

Zal 1. Poster

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Culture of pistils and isolated ovules of *Vicia faba* L. after distant pollination



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Introduction

The application of doubled haploid (DH) technology in plant breeding is based on its potential for producing true homozygous lines in one generation. The utilization of DH in breeding programmes considerably shortens the production of new varieties in plants. *V. faba*, also known as broad bean or fava bean, belongs to the Fabaceae family. *V. faba* is a popular and valuable vegetable species consumed worldwide, however very little works on haploidization has done so far. Moreover, this species is highly recalcitrant to tissue cultures including haploidization methods. This study aimed at the stimulation of the development of haploid cells of the female gametophyte of *V. faba* after distant pollination. As a pollen donor, a distant relative of *V. faba* belonging to Fabace family - *Lathyrus odoratus* was used.

Materials and methods

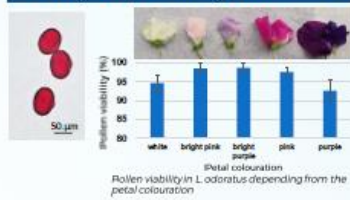
As a plant material, two commercial cultivars (Bartek, Rambos) of *V. faba* were used. Pollen viability of pollen donor - *L. odoratus* - was analyzed with acetocarmine. Selected flower buds of *V. faba* in which self-pollination did not occur, were castrated and hand pollinated with pollen of *L. odoratus*. The pollen germination, after foreign and control pollinations, under a fluorescence microscope with aniline blue was analyzed. Five to seven days after pollination (DAP) pistils and ovules of *V. faba* from foreign and control combinations (K1-K4) were cultured *in vitro*. The development of explants was monitored after 50 days of culturing on two solid culture media (G6, H2).



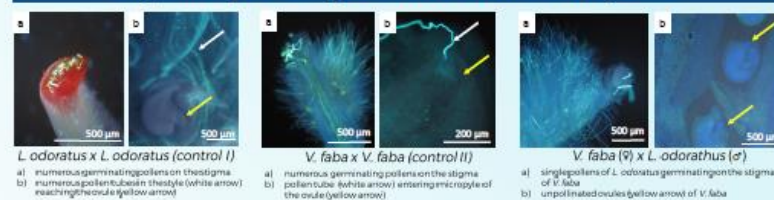
V. faba plants grown in greenhouse conditions a) seedlings b) mature plants
Plants of pollen donor *L. odoratus* grown in open-field conditions
Tagged *V. faba* flowers pollinated with *L. odoratus* pollen (a) viable (b) and aborted (c) pollinated buds 5 DAP

Results

Analyses of pollen viability of *L. odoratus*



Analyses of foreign and control pollinations with anilin blue – 24 h after pollination



L. odoratus x *L. odoratus* (control I)
a) numerous germinating pollens on the stigma
b) numerous pollen tubes in the style (white arrow) each in the ovule (yellow arrow)
V. faba x *V. faba* (control II)
a) numerous germinating pollens on the stigma
b) pollen tube (white arrow) entering micropyle of the ovule (yellow arrow)
V. faba (♀) x *L. odoratus* (♂)
a) single pollens of *L. odoratus* germinating on the stigma of *V. faba*
b) unpollinated ovules (yellow arrow) of *V. faba*

In vitro culture of pistils

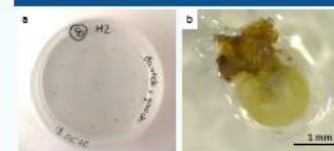


Culture of pistils isolated from flowers of *V. faba* pollinated with pollen of *L. odoratus* a) pistils isolated to *in vitro* conditions 5-7 DAP b) pistil culture after 50 days browning (blue arrow) and callusing on pistils (red arrow)

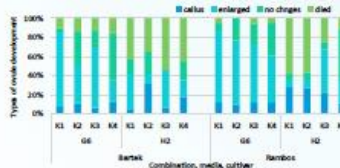


Development in the culture of pistils isolated from flowers of *V. faba* pollinated with pollen of *L. odoratus* and controls depending on the combination (K1, K2 - no pollination; K3, K4 - pollination), media (H2, G6) and cultivar (Bartek, Rambos)

In vitro culture of isolated ovules



Culture of ovules isolated from flowers of *V. faba* pollinated with pollen of *L. odoratus* a) ovules isolated to *in vitro* conditions 5-7 DAP b) culture after 50 days - ovule callusing at the micropylar site



Development in the culture of ovules isolated from flowers of *V. faba* pollinated with pollen of *L. odoratus* and controls depending on the combination (K1, K2 - no pollination; K3, K4 - pollination), media (H2, G6) and cultivar (Bartek, Rambos)

Conclusions

1. Pollen of *L. odoratus* was highly viable (92-98%), thus suitable to use as pollinator
2. Pollen of *L. odoratus* germinates on the stigma of *V. faba* however entering of the pollen tubes into *V. faba* ovules was not observed 24 h after pollination.
3. Pistils isolated from foreign pollinated flowers 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 occur. The callus developed mostly from somatic tissues of pistils.
4. Some ovules isolated from pistils of foreign pollinated flowers to *in vitro* conditions produced callus. The callus developed on the micropylar site of the ovules.

Acknowledgments

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Conference



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2. Poster

Kielkowska A., Skrzypkowski W., Adamus A., Putowska A. 2023. Ovary slice culture and isolated ovule culture in tomato (*Solanum lycopersicum* L.). 11 Konferencja Polskiego Towarzystwa Biologii Eksperymentalnej Roślin (11th Biennial PSEPB), 19-22 września 2023, Poznań, Polska, pp 183

Ovary slice and isolated ovule culture in tomato (*Solanum lycopersicum* L.)

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Introduction


The tomato (*Solanum lycopersicum* L.) is one of the vegetable plants with the highest commercial importance in the world and it ranks as one of the top vegetables in terms of worldwide production. Its global production amounts to nearly 190 million tons annually (FAOSTAT, 2021). For this reason, the development of methods aiming at obtaining doubled haploid lines (DH), to accelerate breeding in this species, is of great importance. Due to the fact that tomato is considered as a species recalcitrant to haploidization, efficient methods for obtaining haploids are still lacking. This study focuses on attempts to induce haploidization in two tomato breeding lines via gynogenesis. To achieve this goal, in vitro cultures of ovaries fragments and isolated ovule cultures of male sterile accessions were employed.

Methodology

The research was conducted on two male-sterile breeding lines of tomato (II-PS and I-MS10). Freshly opened flowers were harvested from the donor plants and sterilized (70% EtOH for 30 sec., 10% chloramine T for 15 min.). Gynogenesis was induced from unpollinated explants. Whole ovaries were isolated from tomato flowers and then ovaries were transversely cut into 1 mm thick slices. Each slice contained several ovules attached to the placenta. These explants were cultured on two media: G2 (MS+NAA+BAP) and G4 (B5+NAA+BAP+2,4-D) in dark or light. In the second experiment ovules were dissected from the ovaries utilizing elaborated method of releasing ovules to the liquid and then transferring to the solid medium. During isolation ovules were detached, as much as possible from the placenta. Excised ovules were cultured on the medium M3 (B5+NAA+2,4-D+TDZ) and medium TG2 (NAA+KIN) in the dark. Ploddy of obtained calluses was analyzed with flow cytometer.


Results

Donor plants



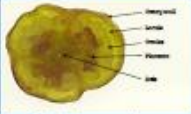
Plants of tomato were cultivated in the greenhouse and subjected to flowering.

Morphology of flowers of donor plants



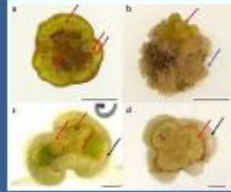
Close up to the anthers and the ovary of tested tomato accessions. In II-PS sterility was manifested by a lack of pollen release from the anthers (functional me). Anthers of I-MS10 did not contained pollen due to abnormalities in meiosis and tapetum development. The explants to the culture were taken from the blooming flowers containing mature embryosacs. Scale bar 1 mm.

Ovary slice culture



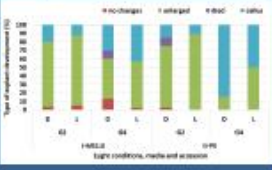
Accession	Media	No. of plated explants
I-MS10	G2	63
	G4	74
II-PS	G2	83
	G4	80
Total		300

On both agar media 300 ovary slices were plated.




The development in tomato ovary slice culture. a) explant in the day of excision b-d) explants after 30 days of culture. Red arrows indicate ovules; blue arrows - callus development; black arrows shows ovary walls. Scale bar 1 cm.

The analysis of the development of explants. Some of the explants enlarged, some showed no changes or died. The formation of callus tissue was also observed (10-84%). The results varied depending on the genotype, light conditions, and growth media.

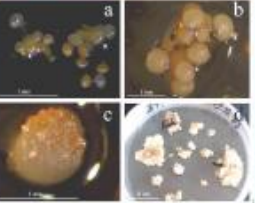


Ovule culture



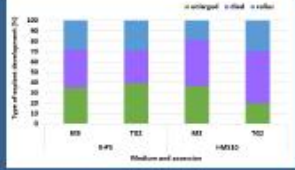
Accession	Media	No. of ovules
I-MS10	MS	643
	TG2	1177
II-PS	MS	742
	TG2	488
Total		3050

On both agar media above 3000 ovules were plated.



The development in tomato ovule culture. a) ovules on the day of excision b-c) explants after 30 days of culture d) calluses developed from ovules after 60 days of culture.

Development of isolated tomato ovules. After a 60-day growth period in darkness, 19-40% of ovules enlarged but not callused, 31-51% of ovules died, and 19-30% ovules produced callus. The effectiveness varied depending on the medium and genotype.



Conclusion

Callus developed mostly from somatic tissues of the ovaries (ovary wall), rarely from the ovules, indicating unsuitability of the applied technique.

Acknowledgements

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Conference

11th biennial PSEPB Conference 19-22 September 2023, Poznań

Conclusion

Obtainment of haploid calluses from the cultured ovules, suggest that the technique of isolated ovule cultures is promising. Further experiments aiming at shoot regeneration will be conducted.

