Zał 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 selfpollination 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).

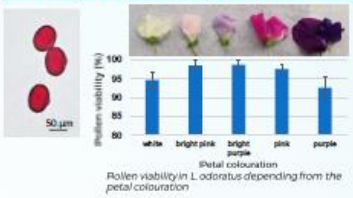
V. faba plants grown in greenhouse conditions a) seedlings b) mature plants

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

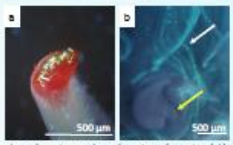
Results

Analyses of pollen viability of *L. odoratus*



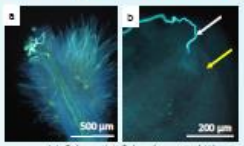
Pollen viability in L. odoratus depending from the petal colouration

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



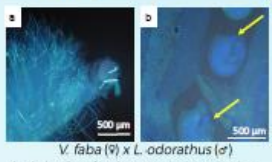
L. odoratus x L. odoratus (control I)

a) numerous germinating pollens on the stigma
b) numerous pollen tubes in the style (white arrow) reaching 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)

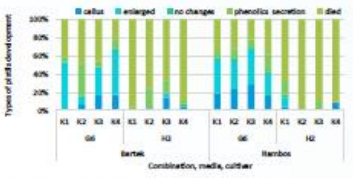


V. faba (♀) x L. odorathus (♂)

a) single pollens of *L. odoratus* germinating on the stigma of *V. faba*
b) unpollinated ovules (yellow arrow) of *V. faba*

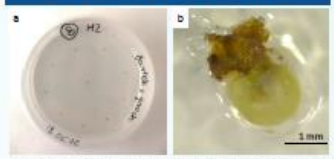
In vitro culture of pistils

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)

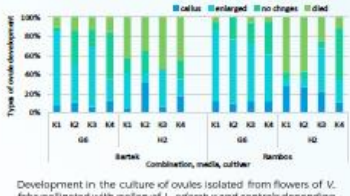


Development in the culture of pistils isolated from flowers of *V. faba* pollinated with pollen of *L. odoratus* and controls depending on the combination (K1, K2 – no pollination, K3, K4 – pollination), media (H2, G6) and cultivar (Bartek, Rambos)

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 occured. 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

Kiełkowska 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