Summary

This study focuses on two of the most commonly cultivated buckwheat species: common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*F. tataricum* (L.) Gaertn). These plants are a natural source of biologically active compounds due to their high phenolic compounds, flavonoids, and protein content. However, the yield of these plants is low and unstable, which limits the cultivation of buckwheat in Poland and Europe. *In vitro* cultures play a significant role in enhancing yield and improving selected traits of buckwheat. Protoplast culture is a valuable tool that, combined with genetic engineering techniques, can be used in breeding programs to expand genetic variability.

The first stage of the research presented in this doctoral dissertation involved conducting experiments to develop conditions for an effective protoplast culture of these two buckwheat species. Therefore, the influence of the following factors was examined (1) donor material for protoplast isolation, (2) immobilization medium for protoplasts, (3) phytosulfokines, (4) auxins and cytokinins, and (5) absorbents and inhibitors of phenolic compounds on cell division activity in protoplast cultures. The study demonstrated that callus with high regenerative potential is a more suitable donor material for protoplast cultures than seedling hypocotyls. Similarly, immobilization of protoplasts in agarose positively influenced their development compared to immobilization in alginate. Furthermore, it was shown that supplementation of the basal medium with phytosulfokine effectively breaks cell division latency in buckwheat protoplast cultures. Using an appropriate cytokinin combination for callus regeneration derived from protoplast cultures enabled plant regeneration within a relatively short period, ranging from 2 to 5 months.

Observed differences in the dynamics of protoplast culture development and regeneration pathways are the basis for the second part of the study, which focused on more detailed analyses. Three time points of culture development were examined: cell divisions, cell colony formation, and minicallus development. These investigations aimed to understand the changes occurring in (1) the spatial distribution of selected cell wall components, (2) proteome, and (3) the expression of selected genes and transcription factors associated with somatic embryogenesis during cell colony development in protoplast cultures.

The spatiotemporal analysis of cell wall component distribution revealed a variable localization of pectin (rhamnogalacturonan I) side chains and extensins, indicating differentiation within the cell colonies. Proteomic analysis showed an increased accumulation

of proteins involved in storage compound accumulation and proteins associated with somatic embryogenesis, suggesting that the culture was preparing for somatic embryogenesis events.

The obtained results may contribute to a better understanding of key processes occurring during the development of protoplast culture, ultimately leading to plant regeneration. From a broader perspective, these findings may support the advancement of future buckwheat breeding programs.