes of *P. vulgaris* on the stigma of *V. faba*; c) *V. faba* ovary with ovules - any of the foreign pollen tubes reached the ovules.




Observations under fluorescence of the stigma of the pistil and the ovaries of *L. sativa* after pollination with pollen of *H. annuus*. a) germinating pollen on the stigmas 24 hours after pollination; b-g) *L. sativa* ovaries and ovules: b-d) ovules with no development; a) globular embryo 48 h after pollination; f) heart-shape embryo 72 h after pollination; g) degenerating ovule 72 h after pollination.

In vitro culture of pistils and ovules



Observations of the culture of pistils from flowers of *V. faba* pollinated with pollen of *P. vulgaris*. a) pistils isolated to *in vitro* conditions 5-7 DAP; b) culture after 21 days; c) development of callus tissue on the surface of the ovary on 30 day of culture; d) browning of the medium surrounding the explants; e) enlarged ovules inside the ovary (arrows).




Pollinated ovaries of *L. sativa* isolated on the culture medium 3-6 DAP, showed no signs of further development.

CONCLUSIONS

1. *Vicia faba* - pollen of *P. vulgaris* germinates on the stigma of *V. faba*, however entering of the pollen tubes in to *V. faba* ovules was not observed 24 h after pollination. Pistils isolated to *in vitro* conditions produced callus, and extracted phenolics to the medium. In some explants enlargement of the ovules inside the ovaries was observed, however embryogenesis did not occurred.
2. *Lactuca sativa* - after pollination with pollen of *H. annuus* rare development of early staged embryos was observed in the *L. sativa* ovaries. Ovaries isolated to *in vitro* conditions did not produced callus tissue either the embryos.

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