Zał 1. Poster

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Research on breaking the cell division latency in *Allium* protoplast cultures

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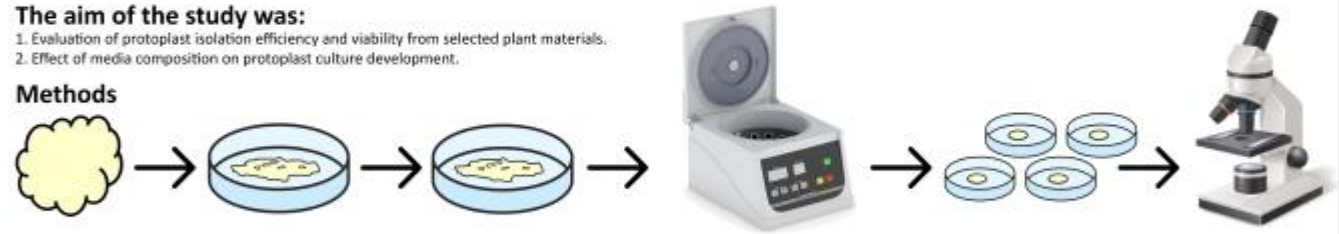
Introduction

As a unique tool for overcoming sexual incompatibility barriers and for efficient genetic transformation of plants, protoplasts are used in a variety of procedures. Various factors such as genotype, physiological state of the initial tissue, culture medium, and environmental conditions are crucial in determining whether protoplast-derived cells are totipotent and can develop into fertile plants. Onion and garlic, as well as other monocotyledonous plants, are still considered recalcitrant to protoplast culture, although considerable progress has been made in this area over the decades. This recalcitrance often manifests itself in the early stages of the cultures with abnormalities in cell wall reconstruction that prevent resumption of mitosis. Mitotic activity is often stopped very quickly, even when the cell wall is restored.

The aim of the study was:

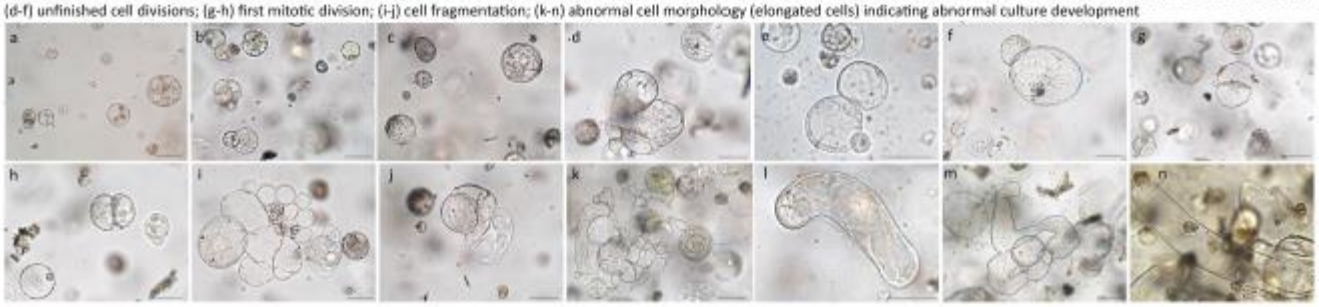
1. Evaluation of protoplast isolation efficiency and viability from selected plant materials.
2. Effect of media composition on protoplast culture development.

Methods

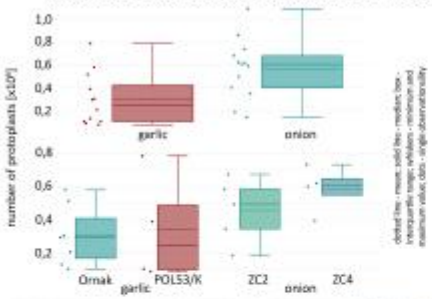


onion or garlic embriogenic callus was cut into fine pieces | 30 min incubation plasmolysis solution | overnight cell wall digestion with shaking (30 rpm) 26°C | purifying protoplasts by density gra dient centrifugation | embedding in a LPM agarose beads | microscope observations

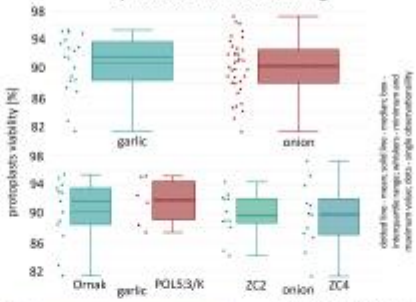
Cultures of garlic and onion protoplasts. Protoplasts isolated from (a) onion and (b) garlic callus embedded in agarose beads immediately after isolation; (c) reorganization of cytoplasm; (d-f) unfinished cell divisions; (g-h) first mitotic division; (i-j) cell fragmentation; (k-n) abnormal cell morphology (elongated cells) indicating abnormal culture development



Efficiency of protoplasts isolation from both garlic and onion callus assessed as number of protoplast per 1 g of fresh weight



Protoplast viability, assessed by fluorescein diacetate staining

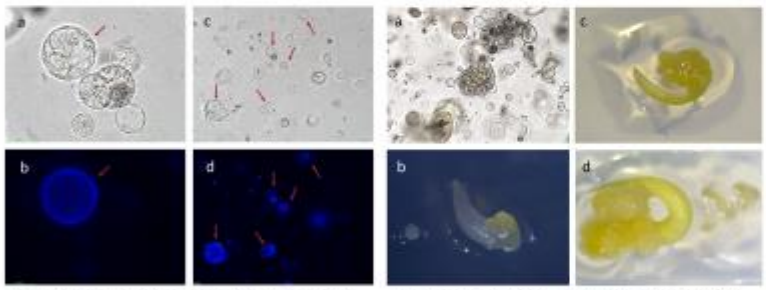


Composition of the media used in the study

Protoplasts were embedded in agarose beads and cultured in liquid CPP media [1] supplemented with growth regulators according the table, and KM media [2] supplemented with phytosulfokines (308 nM) and SAHA (10 µM).

Component	CPP-4	CPP-5	CPP-6	CPP-7	CPP-8
glucose (POCH)	52,4 g	52,4 g	52,4 g	52,4 g	74 g
2,4-D (Merck)	-	2,0	1,8	1,0	1,0
BAP (Merck)	-	-	2,0	1,0	1,0
Jasmonic acid (Duchefa)	-	-	-	-	-
PSK (Peptaltech)	-	-	-	-	100 nM
Putrescine (Merck)	-	-	-	-	8,0
CPPU (Merck)	0,25	0,5	0,25	0,25	0,25
ABA (Merck)	-	-	-	-	10 µM
sorbitol	200	100	200	200	-
honey	30 g	30 g	30 g	30 g	-
pH	5,8				
CPP SOLUTION	Filtering (0,22 µm)				

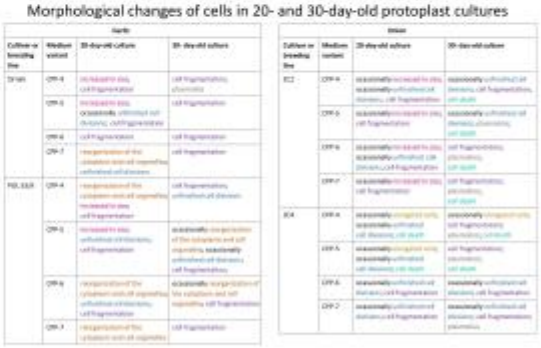
[1] M. Stelmach-Wityk, K. Szymonik, E. Grzebelus Plant Cell Tiss Organ Cult (2024, 157, 24) (31) [2] K.N. Kao, M.R. Michayluk Mb, Planta 1975,126, 105-110.



Cell wall reconstruction tracking using calcofluor-white, (a-b) 24h and (c-d) 72h after protoplasts isolation. The restored cell walls fluoresce to a blue colour (marked by a red arrow)

The addition of SAHA to the garlic protoplast cultures may be linked to the breakage of cell division latency in these cultures; (a) cells aggregates in 20-day-old protoplast culture (b-d) embryo-like structures in 120-day-old protoplasts culture

Morphological changes of cells in 20- and 30-day-old protoplast cultures



Summary

1. The enzyme mixture containing cellulase, macerozyme, and driselase effectively macerated garlic and onion callus, releasing a sufficient number of protoplasts.
2. The released callus protoplasts of all tested materials were characterized by normal morphology and high viability, approx. 90%.
3. The applied CPP-based culture media stimulated developmental symptoms typical for early protoplast cultures such as: increasing cell volume, changing cell shape, reorganizing the cytoplasm, and activating mitosis. However, the formation of multicellular aggregates was not observed in any of the culture variants.
4. KM-based culture media supplemented with phytosulfokine and SAHA induced formation of cell aggregates and callus in garlic protoplast cultures following by embryo-like structures development.

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Zał 2. Poster

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Embryogenic callus induction for protoplast cultures in the genus *Allium*

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Introduction

Somatic hybridization based on protoplast fusion is a complex biotechnological tool that involves combining somatic cells of two different varieties, species or genera to generate new variation. Thus, this technique can produce valuable starting materials for breeding programs that cannot be obtained by conventional methods or their development is time-consuming. Monocotyledonous plants, including garlic and onions, are considered difficult or resistant to protoplast culture. Various strategies are used to stimulate protoplast re-differentiation, *i.e.* isolation of protoplasts from embryogenic tissues such as embryogenic callus.

Aims of the study

Selection of the most suitable garlic and onion accessions for further attempts at protoplast isolation preceded by:

- the set-up of the protocol for an efficient induction of embryogenic callus derived from garlic cloves and zygotic embryos from onion;
- the assessment of regenerative capacity of selected callus lines

Material and methods

Plant material: six garlic and six onion cultivars/breeding lines
Source material for callus induction: garlic cloves and zygotic embryos isolated from onion seeds
Induction media: BDS medium with vitamins [1] solidified with 6 g/l agar and supplemented with 30 g/l sucrose and 2 mg/l 2,4-dichlorophenoxyacetic acid (K1 medium) or 1 mg/l 2,4-dichlorophenoxyacetic acid and 2 mg/l 6-benzylaminopurine (K2 medium);
Histological analysis: callus was embeded in Technovit resin and sections of samples were stained with toulidine blue
Assessment of regenerative capacity : induced callus was cultured on ½ BDS medium with 30 g/l sucrose or on R medium composed of BDS macro- and micro-elements, modified vitamins and 1 mg/l 1-naphthalenacetic acid, 2 mg/l 2-isopentenyl adenine and 100 g/l sucrose

[1] D.I. Dustan, K.C. Short, *Physiologia Plantarum* **1977**, *41*, 70-72

Results

Callus development was observed both on clove fragments and zygotic embryos in all examined garlic and onion cultivars/breeding lines (fig. 1a,d).

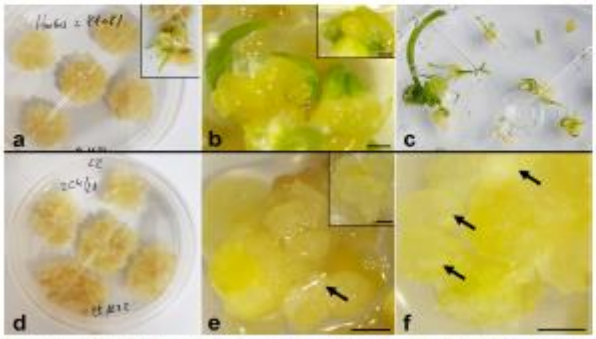

Fig. 1 Regeneration of garlic- and onion-derived callus. **a, d** – yellow, fine-grained donor callus (a – garlic, d – onion) used for plant regeneration; **b** – somatic embryos of garlic on different developmental stages; **c** – somatic embryo to plant conversion observed for garlic; **e, f** – somatic embryos of onion at globular stage (marked with arrows). Scale: 1 mm

Results

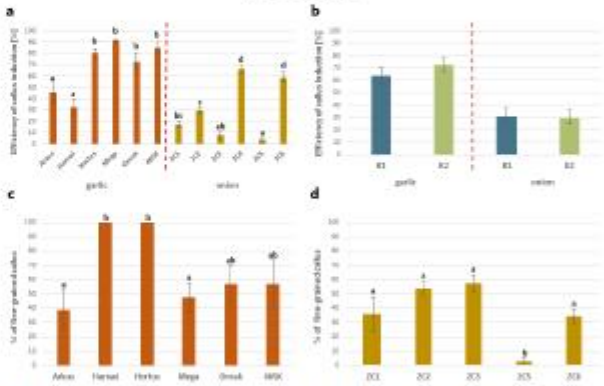

Fig. 2 a – percent (± SE) of induced callus derived from cloves (garlic) and embryos (onion) of twelve *Allium* cultivars/breeding lines; **b** – percent (± SE) of garlic and onion derived explants producing callus on K1 and K2 medium; percent (± SE) of garlic (**c**) and onion (**d**) explants forming fine-grained friable callus. Bars show standard error. Means with different letters are significantly different at 0.05 probability.

Efficiency of callus induction was different and ranged in garlic from 33% (cv. Harnaś) to 92% (cv. Mega) while in onion from 2% (breeding line ZC5) to 66% (breeding line ZC4; fig. 2a).

Similar number of explants produced callus on both tested culture media (fig. 2b), however differences between medium K1 and K2 were observed for individual garlic and onion cultivars/breeding lines.

Among the different types of callus, a preferred friable callus was observed for all examined accessions (fig. 1a,d; fig. 2c,d).

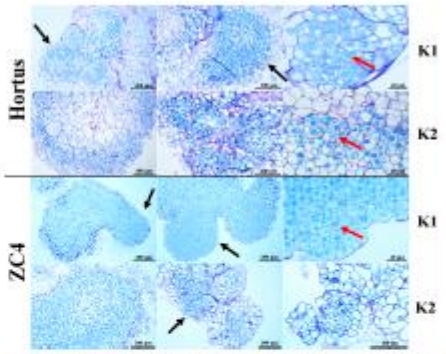

Fig. 3 Histological analysis of garlic (cv. Hortus) and onion (line ZC4) callus proliferated on K1 and K2 medium. Arrows: black – globular stuctures; red – meristematic cells.

Histological observation revealed the presence of globular structures mainly composed of meristematic cells characterized by dense cytoplasm and a round-shaped nucleus (fig. 3).

Conclusions

The source explants and culture media used allowed for efficient callus induction in both garlic and onion. The produced callus showed good regeneration ability, which was manifested by the regeneration of plants through somatic embryogenesis.

The financial support of the Polish Ministry of Agriculture and Rural Development is acknowledged (grant no. KS.zb.802.12.2021 and DHR.hn.802.13.2022)