Summary

Seed longevity in tobacco depending on storage conditions and QTL mapping controlling this trait

Keywords: genetic resources, *Nicotiana*, gene bank, storage, seed longevity, QTL

Plant genetic resources play an important role in preservation of biodiversity in the natural environment. They represent a form of humanity's heritage in light of the steadily declining number of species present in the world. The task of protecting plant genetic diversity is undertaken by gene banks, where plant material is regenerated while maintaining genetic purity and kept in a viable state. Most species are stored long-term in the form of seeds including species from the *Nicotiana* genus.

The aim of this doctoral dissertation was to assess the seed longevity of objects belonging to two species, *Nicotiana tabacum* and *Nicotiana rustica*, which were stored under varying temperature and humidity conditions. Additionally, the identification of genetic regions QTL responsible for seed longevity in tobacco was carried out. The seeds were stored in two gene banks: the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, and the National Centre for Plant Genetic Resources (KCRZG) located at the Plant Breeding and Acclimatization Institute – National Research Institute (IHAR-PIB) in Radzików as well as at the Institute of Soil Science and Plant Cultivation – National Research Institute (IUNG-PIB) in Puławy.

The seed longevity of *Nicotiana tabacum* and *Nicotiana rustica* stored under strictly controlled conditions at the gene bank located at the IPK was compared with the longevity of *Nicotiana tabacum* seeds stored under uncontrolled conditions in the IUNG-PIB laboratory. In the first location, the seeds were stored for a period of 2 to 12 years at a temperature of 20.3±2.3°C and humidity of 50.5±6.3% RH. In the second location, the seeds were stored for the same period, but at room temperature (18–22°C) and air humidity of 45–60% RH.

The seed longevity of *Nicotiana tabacum* objects stored for a longer period, from 14 to 33 years in paper bags placed in vacuum-sealed glass jars (without air) at a temperature of 0°C and seed moisture content of 4% at the KCRZG IHAR-PIB, was also compared. Additionally, *Nicotiana tabacum* and *Nicotiana rustica* seeds stored for a period of 14 to 39 years in hermetically

sealed glass jars at a temperature of -18°C and seed moisture content of 6% in the IPK gene bank were analyzed.

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The seeds of *Nicotiana tabacum* and *Nicotiana rustica* exhibited varying longevity depending on the storage conditions. It appears that seasonal fluctuations in air humidity had a significant impact on reducing the viability of tobacco seeds stored at IUNG-PIB, leading to a faster loss of vigor compared to those stored at IPK. On the other hand, the differentiating factor for the longevity of seeds stored at KCRZG IHAR-PIB and IPK was the temperature.

When comparing the seed longevity results obtained for all three locations, it was found that the most significant factor was the storage temperature. Reducing the temperature from 20°C to 0°C extended the seed storage period from an average of 10 to 30 years. Moreover, reducing the temperature from 0 to -18°C extended the storage period by an additional 20 years. A seed germination rate of over 75% was considered the threshold value for viability.

Two tobacco mapping populations, Florida 301 × Hicks and Beinhart–1000 × Hicks, were used to identify QTLs related to seed longevity traits. The traits considered included: germination capacity, percentage of all germinated seeds, time required to germinate 50% of seeds in the sample, and the Area Under the Curve (AUC) parameter, which measures the surface area under the germination time curve between t=0 and a user-defined endpoint t=x, calculated after 200 hours of germination.

In the Florida 301 × Hicks mapping population, four genetic regions located on four chromosomes were identified as controlling four traits associated with tobacco seed longevity. Meanwhile, in the Beinhart–1000 × Hicks population, 23 additive QTLs were detected, distributed across 11 chromosomes, controlling the variability of the four studied traits related to seed longevity. Epistatic QTLs were detected only for the trait number of germinated seeds. In the control seeds, four QTL pairs located on four chromosomes were identified, while in artificially aged seeds, one pair was located on two different chromosomes.

The results obtained demonstrated significant differences in tobacco seed longevity depending on storage conditions and confirmed the complexity and polygenic nature of this trait. The information regarding tobacco seed longevity can be valuable in planning programs for the regeneration of *Nicotiana* collection accessions maintained in gene banks.