Research project objectives and reasons for choosing the topic

In the face of global climate changes and a growing population of people, scientists stand in front of the task of adjusting plants' breeding and agriculture to climate to meet requirements for food. Lupins are an excellent protein, fiber, vitamins, and minerals source. An increase in the cultivation of Lupins is a potential solution for the ongoing search for a non-genetically modified source of plant protein in the European Union. Lupins are unique research subjects among legumes due to their specific requirement of vernalization-enabling conditions (a period of cold temperatures) to induce flowering. Observed increased rates of winter temperatures in Poland are opening possibilities for winter sowing of crops with moderate frost tolerance. Development of new plant varieties tailored to specific climate conditions, including the ability to withstand sub-zero temperatures that occur during the European winter requires basic research first.

The main goal of this proposal is to investigate and evaluate the effects of temperature and duration of vernalization on flowering time and plant development, frost tolerance, as well as the quality and quantity of yield in the three lupin crops: *Lupinus angustifolius* L., *L. albus* L., and *L. luteus* L. Additionally, the study aims to examine how vernalization influences the photoperiod requirements for these three species.

Research project hypothesis

It is hypothesized that the type of vernalization (affected seed or young plants), the appropriate temperature, and the duration used for vernalization affect the time of flowering and the maturation of pods. It is also assumed that the vernalization treatment can increase the frost tolerance and improve features of the yield structure, as is the case with other crop plants.

Research project tasks and methodology

Selected narrow-leaf, white, and yellow lupin genotypes differing in flowering time and response to vernalization will be phenotyped under controlled conditions. For each genotype, both seeds and young plants will be vernalized (imbibed seeds on Petri dishes in the dark and young plants on the stage of the two pairs of leaves). Two variants of vernalization temperature (4℃ and 8℃), photoperiod (8 h and 14 h) and three vernalization durations (1, 2 or 3 weeks) will be used. At the end of each planned vernalization period, samples will be collected for RNA isolation. The phenotyping will be conducted in two controlled environmental chambers to study the impact of different combinations of vernalization and photoperiod treatments on flowering time and development of the plants.

As the next step, a four-day frost period will be used to investigate whether vernalization can improve overall frost tolerance and whether there are differences, in response, between thermoneutral and vernalization-responsive genotypes. After the eight-day recovery period, frost tolerance will be assessed by evaluating plant damage. A scale of 0 to 5 will be used, where 0 is no damage and 5 is a dead plant.

Samples collected after three weeks of vernalization for each tested combination of photoperiod and vernalization temperature will be sequenced. Differential gene expression analysis will be performed using the obtained sequences to identify candidate genes for further analysis by RTq-PCR. RTq-PCR will be performed on samples from all three time points for both vernalisation temperatures and photoperiods.

A field experiment will be conducted to study the impact of different vernalization variants on plant morphology and yield. The following will be observed: plant height, number of internodes, number of lateral shoots, number of pods, number of seeds per pod, and thousand grain weight. Time to floral bud development, the start of flowering, and pod maturity will be observed to assess the effect of vernalization on flowering and plant maturity time.

To investigate the effects of temperature and duration of vernalization on seed quality, seed samples collected from a field experiment will be evaluated for their protein, fiber, and fat content per dry matter.