Bulgarian Journal of Agricultural Science, 27 (No 5) 2021, 903–910

Modern notions about the pollen sterility in the vine and methods for overcoming it

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Abstract

Topale, S. & Roychev, V. (2021). Modern notions about the pollen sterility in the vine and methods for overcoming it. *Bulg. J. Agric. Sci., 27 (5)*, 903–910

As a result of palynological, cytogenetic and scanning electron microscopy examinations, eight types of pollen sterility in the vine have been identified related to the reasons for their occurrence: genetic; chromosomal; *meiotic*; *genomic*; *chromosomal-genetic; chromosomal-genomic; polyploid* and *total*. In the distant hybrids of F_3-F_5 generations, all genotypic variants of pollen sterility are observed – from partially fertile to semi-fertile and form with normally restored fertility and vitality. As the number of generations increases, the number of self-fertile forms increases and the number of sterile ones decreases significantly. A DRX-M_3-2 seedling with chromosomal sterility of the two gametophytes was found in F_3, in which no grains or seeds were formed, regardless of the pollination method. By crossing distant hybrids of F_3 with 2n=38 with S.V.-12-309 and S.V.-20-366, F_4 generation was obtained, in which DRX-M_4-501,502,503, etc., are fruiting, partially fruiting and infertile with 2n=38, 39. From the crossing of the distant hybrids of F_4 – DRX-M_4-520, DRX-M_4-510 (2n=38) with the cultivars GM-325-58, Cristal and Moldova, F_5 generation was created, in a population of which four artificially created species carrying the new vine genome with n=19, 2n=38, which includes the chromosomes of *Vitis vinifera* and *V. rotundifolia*.

Keywords: types of pollen sterility in the vine; classification; distant hybridization in F_1-F_5 generations; palynological; cytogenetic and scanning electron microscopy examinations

Introduction

Sterility in botany and plant growing means partial or complete inability to form viable and functioning gametes, zygotes, fruits and seeds. It depends on some changes in environmental conditions, as well as a number of internal causes, such as chromosomal and genotypic conditioning, age-related changes and plasma factors. Genetic Male Sterility (GMS) in grape cultivars is of great theoretical and practical importance. Many publications on this problem have long been known, some of which present the results of cytological studies of grape cultivars and interspecific hybrids with sterile pollen in connection with their use for selection purposes (Dorsey, 1914, 1915; Negrul, 1930; Ivanova-Paroyskaya, 1928; 1930; Kolev & Terziyski, 1967; Topale, 1983). Hybrid forms with a combination of functionally female flowers with seedless and almost seedless grains were reported by Negrul (1936), Hizantsyan (1958), Volosovtsev (1967) and Roytchev (2008), according to whom this type of flower should be called “functionally asexual”. Many features of pollen sterility in vines have not yet been studied. In the last few decades, a *new genome has been synthesized* and *artificial synthetic species* have been created in the vine, but the practical realisation of these discoveries in selection programs has not yet been reached (Topale 2008, 2011). In the world literature on viticulture there is no uniform classification of this phenomenon resulting from the reasons for its occurrence. Most often it is determined by several factors, as a result of which it is very difficult to differentiate and determine its varieties. The aim of this study is to propose a new classification of the types of sterility in the vine on the basis of our own palynological and cytogenetic results and scanning electron microscopy observations.

Material and Methods

Grape cultivars and branches grown in the ampelographic collection of the “Magarach” Institute of Wine-Making and Viticulture (Yalta), Russia and the National Institute for Viticulture and Oenology (Chisinau), Moldova were used for the karyological and palynological studies. By applying the method of distant hybridization, a cross between representatives of the subgenus *Euvitis* and the subgenus *Muscadinia* was realized, as a result of which three new generations of grape vine seedlings were created (F_3, F_4, F_5).

For the first time in the world, a new vine genome was synthesized in F_5, including chromosomes from the phylloxera-resistant American grape species *V. rotundifolia* Michx. (n=20) and chromosomes from the European cultivar *V. vinifera* L. (n=19). From the F_5 hybrid plants, the species *V. rotundifera* Dad., *V. vinifolia* Top., *V. nigra* Top. and *V. cruceştiana* Top., which are carriers of the new genome, were selected. They could be used as parents in crosses in order to obtain phylloxera-resistant grape cultivars, with the possibility of growing their own roots without grafting.

Palyno-morphological studies were performed on fresh pollen grains collected at the time of dissolving the anthers of the distant hybrids DRX-M_3,-232; DRX-58-5; DRX-M_3-97; DRX-M_3-205; DRX-M_4-511; DRX-M_3-39; DRX-M_3-52; N.C.-6-15, and also of the triploids XIX-20/48; XIX-28/4; XX-29/8 and the allotriploid DRX-M_3-372. In DRX-58-5, the somatic number of chromosomes has been determined as one of the most important cytogenetic characteristics of each cultivar, explaining the reasons for pollen sterility.

The preparation of pollen for scanning is according to the established methods in electron microscopy (Terziyski & Karageorgiev 1989). The coating of the samples with carbon was performed in JEE – 4X vacuum evaporator. All observations on the micro-morphology of pollen grains from seedless and seeded grape cultivars with different flower types were performed in the Electron Microscopy Laboratory of the Agricultural University – Plovdiv, Bulgaria with JEM 1200 electron microscope and ASID 10 (JEOL news) scanning attachment.

Results and Discussion

The most commonly cultivated grape cultivars (*Vitis vinifera* L.) are characterized mainly by bisexual (hermaphroditic) type of flower, but are also known to be functionally female (Bikan, Nimrang, Almeria, Chaush, etc.). Unlike them and *V. labrusca* L., which are monoecious, all wild grape species distributed in Europe, Asia and North America are dioecious, i.e. female and male flowers are located on different plants. With male sterility are the grape cultivars in which pollen grains are not formed in the anther at all, or they turn out to be non-viable and cannot fertilize the egg. Normal fertile and vital pollen grains in the vine have a barrel shape or are in the form of a wheat grain (Figures 1 and 2). In the extreme manifestations of male sterility, the stamens in the flower do not develop at all or change into petals (Mourvedre grape variety).

Several classifications of pollen sterility types are known for the flowering plants. Taking as a basis the classification of Stout (1921), Rybin (1967) proposes to distinguish 12 types of sterility by reason of occurrence, of which type 4 is due to hybridization; type 6 is due to the presence of polyploidy; type 9 is due to genetic male sterility; type 10 is due to cytoplasmic sterility; type 12 is the result of incompatibility. According to Sears (1947), only three types of hereditary

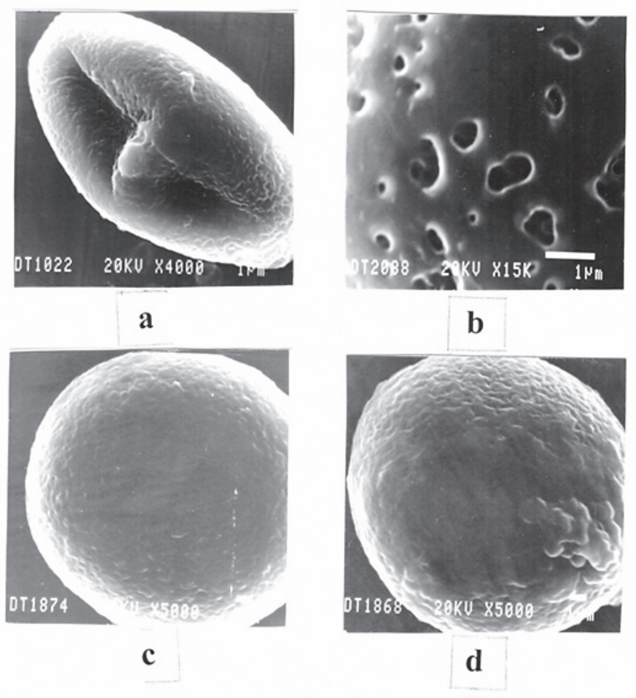


Fig. 1. Scanning electron microscopy photographs of pollen grains of grape cultivars (*Vitis vinifera* L. *ssp. sativa* DC) with different flower type: a – Bolgar, x 4000 – bisexual; b – Kishmish Irtishor, x 15,000 – functionally male; c – Chaush, x 5000 – functionally female; d – seedless hybrid form, x 5000 – functionally female

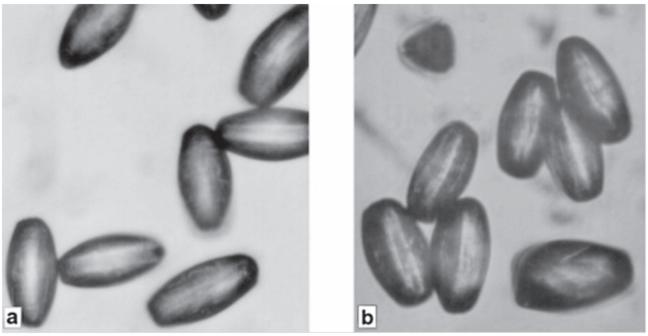


Fig. 2. Pollen grains of: a – *Vitis vinifera* L.- natural species; b – *Vitis rotundifera* Dad. – artificially created species. x **650**

sterility can be distinguished: 1) genetic (GMS); 2) cytoplasmic (CMS) and 3) chromosomal. After taking into account the shortcomings of the existing classifications related to the causality of their occurrence and based on Gabelman (1956), Krupnov (1973) published his own classification which included 8 types of sterility. Thompson (1930, 1940) believes that pollen sterility is directly related to meiosis disorders that occur in prophase_I. Depending on the phase of sterilization, Muntzing (1967) divides it into two types – haploid and diploid. The first type includes cases in which pollen grains and eggs die as a result of their constitution, and the second – when the phenomenon of death is determined by the genotype.

According to our research, eight types of pollen sterility should be distinguished in the vine. The most common is the **genetic sterility**, which occurs in all grape cultivars with a functionally female type of flower, wild species of the genus *Vitis* L. and some distant hybrids of F_1 – F_5 generations (Figure 3). Pollen sterility occurs during the last stage of meiosis – the tetrad phase. Morphologically, this type of sterility is expressed in the special arrangement of the stamens – they are inclined downwards or curved horizontally. Pollen grains are deformed, without grooves and apertures, with a uniform and low-relief ultrasculpture of the exine, orange peel type (Roytchev, 1995; 1997). Genetic sterility is associated with complete destruction of the nuclei and cytoplasm of microspores. It is genetically determined and is observed annually in varieties and distant hybrids with a functionally female type of flower and in sexual hybridizations it is inherited in some of the seedlings of F_1 generation.

Chromosomal sterility is characteristic of distant hybrids of the vine obtained with the participation of representatives of the subgenus *Muscadinia* – species *Vitis rotundifolia* Michx. and is demonstrated by the absence of a pair of conjugated chromosomes due to the odd somatic number – 2n=39 (Figure 4). With this sterility, the pollen grains are almost twice as small as the original species. They are deformed and sometimes have the shape of millet seeds. This is a stable sign and in sexual hybridization it is inherited in some of the seedlings in F_1 – F_4 generation. In the F_5 generation hybrids the analyzed sterility was not observed, because in their microsporogenesis the meiosis is completely corrected (normal) and the pollen grains have the size and shape characteristic of bisexual (hermaphroditic) flowers. These seedlings can be used in crosses without demasculinization (castration) of flowers.

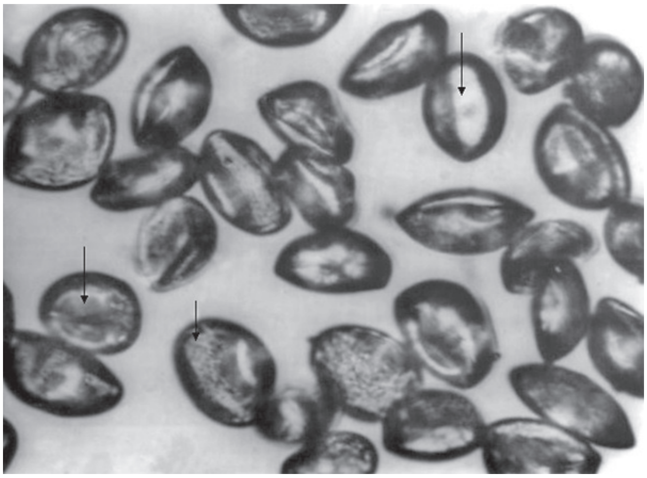


Fig. 3. Sterile pollen grains from the distant hybrid DRX-M3-232. The arrows indicate the lack of content in them. x**560**

It should be noted that chromosomal sterility has so far been characteristic only of the male gametophyte, and the female has functioned normally. Therefore, when crossing or further pollinating hybrids with chromosomal type of sterility (DRX-55, DRX-58-5), they usually form grains with seeds. In 2007, a seedling with chromosomal sterility of the two gametophytes, DRX-M_3-2, called **total sterility**, was discovered for the first time between distant F_3 generation hybrids. The pollen of this plant is morphologically similar in shape and size to that of DRX-55, DRX 58-5. Another feature is that regardless of the method of pollination, no grains and seeds are formed, because one or two weeks after flowering all the inflorescences dry out, so it cannot be used in crosses as a maternal or paternal parent (Figure 5). This hybrid is in the final stage of evolutionary development and should be propagated only vegetative. It can be used as a starting material for colchicine in order to obtain allopolyploids, and is also a valuable object for conducting in-depth

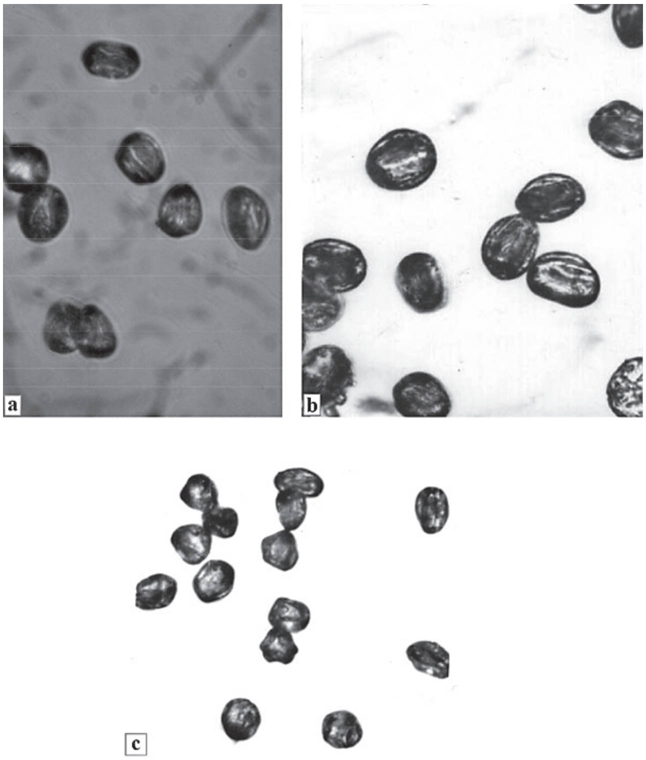


Fig. 4. Sterile pollen grains from the distant hybrid *Vitis vnifera* x V.*rotundifolia* F2-F3: a – DRX-58-5 (F2); b – DRX-M3-97; c – DRX-M3-205. ˣ650

cytogenetic examinations related to the study of the causes of total sterility. Such false hybrids are also called *pseudohybrids*. The establishment of the new type of total sterility of the two gametophytes was registered in AGEPI, Moldova.

Meiotic sterility is due to local disturbances in the meiosis of individual or of multiple anthers, caused by various reasons, including external environmental conditions. This type of sterility is found in bisexual varieties, distant hybrids and wild grape species. It is characterised by the fact that in the anthers, along with the normally formed type (tricolporate), a certain amount of deformed, wrinkled, smaller and sterile pollen grains are always formed (Figure 6). Meiosis is a very fine system of distribution of genetic material and the slightest disturbances caused by internal or external factors during the formation of specialised cells in the process of reduction division, inevitably have a negative impact on the formation of micro- and macrospores and their viability. The disturbed balance between the genetic material in the microspores and the eggs leads to the formation of non-viable gametes, and subsequently zygotes. The seedlings derived from them usually die in infant (juvenile) stage.

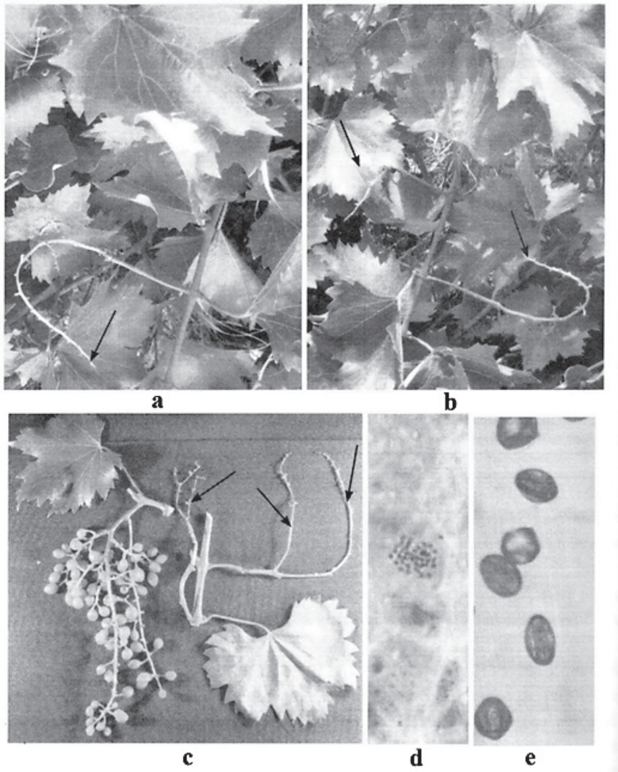


Fig. 5. Total sterility of the gametophyte in DRX-M_3-2: a, b – elongated axes of the inflorescences from which the flower buttons have fallen; c – cluster with normally developed and growing grains (left) and part of the shoot with drying inflorescences (right) 12-14 days after the beginning of flowering; d – number of chromosomes – 2n=39, x 1760; e – sterile pollen, x 650. The arrows show the drying inflorescences

Genomic sterility is caused by the presence of an odd number of chromosomes in polyploid forms (i.e. 3, 5, 7, etc.). In the process of meiosis, they lack bivalents, which mean completely disrupted conjugation and incorrect distribution of chromosomes in the spore cells. As a result of these anomalies, all sorts of hyper- and hypoploid macro- and microspores, which are sterile, arise. This type of sterility occurs only in vine triploids and anorthoploid groups, in which a mixture of sterile pollen grains of different shapes and sizes is morphologically observed. Genomic sterility is characteristic of both male and female gametophytes, so most grains of triploids are parthenocarpic (seedless), and their seeds are underdeveloped rudiments, practically non-viable (Figure 7).

Chromosomal genetic sterility is caused by the absence of a pair of conjugate chromosomes and genetic causes, i.e.

Fig. 6. Sterile and fertile pollen grains from the distant hybrid F_4 – DRX-M4-511. The down arrows show the sterile and the up arrows show the fertile pollen grains, ^{x} 650

complementary action of 2-x factors. This type of pollen sterility occurs in distant hybrids in the F_1 generation with a functionally female type of flower (N.C.-6-15) and in some F_3 and F_4 seedlings. Its characteristic feature is that the sterile pollen grains have a rounded shape with completely destroyed internal content (Figure 8).

Chromosomal-genomic sterility is observed in distant hybrids, which result from a cross between the diploid distant hybrid DRX-55 (2n=39) and the autotetraploid form of the Shabash variety (2n=76) or with autopolyploids in general. In some cases, the unreduced gamete of the distant hybrid with n=39 and the normally reduced gamete of the autotetraploid with n=38 took part in the hybridization, resulting in a plant with a complex hereditary basis comprising 77

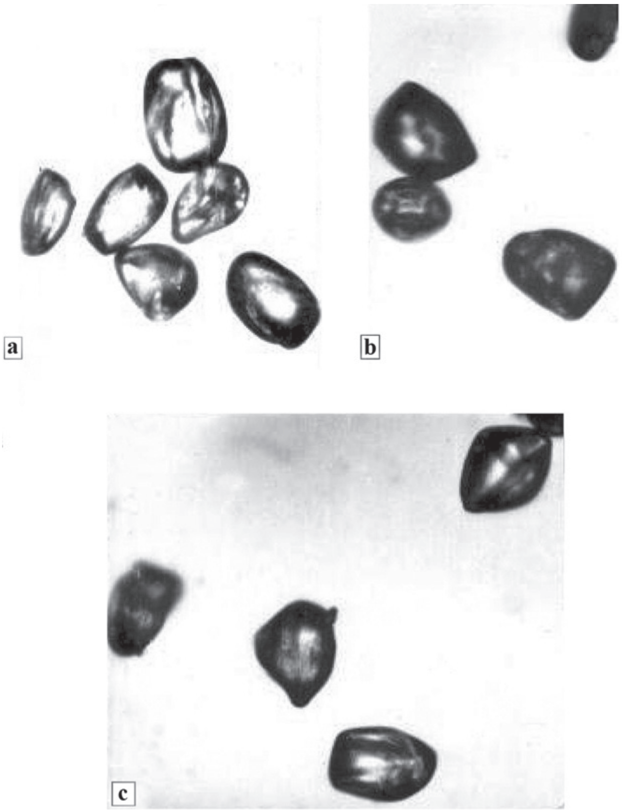


Fig. 7. Sterile pollen grains from vine triploids with 2n=57: a – XIX-20/48; b – XIX 28/4; c – XX-29/8; ^{x} 650

somatic chromosomes. In two other cases, allotriploids with 2n=57-58 were obtained. When the allotriploid enters the reproductive phase, a small inflorescence is formed, consisting of 3-5 flower buttons. The microphotographs of the pollen grains from the allotriploid DRX-M_3-372 convincingly show that, like them, the eggs of the allotriploids (allopolyploids) are probably non-viable (Figure 9). Therefore, triploid vine forms are not promising for use in the selection process, due to serious disturbances in the process of meiosis in the male and female gametophyte. The results of the worldwide experience with the Euro-Asian vine in different territorial areas of viticulture confirm this finding, as there is not a single allotriploid variety in the world vine assortment so far.

The last type of sterility of vine pollen is associated with the presence of **polyploidy** (Figure 10). Numerous comparative studies of diploid and polyploid plants have shown that in some flowering plants, after their transition from diploid to tetraploid state, a decrease in their fertility is observed. In the case of the vine, this negative phenomenon was first studied by embryological methods in the variety Chasselas Grokular by Lazarevski (1934), and later by Rives & Puoget

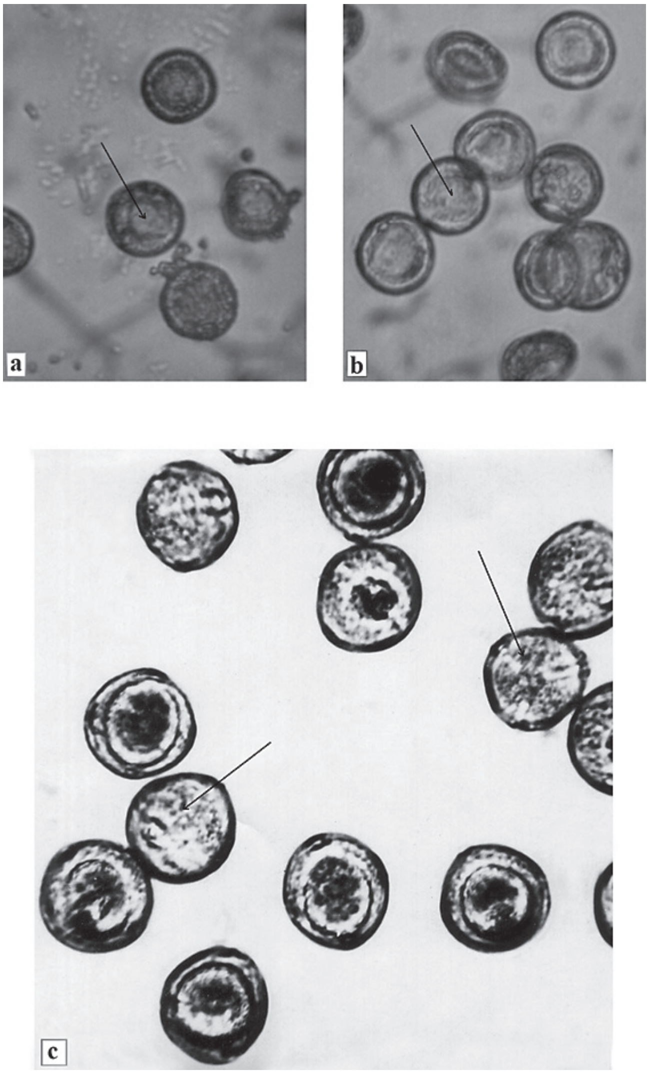


Fig. 8. Sterile pollen grains from distant hybrids *Vitis vinifera* x V. *rotundifolia* with 2n=39: a – DRX-M_3-39; b – DRX-M_3-52 (F_3); c – N.C.-6-15 (F_1). The arrows indicate the lack of content in the pollen grains. a, b – ^{x}550; c – ^{x}650

(1959) and Topale (1988). As a result of cytological disorders in the correct course of meiosis during the formation of eggs in tetraploid mutants, the flower buttons of the catkins fall off. The sterility of pollen, the low rate of germination of pollen grains in artificial nutrient medium and their reduced viability are due to the presence of polyploidy and disorders in the process of meiosis.

Knowledge of sterility is of great practical importance because it allows the correct planting of vines, the

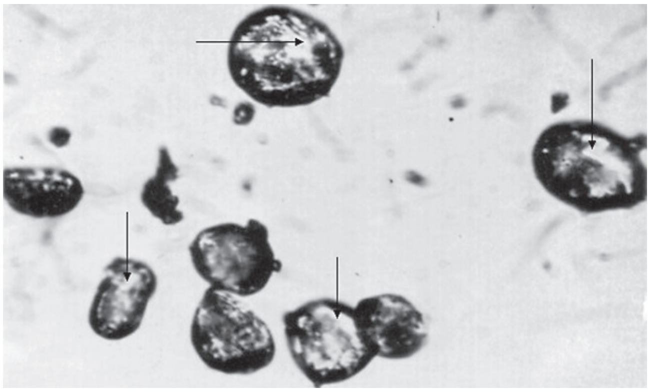
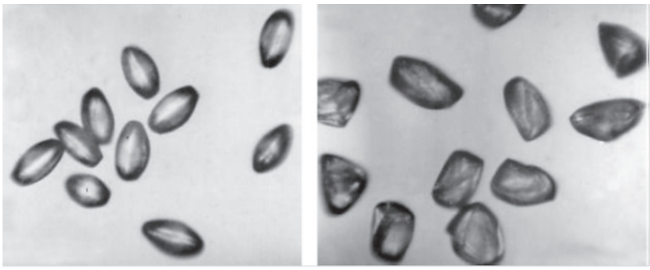


Fig. 9. Sterile pollen grains from the vine allotriplod with functional female type of flower DRX-M3-372 with 2n=58-59 with ^{x}650. The arrows indicate the lack of content in the pollen grains



a b

Fig. 10. Pollen grains from diploid and polyploid grape cultivars: a – Chasselas white (2n=38); b – Chasselas grokular white (2n=76). x650

selection of pollinator varieties, parental pairs in sexual hybridization and to avoid difficulties in demasculinisation of flowers. By crossing the cultivar *Vitis vinifera* L. (2n=38) with wild species of *V. rotundifolia* Michx. (2n=40) the distant hybrids F1: N.C.-6-15 and N.C.-6-16 were obtained. They have a diploid somatic chromosome number of 2n=39 from both parental forms, but are completely sterile. After crossing the distant hybrid N.C.-6-15 (2n=39) with Black rose (2n=38) and other varieties of *Vitis vinifera* (2n=38), the conditionally called F_2 generation hybrids, or the widely known in the literature DRX (BC1) were obtained. Dunstan (1962) indicated that the hybrids DRX-55 and DRX-58-5 have a diploid number of chromosomes 2n=38, and for the same hybrids Topale, Guzun (1980) found another chromosome number – 2n=39 (Figure 11).

By crossing DRX-55, DRX-58-5 (2n=39) with *Vitis vinifera* (2n=38), *V. rotundifolia* (2n=40) and hybrids of

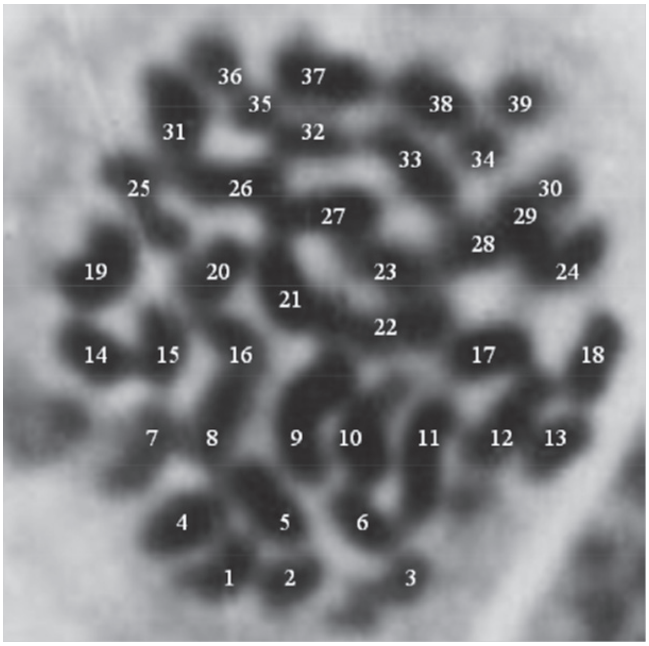


Fig. 11. Metaphase chromosomes from the sterile distant hybrid DRX-58-5, (2n=39). ˣ2660

Seyve Villar (2n=38), distant hybrids were obtained, conditionally called F_3 generation, which are marked with the letter ‘M’ as they were created in Moldova. Cytological analysis showed that the F_3 generation consists of hybrids with a somatic chromosome number of 2n=38 and 2n=39. These data are direct evidence that the DRX-55, DRX-58-5 embryo sacs form three types of viable female gametes (eggs) with n=19, n=20 and n=21.

By crossing the distant hybrids of F_3 – DRX-M3-232 (2n=39), DRX-M3-90 (2n=38) with S.V.-12-309; S.V.-20-366, the conditionally named F4 generation was obtained, including the hybrids DRX-M_4-501, 502, 503 (2n=38), etc., which are fertile, partially fertile and infertile with 2n=38, 39.

From the crossing of the distant hybrids of F_4 – DRX-M_4-520, DRX-M_4-510 (2n=38) with the varieties GM-325-58, Cristal and Moldova, the F_5 generation was created, consisting of hybrids with a somatic number of chromosomes 2n=38. Other types of hybrids have not been found. Based on these results, a conclusion was made to complete the process of synthesis of the new vine genome. Among the seeds in the hybrid population of F_5, four artificially created species have been identified, which are carriers of the new vine genome – n=19, 2n=38, which includes the chromosomes of both species: *Vitis vinifera* and V. *rotundifolia*.

Conclusions

As a result of palynological, cytogenetic and scanning electron microscopy examinations, eight types of pollen sterility in the vine have been identified related to the reasons for their occurrence: genetic; chromosomal; meiotic; genomic; chromosomal-genetic; chromosomal-genomic; polyploid and total – as a consequence of sterilisation of both gametophytes. This classification can be used for practical purposes – correct territorial location of cultivars when creating new vines, sexual hybridization for selection of pollinator varieties and parent pairs according to the selection goal, avoidance of difficulties in demasculinisation of flowers.

In the distant hybrids of F_3 – F_5 generations, all genotypic varieties of pollen sterility are observed – from partially fertile to semi-fertile and forms with normally restored fertility and vitality. As the number of generations increases, the number of self-fertile forms increases and the number of sterile hybrids decreases significantly. It is possible to discover new types of pollen sterility in future studies.

In the F_3 generation, a DRX-M_3-2 seedling was found with chromosomal sterility of the two gametophytes, in which no grains or seeds are formed, regardless of the pollination method, and one or two weeks after flowering all inflorescences dry out. By crossing distant hybrids from F_3 with 2n=38 with SV-12-309 and SV-20-366, F_4 generation was obtained, in which the hybrids DRX-M_4-501,502,503, etc., are fertile, partially fertile and infertile with 2n=38, 39.

From the crossing of the distant hybrids of F_4 – DRX-M_4-520, DRX-M_4-510 (2n=38) with the varieties GM-325-58, Cristal and Moldova, F_5 generation was created, consisting only of hybrids with somatic number of chromosomes 2n=38. Among the seedlings in this hybrid population, four artificially created species have been identified, carriers of the new vine genome with n=19, 2n=38, which includes the chromosomes of the two species – *Vitis vinifera* and V. *rotundifolia*.

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Received: March, 15, 2021; *Accepted:* June, 1, 2021; *Published:* October, 2021