

The world's first (openly available) interspecific reverse hybrid of *Maxima* and *Moschata* pumpkins. Adaptive-Bio-rootstock.

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Abstract

First-generation interspecific pumpkin hybrids are a large-scale commercial product and are used as professional rootstocks for cucurbits(bio-rootstock). Due to their reduced specialization, the tissue and sap of these hybrids are highly compatible, and thanks to the large number of incorporated genes, these rootstocks adapt well to the signals received from the scion plant, producing the substances required by the scion.

Interspecific pumpkin hybrids also have their drawbacks. F₁ hybrids are often sensitive to cool climates and are not prone to rapid growth and flowering. Not all melon varieties are ideal for grafting. In cold climates, open ground, and areas with high virus and insect infestations, grafted plants may be more susceptible to disease. Rootstocks require watering and a mild, greenhouse-like climate. Under these conditions, they achieve vigorous growth and timely flowering, demonstrating conditional immunity to pathogens and nematodes.

Adapting professional rootstocks to harsher climates or terroirs is impossible, as they do not produce viable seeds. Therefore, professional rootstocks are adapted to the conditions in which pure lines are grown, often in specialized greenhouses for hybrid production, except the initial adaptations of pure lines in the field.

Therefore, producing interspecific pumpkin hybrids in the second generation can offer many advantages. Such a crop can be more fertile, especially if it is obtained by reverse hybridization from a fertile interspecific hybrid adapted as a pollen donor. Also, when producing a reverse hybrid, the hybrid's genetic diversity increases. Furthermore, when the resulting hybrids are further crossed with other hybrid lines, the genetic material of all lines will be combined into a common gene pool for the resulting pure line. Essentially, this is the classic formation of a complex new variety, but in this case, it is the formation of a new species(Synthetic population) —*Moschata/Maxima*. This variety, possessing nominal fertility, can cross-pollinate multiple times without deep cleavage or loss of quality, and after extensive selection, it can pupate(stabilize into a distinct variety) into a similar variety (or more accurately, species). Such an interspecific hybrid will likely be pollinated by its ancestor crops; establishing a stable species barrier requires many generations, and in the early stages, it is necessary to pollinate the crop manually or grow it away from other cucurbits.

But the key factors are fertile offspring and adaptability(Adaptive breeding), as well as the potential for subsequent crosses with other cucurbits, such as squash, *Ficifolia* (*Cucurbita ficifolia*), or some rare pumpkin species. For example, introducing squash genes into a hybrid can improve compactness and speed growth and flowering, ideal for more northern latitudes. Such hybrids are not formed from varietal squash and pumpkins due to their genomic differences, but crossing hybrid interspecific forms offers the potential to produce a similar hybrid. Also, phycephalia and some other distantly related pumpkins do not form hybrids under natural conditions, but working with interspecific hybrids may offer the potential to produce a more robust hybrid, potentially a professional melon rootstock, with qualities unavailable to modern first-generation interspecific hybrids.

Keywords: Interspecific hybridization, Incompatibility barriers, Synthetic population, Recurrent selection, Transgressive segregation, Bio-rootstock, Intensive cucurbit farming, Adaptive breeding, Open-source breeding, Plant genetics, Plant growth Physiology, Agriculture, Organic Breeding, Low Input Agriculture, Sustainable Farming, Permaculture

Another important advantage of subsequent generations of interspecific hybrids is the inclusion of new pure lines in their genome. Expanding genomic diversity within a plant can further enhance the non-specificity and flexibility of its physiology, thereby further improving the survival and growth of the scion. Even with greater fertility, such hybrids may have superior rootstock properties, as there is no direct correlation between fertility and rootstock quality. This suggests optimism for the future of this research.

Our work involves several stages: preparing the genetic material and directly producing the reverse hybrid. The genetic material was obtained by crossing different Maxima and Moschata cultivars, producing intraspecific hybrids, and adapting and selecting these hybrids in subsequent generations. The interspecific reverse hybrid was produced using pollen from the intraspecific hybrids (in the third generation) and female flowers from a commercial intraspecific hybrid of Maxima and Moschata. Pollination was carried out with immature flowers and pollen, using a number of additional techniques to reduce the interspecific barrier.

In September 2025, we obtained approximately 50 seeds from two pollinated pumpkins. All pumpkins used in the experiment were grown outdoors in central Ukraine, in a cool climate, in an area contaminated with the Cucumber Mosaic Virus and insects, as a method of adaptation and resistance testing. We germinated two seeds from the received batch (to assess germination before mass sowing), one of the two seeds turned out to be viable and produced sprouts, a rough estimate of germination is 50%, the seeds were stored at room temperature under normal conditions:



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Textured leaf, growth corresponds to the size of the seed, without deviations from the norm.



The figure demonstrates the combined morphology of Maxima and Moschata. In this case, genetic testing was unnecessary, as the seeds were obtained from the fruit of a professional rootstock, the plant was completely consistent in appearance with the intraspecific rootstock, and the small, wrinkled fruits were not pollinated with varietal pollen without special techniques.



As the figure shows, the work was not conducted in a laboratory setting(Open-source breeding), which adds reliability to the reverse hybridization method, although the actual preparation of the genetic material and the crossing process were not trivial. For this reason, the work can be classified as amateur, but only partially, especially considering that similar specimens had not been obtained previously.

Since the seeds were germinated on a damp cloth, the seedling, already with a long stem, was planted and grown on a windowsill with almost no light. Its stem is slightly elongated, similar to the behavior of varietal pumpkins under these conditions. However, what is characteristic of our specimen is the bluish-white waxy coating on the cotyledons, the dense hairs on the stem and leaves, and the particularly unique first leaf, resembling a maple, with strong veins and a textured leaf between them.

The potential of fertile interspecific complex pumpkin hybrids is difficult to overestimate. The first step was successful, and dozens of projects are expected in the future to develop specialized fertile hybrids with specific properties to meet all possible agricultural needs.

Methods

The development of the fertile interspecific *Cucurbita maxima* x *Cucurbita moschata* bridge was achieved through a multi-stage hybridization protocol designed to bypass complex progame incompatibility barriers. The methodology centered on the creation of a broad genetic plateau using pre-adapted intraspecific hybrids of *C. maxima* and *C. moschata*. To initiate interspecific embryogenesis on professional rootstock lines, a high-intensity bio-mechanical induction technique was employed. This involved the application of a heterogeneous gametophytic substrate, consisting of intact young and mature pollen integrated with a modified lysate produced via controlled mechanical maceration of the male gametophyte. The process was augmented by the introduction of exogenous biochemical mediators to neutralize maternal immune responses. Pollination was strictly coordinated with microsurgical interventions, including hormonal regulation of the floral apparatus and targeted scarification of the stigmatic and stylar tissues, followed by the strategic application of the pollen-mediator complex directly into the incisions. This approach enabled the consolidation of divergent genomes and the subsequent stabilization of fertile F2 populations.