Effect of Hybridization on the Development of Additional Embryos in Citrus

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Abstract
Results of study of pollen effect on the degree of polyembryony in the citrus are analyzed. The pollen of Citrus wilsoni and Citrus ichangensis were used as a pollinizer, lemons Lemon gruzinski, Lemon mayer and indigenous variety of orange (citrus sinensis var.) as a mother-plant. It was established that potential of development of additional embryo and stimulating effect of pollen on the degree of polyembryony are caused by specific biological characteristics of plants and according to genotype features are often distinctive. Nucellar savings hereditary features of pollinizer at the level of organism were not recorded. We can suppose that pollinizer has only inducing influence on additional embryo production on corresponding somatic cells and doesn’t take part in determination of their genetic characteristics. Hence, consideration about hybrid nature of nucellar savings should be groundless.

Key words: Aponixic, Nucellus, Polyembryonic plant.

Introduction
The sexual reproduction created the possibilities of combination and recombination inherited changes corresponding to cytogenetic basis which broadened the opportunity of selective activity. That is the classic method – hybridization that takes an important place in the creation of plant species as far as the hybrid generation has the wide standard of reaction, the high potential of adaptation towards environment. Besides the combination changes, in the generation of citrus there are zygotic and nucleus changes as well [7, 9, 13].

Some families of metasperm plants are characterized by apomictic reproduction potential. Development of new organisms via somatic cells of seed-buds enables them to maintain unchanged genotype of mother plant in the further generations. Such generations, by cloning of mother genotypes, remain balanced proportions of adaptively available gene combinations within species and consequently are not submitted to segregation. Evolutionary priority of apomictic reproduction should be explained by such mechanisms [1].

The citrus are characterized by apomictic of facultative type. Along with zygotic embryo apomictic embryo are developed in the seed. Most species of this genus are characterized by polyembryony of induced type [6, 9, 10, 11,12]. Additional embryos in citruses are not zygotic and are developed from structurally changed nucellus somatic cells [9] or from subepidermal tapetal cells [4]. Genetic differences of zygotic and nucellar embryo were also registered using protein markers [5,13].

In scientific literature there are various opinions in regard to the effect of pollinizer plant on the degree of polyembryony in the citrus. Some authors consider that development of additional embryo depends on mother plant, as well as on pollinizer [8], in the opinion of others – only on mother plant [5], and others consider that it mainly caused by biological features of pollinizer [2,3].

The aim of our work was to study polyembryony degree in citrus cultivars (Lemon mayer, Lemon gruzinski and local variety of orange – citrus sinensis var.) at their pollination by wild forms of citruses (Citrus wilsoni and Citrus ichangensis), and to analyze the results of the effect of pollinizer plant on the polyembryony.

Materials and methods
As a material for our experiments we used lemons Lemon mayer, Lemon gruzinski, local variety of orange, Citrus wilsoni and Citrus ichangensis). For conducting of hybridization unblownflowers were opened by tweezers on the mother plant branches, pollen tubes were removed and gauze bags were put on them. After some days, when the liquid appeared on the stigma, pollination was carried by brush.
Pollination was carried out in the morning hours. After pollination gauze bags with corresponding marks were put on the branches with flowers. After fruit-set gauze bags were removed.

After fruitage seeds were taken, and their stratification was carried out. In early spring they were sowed in the wood boxes at intervals of 10 cm on prepared specially soil. Every sprout was analyzed. Data obtained by free pollination were used as a control in every case [8].

Results and Discussion.

_Citrus wilsoni_ and _Citrus ichangensis_ belong to the wild forms of citruses. They are characterized by sharp features and dominate at hybridization with cultivars. As a result, it is possible to distinguish accurately by outward signs hybrid (zygotic) embryos from nucellar ones (clones of mother plant) grown from young sprouts of one seed. According to experimental results _citrus sinensis_ var. revealed especially high potential of development of additional embryo (44,3%), this parameter for lemon species was lower; for _Lemon Mayer_ it equals 3,6 %, and for _Lemon gruzinski_ – 2,8%.

At the usage of various species asa mother plant effect of pollenizer plant on the polyembryony degree is different. While using _Citrus wilsoni_ as pollenizer certain stimulation of polyembryony was observed in lemons, compared with free pollination. Namely, for _Lemon Mayer_ it equals 4,6 %, but in the case of control – 3,6%; for _Lemon gruzinski_ it is 3,2%, and for control – 2,8%. Analogous results were recorded at pollination of lemons by _Citrus ichangensis_. But stimulating effect of pollenizer plant on polyembryony is more aloud: _Lemon Mayer_ – 7,7% and control – 3,6%; _Lemon gruzinski_ - 3,9% and control – 2,8%.

In the case of local variety of orange ( _citrus sinensis_ var.) is observed: at free pollination polyembryony degree is 44,3%, whereas at the usage of _Citrus wilsoni_ (9,3%) and _Citrus ichangensis_(10,0%) as pollinizer this parameter is lower considerably. Probably, in oranges in the case of self-pollination (and more probably at free pollination) inducing effect of pollen tube entrails on additional embryo production is higher than at pollination by different genotypes. Above mentioned regularity considers fruit-set of two-embryonic seeds, as well as polyembryonic seeds (Fig.1,2).

![Fig. 1. Citrus ichangensis pollen effect on polyembryony degree at its using in hybridization (%).](image1)

![Fig. 2. Citrus wilsoni pollen effect on polyembryony degree at its using in hybridization (%).](image2)

It should be mentioned that while using _C. ichangensis_ as pollinizer its inducing affect in additional embryo production is higher in every variant of hybridization than in the case of using C.wilsoni. There are various opinions in scientific literature about genetical nature of apomictic embryo. According to our results in nucellar sowings hereditary features of pollenizer at the level of organism...
were not recorded. We can suppose that pollinizer has only inducing influence on additional embryo production on corresponding somatic cells and doesn’t take part in determination of their genetic characteristics. Hence, consideration about hybrid nature of nucellar sowings should be groundless.

**Conclusion**

According to our results in nucellar sowings hereditary features of pollinizer at the level of organism were not recorded. Consequently, according to above mentioned, potential of additional embryo production and stimulating effect of pollen on the degree of polyembryony could be reasoned by specific characteristics of citrus plants.

**References**