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PRACA DOKTORSKA

Kompleksowa analiza epigenetyczna procesów reprogramowania komórkowego w tkankach hodowanych *in vitro* oraz *in vivo*- badania porównawcze gatunków *Fagopyrum*

SUMMARY

The first stage of the research focused on the study of differentiation processes in embryogenic callus cells (EC) of *F. esculentum* and morphogenic (MC) and non-morphogenic (NMC) callus of *F. tataricum* at selected days of culture. EC and MC were induced from immature zygotic embryos. *F. esculentum* EC is characterized by a structure typical of embryogenic callus tissue. It consists of embryogenic masses and parenchymatous cells, maintaining regeneration stability for up to two years of cultivation, with a morphology characterized by a dense, spherical structures. *F. tataricum* MC is capable of organogenesis or somatic embryogenesis for approximately ten years of cultivation, showing a high level of genome stability. NC appears on the surface of MC after approximately two years of cultivation as a result of endoreplication. It is characterized by aneuploidy, rapid growth rate, a high level of oxidative stress, and a complete loss of morphogenetic capacity.

The aim of the research in the first stages was to determine changes in the levels of selected epigenetic markers, including DNA methylation, histone methylation and acetylation, during dedifferentiation and re-differentiation of cells of the above-described calli on the selected days of culture. It was demonstrated that a reduced level of H3K4me2 correlates with cell dedifferentiation in *F. esculentum* EC and *F. tataricum* MC. In contrast, the reduced level of DNA methylation is most likely associated with the acquisition of embryogenic potential and the re-initiation of proembryogenic cell complexes in *F. tataricum* MC. Among all the studied modifications, histone acetylation, particularly H4K16ac and H4K5ac, showed the greatest variability in NC, which can be linked to the endoreduplication processes characteristic of the development of this type of callus.

The obtained results allowed the selection of one epigenetic modification (H3K4me3) for chromatin immunoprecipitation studies. The aim of this research was to analyze and compare gene expression levels and H3K4me3 levels in cell wall-related genes between *F. tataricum* MC and NC. Bioinformatic analyses of DNA sequencing of tissues subjected to chromatin immunoprecipitation revealed that the genomic content of H3K4me3 is higher in NC. However, in genes encoding specific enzymes responsible for the reorganization of cell wall components, such as pectin methylesterases and peroxidases, NC exhibited a reduced level of H3K4me3, which was associated with the lack of morphogenetic ability of this type of callus. In MC, the level of H3K4me3 in the studied cell wall genes increased with the progression of culture, which may indicate increased activity of enzymes involved in the transformation of cell wall components during cellular dedifferentiation processes and disintegration of proembryogenic cell complexes. It was concluded that the H3K4me3 modification plays a key role in activating genes involved in cell wall biosynthesis and reorganization.

In the second part of the research, analyses of DNA methylation levels during flower development *in vivo* were conducted. Methylation levels were examined in the elements (petals, nectaries, ovaries, stigma) of closed and open flowers of the cross-pollinated species *F. esculentum*, which characterizes with heterostyly (Pin and Thrum morph flowers), and the self-pollinated species *F. tataricum*. Compared to closed flowers, open flowers showed a decrease in DNA methylation in the stigma, petals, and ovaries (except for the ovary in Thrum type). Significant differences in DNA methylation levels were also found between Pin and Thrum morphotypes, as well as in *F. tataricum* flowers. It was also demonstrated that the decrease in DNA methylation correlated with reduced expression of genes encoding DNA methyltransferases. In open Thrum flowers, *MET1* expression was over 45 times lower than in closed flowers, while in closed and open Pin flowers, the change in *MET1* expression level was not statistically significant. Reduced transcription of other analyzed genes (*CMET3, DME1, DME3, ROS1*) was observed in open Pin flowers. Additionally, the *ROS1* gene, encoding DNA demethylase, showed reduced expression in open *F. esculentum* and *F. tataricum* flowers. Since there is no available literature data on epigenetic changes during flower development in *Fagopyrum* species, it can be assumed that a higher level of DNA methylation and the expression levels of genes encoding DNA methyltransferases and demethylases in closed flowers of both *Fagopyrum* species indicate that methylation is involved in the processes accompanying their development.

Keywords: callus, chromatin immunoprecipitation, dedifferentiation, DNA methylation, epigenetics, flowers, gene expression, heterostyly, H3K4me3, redifferentiation