

Summary

The genus *Fagopyrum* is a group of dicotyledonous plants belonging to the family *Polygonaceae*. The genus includes 22 species, both annual and perennial, which grow mainly in the Eurasian highlands. The most important cultivated species are common buckwheat (*F. esculentum*, also called sweet buckwheat) and Tartary buckwheat (*F. tataricum*, also called bitter buckwheat). The main reasons for the low prevalence of buckwheat cultivation in Poland and Europe are low yields, differences in dietary habits and low awareness of food producers and consumers about the nutritional value and other beneficial properties of buckwheat. Protoplast-based biotechnology and plant regeneration systems present a promising approach to overcome these limitations, facilitating the study of cellular reprogramming, totipotency, and somatic hybridisation under controlled conditions.

This doctoral thesis focuses on *F. tataricum* and *F. esculentum* as model systems to investigate protoplast development, morphogenesis processes, and *F. esculentum* (+) *F. tataricum* hybrid cells' *de novo* cell wall reconstruction, addressing critical gaps in these processes. The study aimed to optimise protoplast isolation and culture conditions by evaluating source material, immobilisation methods, and culture media additives; enhance plant regeneration via cytokinin-mediated morphogenesis; characterise *de novo* cell wall reconstruction dynamics in parental and hybrid cells obtained via protoplast electrofusion; and determine the role of phenolic regulators in developmental outcomes.

The findings, detailed across four scientific publications, highlighted how culture conditions directly influence cellular behaviour and regeneration. *F. tataricum* morphogenic callus and *F. esculentum* embryogenic callus were demonstrated to be better sources of protoplasts compared with seedlings hypocotyls, yielding higher isolation efficiency. The immobilisation of the isolated protoplasts in a low-melting-point agarose matrix, cultivated in medium supplemented with α -phytosulfokine, enhanced cell division, colony formation and the subsequent development of microcalli. Most notably, during the regeneration stage, cytokinin types and concentrations were critical to achieve morphogenesis, with thidiazuron accelerating somatic embryogenesis in *F. esculentum*, leading to whole plant regeneration within two months; while benzylaminopurine–kinetin combination promoted both somatic embryogenesis and organogenesis in *F. tataricum*, yielding regenerated plants within three months.

A novel electrofusion protocol successfully generated *F. esculentum* (+) *F. tataricum* hybrid cells, which were manually collected following an optimised sorting method, enabling a comparative analysis of cell wall regeneration. Immunolabelling of cell wall components revealed that *de novo* cell wall reconstruction in hybrid and parental cells occurs within 48 hours. It also showed species-specific dynamics in arabinogalactan proteins, extensin, xyloglucan, and pectins, with hybrid cells exhibiting parental-like patterns but a delayed methylesterified homogalacturonan deposition in *F. esculentum*, suggesting potential divergence in pectin metabolism during early wall assembly.

Finally, this study demonstrated that controlling phenolic and flavonoid contents using phenylalanine ammonia-lyase inhibitors (AIP, AOPP, OBHA) and adsorbent (PVP) directly impacts *F. tataricum* protoplast and callus responses during growth and regeneration. Low concentrations of AIP and PVP promote cell colony formation during protoplast culture and diploid plant regeneration, whereas AOPP and OBHA yield both diploid and tetraploid plants. On the other hand, plant regeneration from proembryogenic cell complexes with higher concentrations of AIP exhibited reduced oxidative stress, glutathione/oxidised glutathione ratio, and upregulated expression of embryogenesis-related genes, while PVP primarily adsorbed phenolics, altering nutrient availability.

Beyond advancing theoretical knowledge in plant biotechnology, the thesis provides transformative insights into protoplast technology, linking culture conditions to cellular responses and regeneration products. It also establishes the first reproducible protocol for *Fagopyrum* hybrid cell production and delineates the molecular and metabolic basis of phenolic-mediated regeneration bottlenecks.