USE OF INDUCED APOMIXIS IN THE BREEDING OF FIGS

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The tendency of certain varieties and species of figs toward induced facultative apomixis when isolated flowers are exposed to various inducing agents (foreign pollen and biologically active compounds) has been established experimentally and utilized in practice. The degree of trait variation in apomictic plants was studied over two generations after treatment with various inducing agents. Valuable plant forms were isolated.

Introduction. Apomixis is a very common phenomenon in angiosperms but complex in its manifestations. Many problems related to apomixis still have not been solved. It has been repeatedly pointed out that thorough study of apomixis and its practical utilization in breeding and seed production are necessary. Nevertheless, the significance of apomixis in the breeding and evolution of plants has been somewhat underestimated, so that different types of apomixis have not been adequately investigated and utilized: experiments have been conducted for only a small number of species, there have been comparatively few embryological studies of apomixis, etc.

Apomixis is now utilized in several ways in the breeding of crop plants. The greatest amount of work has been done on the production of haploids for creation of homozygous diploid forms. Utilization of induced diploid apomixis in the breeding of cross-pollinated plants with normal meiosis and sexual reproduction that are also capable of propagating vegetatively has been on a considerably smaller scale. Use of induced apomixis for such plants results in development of a wide variety of forms in their progeny, these often carrying valuable recessive traits. Many years of experimentation at the Nikita Botanic Garden with figs have confirmed this.

Experimental Subjects and Method. We first observed the tendency of the common fig (*Ficus carica* L.) toward induced apomixis at the Nikita Botanic Garden after pollination of its flowers with white mulberry pollen [1, 2]. We subsequently conducted many experiments to study the tendency of a large number of cultivated varieties and wild forms of two fig species (the common and Afghanistan figs) toward autonomous and induced apomixis.

The tendency toward autonomous apomixis was studied in 120 common fig varieties over a period of several years, isolating the female inflorescences to keep them from being pollinated. We also determined the degree of pollen and ovule fertility and made crosses to establish the presence of apomictic elements in the varieties under investigation.

The tendency toward induced apomixis was studied in 63 fig varieties and forms. Pollen from more than 25 species belonging to different families (applied to the flowers when they were fully open) was tested for induction of apomixis. In later years, we employed various biologically active substances: growth stimulators, vitamins, biogenic stimulators, antibiotics, kinetin, adenosine triphosphate, and other compounds, which were mixed with a filler (talc) and also applied to the flowers in different doses. The closed structure of the fig inflorescence, with the florets located on the inner side of the scape, necessitated use of a readily dispersed material (talc) as the filler, in place of the lanolin employed for other plants.

Chromosome counts were made with permanent preparations of the rootlets of the young apomictic seedlings; temporary preparations of the young leaflets were stained with a mixture of acetic acid and iron hematoxylin.

The developmental characteristics of the apomictic embryos of the common and Afghanistan fig were studied at the Cytology and Embryology Laboratory of the Nikita Botanic

arden by A. I. Zdruikovskaya and later by R. S. Romanova.

Experimental Results and Discussion. Numerous experiments conducted under botanic-arden conditions (in the Crimea) established that autonomous apomixis was lacking in all he fig varieties tested and that meiosis and sexual reproduction proceeded normally.

In addition, experimentation revealed a tendency toward induced apomixis in seven va-ieties of common fig and two forms of Afghanistan fig. Development of apomictic embryos nd seeds was stimulated by pollen from the lily, pubescent oak, mulberry, paper mulberry, nd certain other members of the mulberry family, to which the fig belongs. Under the in-luence of such pollen applied profusely to the stigmata of fig flowers, seedlings devel-ped from the apomictic seeds and matured when cultivation conditions were favorable.

Experiments involving use of various biologically active substances (some of which ad mutagenic properties) for induction of apomixis showed that only some of them caused evelopment of apomictic embryos and viable seeds when applied to isolated flowers during he flowering period [3, 4]. Of the substances tested, the strongest positive effect was xerted by α-naphthylacetamide (NAA), mixtures of this compound with vitamins B_1, B_2, PP, nd C, the potassium salt of penicillin, adenosine triphosphate, kinetin, and succinic and spartic acid, the latter two compounds having been tested as apomixis inducers only dur-ng the last two years of the study. The greatest apomictic-seed germination rate (95-8%) was observed when the flowers were treated with biogenic stimulators and kinetin.

It was not always possible to obtain seedlings from seeds containing apomictically eveloped embryos, even when the strongest substances were used as inducing agents. Un-avorable conditions for tree growth, especially soil drought and dry air, caused early hedding of the inflorescences or young fruits. Application of a small amount of foreign ollen or an active compound was inadequate for tree ripening of the inflorescences, which ontained up to 1500 florets each. Application of an excess of a biologically active com-ound often caused accelerated ripening of the compound fruit (within 8-12 days after ap-lication), which did not promote development of apomictic embryos or ripe seeds.

Embryological investigations conducted by G. S. Romanova in 1971-1975 (whose results are still unpublished) showed that the apomictic fig embryos produced under the action of NAA and adenosine triphosphate developed from the cells of the ovular apparatus (from the ova and, less frequently, the synergids). Zdruikovskaya [5] and Romanova (unpublished data) detected parthenogenesis and nucellar embriony in the Afghanistan fig.

Counting of the chromosomes in the apomictic seedlings of the common fig showed that they were all diploid (2n = 26). It was thus established that induced diploid partheno-genesis takes place in the common fig. In view of the fact that apomixis is facultative in the fig, while meiosis and fertilization take place normally, it can be assumed that, when inducing agents are applied to the flowers, their action results in development of processes in the ovules leading to postreduction doubling of the chromosome number in the ova and subsequent nuclear division accompanied by cytokinesis. Similar postreduction restoration of the diploid state in the ovum has been observed in other crops with a ten-dency toward apomixis.

The apomictic seedlings of the triploid Afghanistan fig were wholly viable triploid and tetraploid plants with 39 or 52 chromosomes; there were also numerous aneuploid forms that died during the early stages of their development. Since two types of apomixis were detected in the Afghanistan fig, triploid apomictic embryos could develop as a result of nucellar embriony or unreduced parthenogenesis. The tetraploid apomictic embryos appar-ently developed from the ova as a result of postreduction doubling of the chromosome num-ber under the influence of the inducing agents.

Experiments on the experimental production of apomictic seedlings in the common and Afghanistan fig made it possible to raise 3000 apomictic seedlings over three generations.

Morphological and pomological study of apomictic seedlings from seeds exposed to var-ious inducing agents established that there was great trait variation in the plants of all three generations.

In order to account for the high degree of variation of apomictic seedlings (haploids in corn and diploids in figs), it has been hypothesized that its main causes are appear-ance in the haploids of traits that are in the recessive state in diploids and production of a different chromosome complement by each haploid [7]. During subsequent postreduction doubling of the chromosome number in the cell nuclei under the action of the inducing

agent, the individuals produced will manifest the same degree of variation as the haploids but will have greater viability.

The first-generation common-fig apomictic seedlings differed in the sizes of all their organs, the shape, color, flowering time, and ripening time of their fruits, yield, and fruit quality. We isolated 12 economically valuable dry-fruit, preserve, and table-fruit varieties, of which five were submitted for state varietal testing. Two apomictic seedlings, of the dry-fruit Smena and Aromatnyi Nikitskii varieties, have been approved as a result of testing.

Depending on the initial variety and type of inducing agent, the second-generation apomictic seedlings were more or less valuable and diverse. When foreign pollen was used as the embryo-development stimulator, the second apomictic generation exhibited signs of depression or resembled wild-type plants. Wild-type apomictic seedlings appeared in the first generation for some initial varieties (Calimyrna). The second-generation apomictic seedlings exhibited weak annual growth, slender shoots, small leaves and fruits, uniformity of fruit coloration, and a mediocre flavor.

The apomictic seedlings obtained as a result of treatment of flowers with biologically active substances (NAA, the potassium salt of penicillin, etc.) displayed a totally different pattern in the second generation. These plants were characterized by great diversity of traits, including large differences in growth vigor and fruit coloration, size, shape, and quality. Individual strong seedlings gave very large fruit yields, with the fruits and leaves having an unusual shape. Some plants completely lacked downiness on the skin of the fruit, a trait typical of all fig varieties. The flowers often had a modified form and the fruits contained very small seeds. All this indicated that the inducing agents undoubtedly affected the formation of the apomictic seedlings, an effect especially pronounced in the second generation. Inducing agents apparently not only delay the shedding of unpollinated inflorescences but also stimulate doubling of the chromosome number in the ova and cause development of apomictic embryos (on which they then have a mutagenic action). The closed inflorescences of the fig promote prolonged retention of substances introduced into them and hence facilitate their action on the developing embryo.

Conclusions. A study conducted at the Nikita Botanic Garden established that certain fig varieties and species have a tendency toward induced facultative diploid apomixis.

Types of pollen and biologically active substances that cause development of apomictic embryos and viable seeds in figs were isolated.

Highly polymorphic apomictic seedlings with economically valuable traits were raised. Forms with valuable recessive traits were detected.

It has been hypothesized that the agents used to induce apomixis in figs in our experiments not only promoted doubling of the chromosome number in the ova during the post-reduction period and stimulated further development of the apomictic embryos but also had a definite mutagenic effect.

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