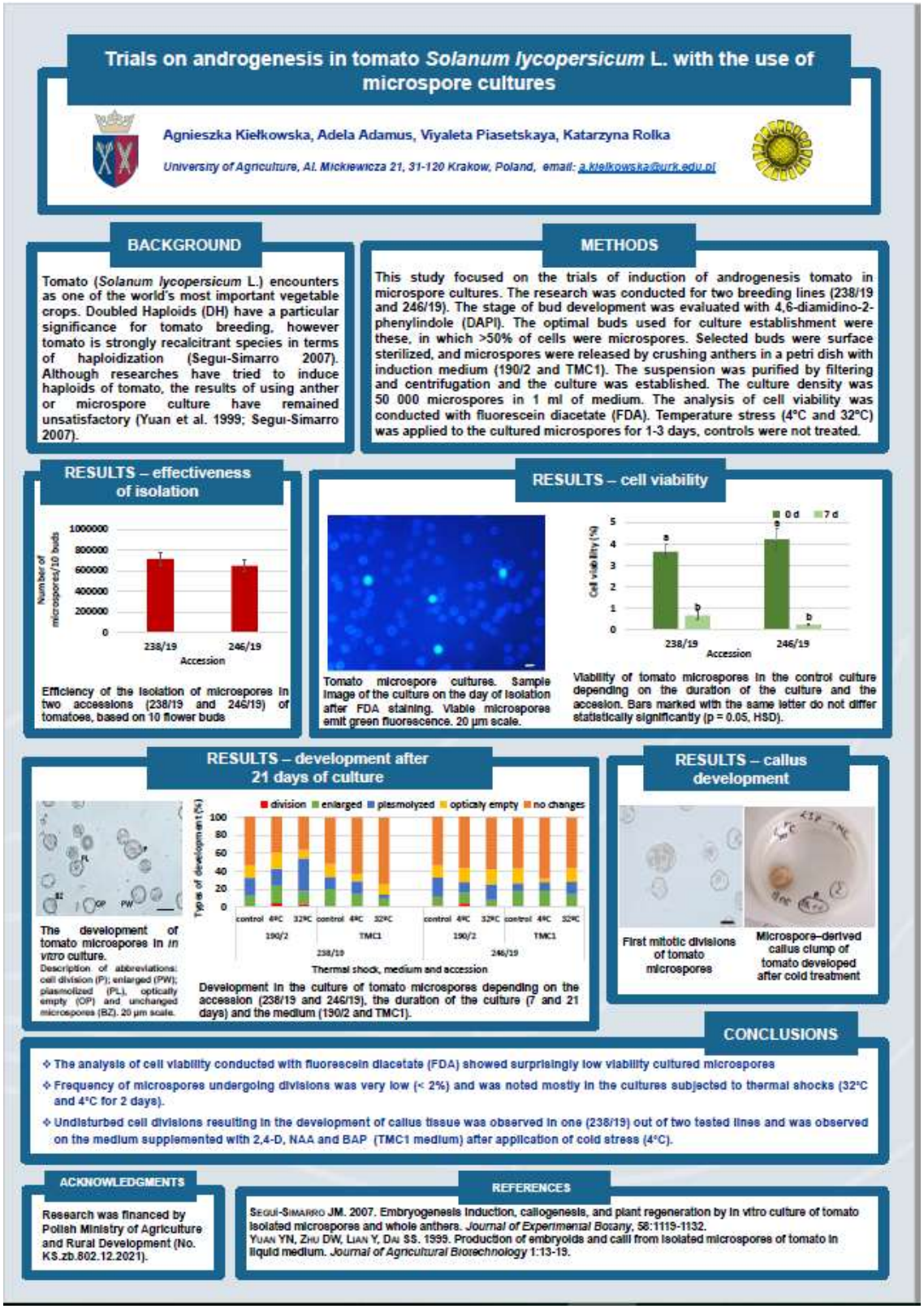
Zał 1. Poster

Kiełkowska A, Adamus A., Piasetskaya V, Rolka K. 2022. Trials on androgenesis in tomato *Solanum lycopersicum* L. with the use of microspore cultures. XXXIV Konferencja Embriologiczna, 20-23 maja, Zakopane , Acta Biologica Cracoviensia vol. 62 suppl. 1 pp. 106



Zał 2. Poster

Kiełkowska A, Adamus A., Chachlowska D. 2022. Trials on gynogenesis in tomato (*Solanum lycopersicum* L.) in ovary and isolated ovule cultures. VI[th] Polish Congres of Genetics, 27-30 czerwca 2022, Kraków, pp 273-274

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

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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 ovules were dissected from the ovaries. During isolation ovules were detached, as much as possible from the placenta. Both ovaries and ovules were cultured on two solid media: G1 (BS+BAP+2,4-D) and G4 (BS+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 completly 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

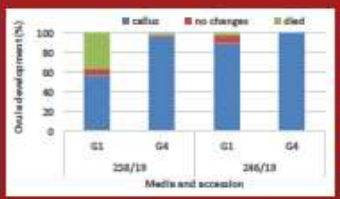
Ovary breaking and ovule growth was not observed, suggesting that the technique is ineffective.

Ovule culture

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

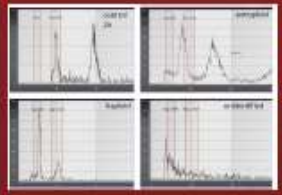
Accession	Media	No. of ovaries used for isolation of ovules
238/19	G1	115
	G4	115
246/19	G1	125
	G4	125
	Total	480

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, 1-37% of the cultured ovules get browned and died up to 4[th] 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.

Acknowledgements

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Conference

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Zał 3. Poster

Kiełkowska A, Adamus A, Skrzypkowski W, Kiszczak W, Podwyszyńska M, Marasek-Ciołakowska A, Góraj-Koniarska J, Burian M. 2022. Trials on the haploidization in *Vicia faba* L. and *Lactuca sativa* L. after distant pollination. 8th Central European Congress of Life Sciences EUROBIOTECH 20-22.06.2022, Kraków, pp 125