Study of galantamine biosynthesis in *Hippeastrum papilio* (Ravenna) van Scheepen

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Ph.D. Dissertation

Plants are a source of biologically active substances of great diversity and importance to humanity, thanks to their rich palette of biological activities. Often, medicinal plants are used from their natural habitats for the production of pharmaceutical products, and thus many of them fall into the category of "endangered species". In addition, the amount of the desired chemical compound in plants often varies or is unsatisfactory. For this reason, the topic of environmentally friendly and responsible industrial production of raw materials from medicinal plants is increasingly relevant. Therefore, it is of great importance to study new approaches to growing medicinal plants, in which the conditions for biosynthesis and accumulation of both biologically active substances and biomass are optimized, leading to cost-effective methods for industrial production.

The Amaryllidaceae family consists of three subfamilies: Agapanthoideae, Allioideae, and Amaryllidoideae, and is thought to have originated in Africa. Amaryllidoideae Burnett is the largest of the three subfamilies. Based on molecular phylogenetic studies, the subfamily is further subdivided into 14 tribes, which belong to several clades. Better known representatives of the subfamily are the genera *Galanthus*, *Leucojum*, *Narcissus*, *Lycoris*, *Hippeastrum*, etc., which, in addition to being ornamental plants, are also known as sources of bioactive substances – Amaryllidaceae alkaloids (AAs), some of which are of great medical and economic importance. Galantamine is an acetylcholinesterase inhibitor, the only alkaloid in this family that is approved by the US Food and Drug Administration (FDA) as a medication for the symptomatic treatment of Alzheimer's disease. Currently, more than 55 million people worldwide live with dementia, with approximately 10 million new cases diagnosed each year.

For industrial production of biologically active substances (BAS), cultivation of medicinal plants is preferred, which ensures sustainability and predictability of the raw material on the one hand and reduces anthropogenic pressure on their natural habitats on the other. The phytochemical composition of plant species is unstable and can be influenced by several factors: genetic predisposition (genotype, ploidy level), physiological (organ, maturity, age), and agronomic (nutrient solution, stress factor).

Hippeastrum papilio biosynthesizes a significant amount of galantamine, but there are no detailed quantitative studies on its content in different organs and the dynamics of its accumulation. Data on the influence of factors such as ploidy, age, macronutrients, and elicitors on the processes of galantamine biosynthesis and accumulation in *Hippeastrum papilio* are also not found in the literature.

The effect of ploidy on galanthamine biosynthesis, as well as the morphological and cytological traits of *Hippeastrum papilio* plants, was studied. The results revealed significant differences between diploid and autotetraploid *H. papilio* plants. The length and width of stomata in autotetraploids were larger than those observed in the diploid leaves. The biomass of one-year-old autotetraploid *H. papilio* plants was reduced by 53.99% for the plants' fresh weight. The content of galanthamine and haemanthamine was found to be 49.73% and 80.10%, respectively, higher in the leaves of autotetraploids, compared to the diploid ones.

The biosynthesis and accumulation of alkaloids are tissue – and organ-specific processes. Histochemical localization of alkaloids in *H. papilio*'s plant organs revealed their presence in all studied samples. Alkaloids were observed in vascular bundles, vacuoles, and intracellular spaces, while in other plant tissues and structures, they depend on the plant organ. The bulbs and roots showed higher alkaloid content compared to the leaf parts. The highest alkaloid content was found in the inner bulb part.

The effect of three different Fertilizers on three different ages of roots, bulbs, and leaves of *H. papilio* showed that extra fertilizing with N, K, and Ca does not positively influence galanthamine and biomass accumulation. Despite the lower biomass accumulation per individual, plants grown for two seasons (age 1) showed a comparable galanthamine yield per square meter at the end of the vegetation period to those grown for three seasons (age 2), due to their higher cultivation density. The dynamics of alkaloid and biomass accumulation, studied in plants from age 1 during the vegetation season, revealed that the highest galanthamine content in the plant organs occurs at the beginning of the vegetation period.

In order to enhance the galanthamine and haemanthamine accumulation in *H. papilio*, elicitation with salicylic acid (SA) was tested in one-year-old hydroponic cultures. Treatment with 100 μM/L SA during 148 h enhanced GAL biosynthesis by 19% compared to the initial level. A similar trend was observed for HAEM accumulation.