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Sexual and Asexual Breeding in Panax Ginseng C.A. Meyer

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Panax ginseng C.A. Meyer is the most famous plant of Far Eastern region and oriental traditional medicine. It's long-lived herbaceous perennial, endemic of Manchuria floristic region. Natural populations of ginseng were exploited for a long time. It is considered that in the early 20th century *Panax ginseng* occupied a wide territory of the Far East, ranging from latitudes 40 to 48° north and longitudes 125 to 137° east of Greenwich. As a result of intensive use of wild roots, there are almost no ginseng plants left in natural habitats. Three Russian wild ginseng populations are now close to extinction. Primorskij Krai of Russia is the only country where *P. ginseng* is still growing in the natural habitat. Yet, all natural populations are badly exhausted, unruled extirpation of ginseng as well of poor competitiveness of this relict species in current conditions and its inability to resist nature and anthropogenic factors. The different protection measures including the reintroduction in the favorable habitats are in need to restore this famous plant to its former range.

The mating system of this plant was investigated in detail to assist in design of ginseng conservation program. This work became especially necessary when the low genetic polymorphism was discovered in natural as well as in cultivated ginseng populations. Ginseng mating system was described as predominantly self-pollinated. However, our investigations show that in different conditions, ginseng plants could realize seed production via autogamy, xenogamy ensured by insect pollen transfer or apogamy without pollination.

Our experiments on xenogamy, autogamy and apogamy were carried out with cultivated and wild growing ginseng plants. The probability of sexual and asexual seed development in simulative ginseng population was evaluated by allozyme analysis. Chi-square tests of homogenity for genotype frequencies were computed as well. Ovules and seeds were prepared by standard histology methods. Serial sections were stained with haematoxyline/eosin or with haematoxyline/alcian blue and visualized using Zeiss Axioplan microscope.

In greenhouse tests on selfing, crossing and agamospermy were resulted in seed production by *Panax ginseng* plants with 40.9%, 26.5% and 22.9% yields, respectively. In open habitat seed production of agamospermy was degree up to 2.8%. Probably, apomictic embryo formations are one of possible stress react[a]ion in ginseng plants.

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We studied structure of ovary and ovule and their changing after cross- and self-fertilization as well as developing of embryo without pollination (fertilization). *P. ginseng* ovary is two-locular and each loculus has two ovules, upper and lower. In mostly causes upper ovules have smaller sizes compared to lower ones and they are not developed into seeds. Deformed embryo sacs were observed in upper ovules. Before the time of pollination in rare causes normal ovules embryo sac are formed in upper area. Early that assumption was considered as erroneous. Lower ovule consists of one integument which established the embryo sac with a narrow and long micropylar canal. The ovule is completed with a strand consisted of trachedial cells and with a small obturator hanging over the micropyle. Histological analysis has shown the absence of any abnormality in embryo sac formation. Sometimes lower ovules with destroyed embryo sac were observed. Before fertilization the polar nuclei fused, forming the secondary endosperm nucleus. About 16-24hr after beginning of the pollen germination, the pollen tube moves into the tissue of the stigma and the content of the pollen grain with two sperm cells flow out to the pollen tube. The double fertilization was asynchronous. The fusion of the first sperm cell with nucleus of the central cell of the embryo sac gave rise to the endosperm, afterwards secondary sperm cell fertilized of egg cell. Cross-pollination zygotes were getting of to dormancy for more short time, than self-fertilized zygotes. Rates of zygotic and parthenogenetic embryo development were different. Zygotic embryos were developing more quickly as compared with somatic embryo. Often ginseng plants formed fruits consisting of 3,4,7- seeds.

The offspring obtained from these seeds were viable that allowed us to investigate the genotypic segregation of three polymorphic allozyme loci, encoding phosphoglucomutase (*Pgm-2* and *Pgm-3*) and glutamate pyruvate transaminase (*Gpt-2*). In most cases progenies resulting from fifteen control crosses as well as from five self-pollinated plants indicated the genotype segregation according to Mendelian patterns at all three genes. However, three parthenogenetic progenies have shown autosegregation with an excess of heterozygotes for all marker genes and demonstrated a proportion of three genotypes in a 3:8:3 ratios for the summarized sample. This result suggests that diploid megaspores produced seeds in these experimental plants via one of parthenogenetic path similar to meiotic diplospory.

Thus, our data show that ginseng parthenogenesis is one of a possible ways of self-fertilization and has consequences similar to autogamy effect. Probably asexual breeding of *P. ginseng* is connected with evolutionary history of the species that also involved human activity. The results have several conservation implications for improving of ginseng conservation program.