The project's primary goal is to explore genetic factors responsible for Pampa-type cytoplasmic male sterility (CMS) and fertility restoration in triticale, studying the mitochondrial and nuclear genomes of sterile and fertile lines. Planned studies include (1) Genotyping the RIL F8 mapping population with DArTseq markers and phenotyping the BC1F1 plants utilizing visual bonitation scale and seed settings; (2) Construction of the triticale genetic map utilizing MultiPoint UltraDense commercial software and mapping of the QTLs responsible for maintaining sterility and fertility restoration using WinQTL Cartographer program; (3) Comparative transcriptome analysis of the CMS and maintainer lines using RNAseq analysis. The anthers at the tetrad (Td), uninucleate (Un), binucleate (Bn), and trinucleate (Tn) stage of microspores development will be collected. Differentially expressed genes (DEGs) related to pollen development in sterile and fertile materials will be identified. The RNAseq results will be validated by the Real-time quantitative PCR (qRT-PCR) technique; (4) Identification of DEGs unique to tapetum of sterile and fertile materials. Tapetal tissue will be dissected using a laser microdissection (LM) system after fixation and section of frozen anthers at the uninucleate stage with cryostat. (5) Cellular location of DEGs associated with tapetum degradation via in situ hybridization method and visualization using transmission electron microscopy; (6) Development of the markers for selection purpose based on the QTLs of the trait identified using QTLs mapping approach.

Justification for tackling scientific problems: Triticale is a relatively new cereal species made from the cross of wheat and rye. This species enjoys unflagging popularity in Poland, resulting from its numerous advantages. Triticale combines the relatively high nutritional value of grain and tolerance to soil quality and acidification. Commercial plant breeding has great hope in the possibility of increasing the grain yield and green mass by introducing hybrid varieties. The systems of hybrid seed production are based on the availability of cytoplasmic male sterility (CMS) sources. In triticale, *Triticum timopheevi* sterilizing cytoplasm from wheat is the most frequently used. The system is characterized by a low frequency of maintainers, unstable male sterility in different environments, and incomplete fertility restoration. Contrary to CMS-Tt, the CMS-Pampa cytoplasm from *Secale cereale i*s a more reliable source of male sterility for triticale. Suitable sources of restorers complement it. However, the genetic background of the phenomenon in triticale is not recognized. Knowledge of its genetic basis is essential to understand the mechanisms of both CMS and pollen fertility restoration in triticale with Pampa sterilizing cytoplasm. This can be achieved by identifying genes responsible for maintaining sterility and restoring pollen fertility and their location on chromosomes. Such studies provide the basis for identifying markers that may facilitate plant selection and further studies on the Pampa CMS phenomenon in triticale.

The most important expected effects: (1) Chromosomal location of the quantitative trait loci (QTL) responsible for pollen fertility restoration in triticale with CMS Pampa; (2) Determination of the contribution of rye and wheat genomes to the expression of the fertility restoration trait. Comparing the results to equivalent data for rye with CMS Pampa and triticale with CMS *T. timopheevi*; (3) Establishing the mechanisms involved in pollen abortion and closely related to tapetum based on changes in gene expression at various developmental stages of anthers collected from CMS and maintainer (fertile) lines; (4) Identification of the markers for the selection of CMS and restorer lines.