

## A PROSPECTIVE METHOD OF INTERSPECIFIC HYBRIDIZATION IN THE *CUCURBITA* GENUS

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**Summary.** A new method of interspecies crossing representatives of the *Cucurbita* genus was tested, which involves simultaneously applying an aqueous mixture of growth regulators to the base of the pistil of female flowers during the pollination of the maternal hybridization partner. This experimental method made it possible to carry out a successful crossing and obtain a full-fledged hybrid F<sub>1</sub> progeny (*Cucurbita pepo* var. *giraumtonia* Duch. / *Cucurbita maxima* Duch.). From the hybrid seeds planted in the field, it was possible to obtain hybrid forms of plants, which, according to the morphology of the leaf and the shape of the fruit, had intermediate traits of the hybridization partners. It was established that interspecific F<sub>1</sub> hybrids had a bushy plant form inherited from the maternal component. The fruits of interspecies hybrids had either an elliptical or broadly elliptical shape of green color with small and dense dots and with a darker and denser color of the ribs. Leaf blades in interspecific hybrid plants were characterized by heart-shaped or triangular shapes, with a complete absence or very shallow incisions, intense green color, sometimes with the presence of silver spots on the upper side. The procedure of artificial pollination of each hybrid plant was carried out. The final results regarding the analysis of hybrid plants will be carried out after the completion of all phenological phases of development.

**Keywords:** zucchini (*Cucurbita pepo* var. *giraumontia* Duch.), large-fruited pumpkins (*Cucurbita maxima* Duch.), interspecific hybridization, hybrid progeny, bushy form, growth regulators.

### Introduction

Three types of pumpkins are grown in Ukraine: large-fruited (*C. maxima* Duch.), nutmeg (*C. moschata* Duch.) and hard-skinned (*C. pepo* L.). Although, the genus *Cucurbita* (2n = 40) is quite diverse in the *Cucurbitaceae* family and has 27 different species. The crossing of different species and genera of pumpkins is used in cases where within one species there is a shortage of certain traits and properties that are desirable for combination in one variety or hybrid, but which are manifested in other species, even if they are not used as cultivated plants. The practical value of remote hybridization lies in the recombination of genetic material from different genotypes – both cultivated and wild. In exceptional cases, the appearance of outstanding transgressive forms is possible, that is, combinations of genes that have not previously been found in nature. Such forms play an important role in the evolution of species and are of considerable value in practical breeding, on their basis fundamentally new varieties and F<sub>1</sub> hybrids are created. Attempts to enrich the gene pool of some pumpkin species at the expense of the gene pool of other species have a long history.

It was established that the species *C. moschata* and *C. maxima* cross most easily and these species with *C. pepo* are more difficult [1]. Hybridization between different species often leads to the emergence of embryonic lethal mutants due to a failure in the formation of the endosperm. The endosperm of flowering plants plays a vital role in providing nutrients and transferring hormones from the tissues of the maternal form to the embryos [2]. Various mechanisms, such as the endosperm

stable maternal and paternal count (2:1) [3], polar nuclei activation index [4], and genomic imprinting [5], contribute to hybridization barriers associated with abnormal endosperm differentiation. Crossbreeding between representatives of the genus *Cucurbita* also faces problems related to overcoming incompatibility barriers during interspecific hybridization, abortion of seed hybrid embryos, and low seed viability [6]. These obstacles prevent the use of desirable genetic traits of wild and exotic pumpkin species and limit the genetic diversity available for increasing yield and resistance to biotic and abiotic factors of cultivation [7]. The biotechnological method of growing (rescuing) immature embryos *in vitro* allows to partially improve the situation of overcoming incompatibility barriers during interspecies hybridization in the genus *Cucurbita*. Several conducted studies have demonstrated the high efficiency of this method on pumpkins, demonstrating its high potential in solving the problems of transgressive breeding and expanding genetic diversity [8, 9, 10]. This method not only helps to obtain valuable genetic material, but also makes it possible to create interspecies hybrids with a complex of desired traits that cannot be obtained by traditional breeding methods [11]. For example, Rakha et al. successfully used the biotechnological method of growing hybrid embryos in *in vitro* culture to overcome the barriers of incompatibility between three species of *Cucurbita* (*C. moschata*, *C. ficifolia* and *C. martinii*), which ultimately gave researchers the opportunity to create new varieties of pumpkin with improved fruit quality characteristics [12]. Sisko et al. reported a variable rate of regeneration success among embryos obtained from different combinations of crosses: the success in regeneration of interspecific hybrids *in vitro* culture obtained from the cross of *C. maxima* and *C. pepo* was 15.5%, for *C. pepo* and *C. moschata* it was 20 %, and for *C. pepo* and *C. moschata* it was 37.5% [13].

Based on the above, the goal of our research was to develop an experimental approach that allows obtaining viable seeds from interspecies hybridization of representatives of the genus *Cucurbita* under the conditions of full maturation of hybrid seed embryos *in planta*. The basis of the conducted search experiment was the method of inducing the growth of apomictic seeds, which involved the combined treatment of formed female flowers with growth regulators and pollination by another plant species that is phylogenetically close, but incompatible with the recipient species of foreign pollen. A similar approach was successfully applied at the Institute of Vegetable and Melon Growing of the NAAS for the formation of apomictic seeds *in planta* of sweet pepper and cucumber [14, 15].

### Materials and methods

The experiment on interspecies hybridization was laid in 2023 at the experimental base of the Institute of Vegetable and Melon Growing of NAAS, located in the agro-climatic zone of the Eastern Forest-Steppe of Ukraine (Selektsiynе village, Kharkiv region, Ukraine). A large-fruited pumpkin (*C. maxima* Duch.) of the Narodnyi variety of Ukrainian breeding was used as the paternal form. To obtain hybrid seeds, zucchini plants (*C. pepo* var. *giraumtonia* Duch.) were used as the maternal form. In particular, 3 hybrids of foreign breeding Defender F<sub>1</sub>, Patriot F<sub>1</sub> and Rimini F<sub>1</sub> (A.L. Tozer Ltd, Great Britain) were involved in the experiment.

The experiment was conducted in field conditions. Sowing seeds of hybrids F<sub>1</sub> of zucchini and large-fruited pumpkin was carried out manually according to the scheme of 70 x 70 cm, 30 pieces each. a certain type of plant. The area of the accounting plot is 9.8 m<sup>2</sup>, repetition is one time. Plant care was carried out according to the technology of growing zucchini for the above-mentioned agro-climatic zone of the Eastern Forest-Steppe of Ukraine [16].

In the experiment, two options for crossing zucchini and pumpkin plants were studied. The first, experimental option, provided for the production of hybrid seeds by the combined application of an aqueous mixture of growth regulators on the base of the pistil and pumpkin pollen on the stigma of female zucchini flowers. The second, control option, involved only applying pumpkin pollen to the stigma of female zucchini flowers. In more detail, two variants of interspecies hybridization consisted of the following manipulations for crossing squash and pumpkin plants:

- a day before opening, isolation of female zucchini flowers was carried out with parchment insulators;
- the next day, insulators were removed and pollen taken from pumpkin plants was applied to the stigma (experimental and control options);
- with the help of a micropipette, up to 50 µl of an aqueous mixture of growth regulators was applied to the base of the pistil of female flowers (experimental version only);
- female zucchini flowers processed by the above methods were isolated from the rest with parchment insulators for 5 days – a sufficient period of time, after which the stigmas lost their ability to perceive pollen from plants of their species.

At the end of the reproductive phase of plant development, an accounting of the obtained seed-fruits in the biological phase of development was carried out, the seeds were extracted from the fruits, the number, weight and appearance of the formed seeds were counted.

### Results and discussions

In 2023, squash plants were pollinated with large-fruited pumpkin pollen 36 times for each hybrid genotype. As a result of the application of the experimental variant of interspecies crossing with the additional application of a mixture of growth regulators, fully formed seeds were found in all three hybrid samples. Namely, the Defender F<sub>1</sub> hybrid formed 4 fruits with a total number of 183 seeds, the Patriot F<sub>1</sub> hybrid formed 2 fruits with a total number of 100 seeds, and the Rimini F<sub>1</sub> hybrid formed only 1 fruit with the presence of 8 seeds. The percentage of formed fruits with potentially hybrid seeds for the parent form of the Defender F<sub>1</sub> hybrid was 11.11 %, for the Patriot F<sub>1</sub> hybrid – 5.56 %, and for the Rimini F<sub>1</sub> hybrid – 2.78 %. Therefore, there was a different reaction of the genotype of the maternal form to the applied conditions of pollination with pumpkin pollen. In the control version of crossing, which involved only applying pumpkin pollen to the receptacles of the mother forms, the formation of one fruit was found in the Defender F<sub>1</sub> hybrid with the presence of 145 seeds and the Rimini F<sub>1</sub> with the presence of 95 seeds. The formed seeds were incomplete, with missing endosperm and lacked the ability to germinate both in the laboratory and in the field.

In 2024, at the time of writing theses (August 1, 2024), the plants of the original parental forms and interspecific hybrids derived from them were in the phase of beginning fruit formation. This phase made it possible to conduct a comparative analysis of the differences between the plants of the maternal and paternal components and the F<sub>1</sub> hybrids obtained on their basis according to the morphological and identification features of fruits, leaves and the shape of the bush.

The plants of the maternal forms – squash-zucchini hybrids Defender F<sub>1</sub>, Patriot F<sub>1</sub> and Rimini F<sub>1</sub> are characterized by a compact form with a bushy habit. The stems are green with no branching. The morphology of the leaf blade is distinguished by its triangular shape, green color, silvery patches and medium incisions. Zucchini plants of the above hybrid genotypes were characterized by a high load of plants with an ovary and a friendly yield. Fruits in the phase of technical ripeness are characterized by a cylindrical shape, elongated, smooth, very weak ribbed, dark green in technical ripeness. The parental form, the Narodnyi large-fruited pumpkin variety, is characterized by the following morphological and identification features: the plants are long-veined, the main stem is cylindrical in shape with stiff hairs (pubescence is characteristic of the species) and reaches a length of 2.5–3.0 m; leaves are green, round-heart-shaped, large (25–30 cm), not incised; in the phase of biological maturity, the fruits are transverse elliptical or globular (fruit index 0.8–1.0), medium or large (35–60 cm) in size. The surface of the fruit is weakly segmented, the color of the fruit when fully ripe is pink with an orange tint, with a pattern in the form of cream intersegmental stripes, the skin is thin (4–6 mm).

As an example, Figures 1 and 2 show the morphological features of the fruits and leaves of the original forms and the hybrid form obtained as a result of interspecific crossing of the zucchini hybrid Defender F<sub>1</sub> with the large-fruited pumpkin variety Narodnyi. It was established that the F<sub>1</sub> hybrids

(*Cucurbita pepo* var. *giraumontia* Duch. / *Cucurbita maxima* Duch.) had a bushy plant form inherited from the maternal component.

The fruits of interspecific hybrids had either an elliptical or broadly elliptical shape of green color with small and dense dots and with a darker and denser color of the ribs. Leaf blades in interspecific hybrid plants were characterized by heart-shaped or triangular shapes, with a complete absence or very shallow incisions, intense green color, sometimes with the presence of silver spots on the upper side.

Self-pollination of each hybrid plant was carried out. The final results regarding the analysis of hybrid plants will be carried out after the completion of all phenological phases of development.



**Figure 1. Morphological peculiarities of leaves and fruits of the original hybridization partners – the zucchini hybrid Defender F<sub>1</sub> and the large-fruited pumpkin variety Narodnyi: 1 – phenotype of the leaf blade of zucchini (left) and pumpkin (right) plants; 2 – the shape of the zucchini fruit in the phase of technical ripeness; 3 – the shape of the pumpkin fruit in the phase of biological maturity.**



**Figure 2. Morphological peculiarities of the structure of the leaves and the shape of the fruits of interspecific hybrid F<sub>1</sub> (*Cucurbita pepo* var. *giraumtonia* Duch. / *Cucurbita maxima* Duch.) compared to the maternal form – the zucchini hybrid Defender F<sub>1</sub> (*Cucurbita pepo* var. *giraumtonia* Duch.): hybrid sample D-10 (left) and zucchini hybrid D-8 (right)**

### Conclusions

It was established that carrying out a modified procedure of interspecies crossing between a zucchini (maternal form) and a large-fruited pumpkin (parental form) with the additional application of an aqueous mixture of growth regulators to the base of the pistil of female flowers of the zucchini during pollination with the pollen of the parental component contributed to the formation of viable hybrid seeds *in planta*. The preservation of hybrid seed embryos did not require the use of a biotechnological method of saving hybrid embryos by growing them on artificial nutrient media *in vitro*. From the hybrid seeds planted in the field, it was possible to obtain hybrid forms of plants, which, according to the morphology of the true leaf and the shape of the fruits, had intermediate signs of hybridization partners. From the seven fruits obtained as a result of interspecies crossing in 2023 with the participation of three zucchini hybrids Defender F<sub>1</sub>, Patriot F<sub>1</sub> and Rimini F<sub>1</sub>, seven

families of plants of interspecies hybrids F<sub>1</sub> were grown. Within a separate family, there were certain differences in the shape and color of fruits and leaves in hybrid plant populations compared to hybridization partners. It is likely that during hybridization there was recombinational variability within the combination of genetic material from both crossing partners. Preliminary cuttings of the fruits of hybrid plants obtained as a result of free pollination confirmed the presence of formed seeds with traits of a well-formed endosperm.

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