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Przedstawiam abstract mojej rozprawy doktorskiej, zatytułowanej: Badania embriologiczne *Tinantia anomala* (Torr.) C. B. Clarke, wykonanej i napisanej pod promotorstwem dr hab. Krystyny Winiarczyk.

Abstract

Tinantia anomala is an annual herb. This plant has heteromorphic leaves (that form sheaths at their bases and surround the stem) and specific zygomorphic flowers with strikingly polymorphic stamens. The presence of bright trichomes, which mimic a multitude of pollen grains, indicates that *T. anomala* has adapted to lure pollinators (floral mimicry). The plant reproduces generatively by self- or cross-pollination and produces a lot of seeds during the vegetation period. Furthermore, *T. anomala* can reproduce in a vegetative way by forming underground bulbs.

Given the interesting structure of flowers and little information describing this species in the scientific literature, *T. anomala* has become a subject of embryological research conducted as part of this work. The main objective of the research was to explore the stages of micro- and megasporogenesis in *T. anomala*, because these process have been documented before. The second task of the research was to investigate the biology of flowering (including the way of pollination, the progamic phase, and fruit and seed set) and to make an attempt to determine the role of polymorphic stamens. In this task, the viability of pollen grains from each anther was tested and their ability to germinate in vivo were tested. During the realization of these targets, different microscopy techniques has been used (LM, FM, CLSM, DIC, SEM, TEM) and molecular methods (DNA isolation, PCR, sequencing the genome, 2D

electrophoresis, MALDI-ToF assay) were used. Genetic studies were undertaken because of the ambiguity of morphological classification of the species. In this part of the work, three specific fragments of the *T. anomala* chloroplast genome were sequenced (the rbcl and nadhF genes and the trnL-trnF intergenic spacer) as well as genome fragments of other four species of the family Commelinaceae. The comparative analysis of sequence data available in the GenBank NCBI confirmed the taxonomic position of the tested species. The 15 nucleotide sequences obtained were deposited in the database.

In embryological studies of *T. anomala*, the stages of micro- and megasporogenesis were explored. The anatomy of flower generative organs also was described. The anthers in the *T. anomala* flower vary in shape, size, and color, and the staminal trichomes differ in color and morphology. The results indicate that the dimorphic anthers have the same anatomy and produce fertile pollen grains with three germinal apertures without any furrow. *T. anomala* formed two-celled male gametophytes that exhibit high viability (over 90%). Pollen grains have also a high ability to germinate (ca. 98%) in pollen tubes in both cases: after self-pollination and after transfer of pollen grains to a foreign flower. The pollen grains differ not only in color but also in the protein composition. The protein fraction isolated from anthers was separated by 2-Dimensional Electrophoresis and, as a result, more than 200 different polypeptides were identified. Among them, 40 proteins were specific to the upper anther and 8 proteins were specific to the lower anther. Other proteins were common in both types of the anther. After determining the function of 15 randomly selected proteins using mass spectrometry assays, we found that these polypeptides are involved in the metabolism and energy changes, and that they are structural proteins. Moreover, mitochondrial transport of a significant amount of calcium (protein At4g36820) was shown in both types of the anther.

During the research on microsporogenesis, the presence of raphides (needle-shaped crystals of calcium oxalate) occurring in the ameboidal tapetum was reported. Ultrastructural studies of the raphides have shown that they are produced in tapetal vacuoles, forming clusters between meiocytes. During the degradation of a tapetum, vacuoles disintegrated and released raphides. The number of the crystals significantly decreases during microgametogenesis. Probably, substances derived from the decomposition of the crystals are then used to create the thick sporoderm which surrounds *T. anomala* pollen grains. The studies on microsporogenesis resulted in a discovery of unique structures that appear directly before anther opening. These are short-term rings, built of a different number of segments, which are formed between pollen grains. The potential role of these rings can be separation

and protection of the pollen grains during dehiscence. The pollen grains of *T. anomala* were hydrated before the release from the anther, which is a rare process among plants. During the studies on the development of ovules, significant growth of integuments (especially the outer integument) was reported. Integuments divide the ovule into a smaller micropylar part, where the embryo sac is developing, and a much larger chalazal part. Megasporogenesis in *T. anomala* progresses properly, and the embryo sac develops according to the type of Oenothera. The Oenothera type is rare in the family Commelinaceae. The egg apparatus consists of two synergids (with clearly visible filiform apparatus) and an elongated egg cell. The central cell has a considerable size and a large haploid nucleus. No antipodes form in *T. anomala*. The progamic phase and fertilization takes place smoothly and, after these stages, fruits (capsules) are formed with seeds inside. The anatomical analyses have shown that seeds have a thin testa with a specific sculpture, large amounts of cellular endosperm, and an embryo located in a separate chamber. Under favorable conditions, the seeds germinate quickly in young plants.

Observations of the flowering biology conducted for 4 years have shown that *T. anomala* can produce a new generation of plants quickly and without any obstacles. Under greenhouse conditions, the plant was flowering and setting fruits throughout the whole year, not showing winter dormancy. The dominant life strategy in *T. anomala* is self-pollination, but cross-pollination can appear optionally. The different strategies of reproduction indicate that this evolutionarily young species is still looking for its own ecological niche and has high potential to colonize new areas.