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The Study of Polyploid Mutant Forms of Grapes

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Review Article

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Abstract

These days, the selection of grapes with the purpose of obtaining new initial polyploidy material is done with various inductions of mitotic genomic mutations with the help of colchicine and other mutagens. Duration and complexity of the selection process called for a new method that would increase efficiency and accelerate selection of polyploid forms of grapes. The article describes the impact of various concentration of colchicine aqueous solution during inflorescence at meiosis stage of 3 grape cultivars. The study revealed that mutational efficiency constitutes 2.2-28.0%. This is a new technique for the receipt of meiosis of genomic mutations at meiosis stage in grapes. Behaved cyto-anatomical and histological analysis of polyploid mutant forms of grapes. Revealed cytogenetic and histological mutational variation in forms. Genetic analysis of variability is given.

Keywords: Autotetraploids induced polyploidy; Meiosis; Mitosis; Gametes; Hromosom histology; Anatomy; Cytology

Introduction

In order to produce experimental polyploid forms, almost all the methods used are based on the influence of mutagenic agents on actively dividing somatic cells in the selection of grapes [1-6]. As a result, the obtained genomic mutations are mitotic autotetraploids according to genetic properties. Under the influence of natural mutagenic factors, spontaneous mitotic polyploids usually occur in grapes as a result of non-disjunction of chromosomes in anaphase. Instead of two nucleis it is formed single, in which the number of chromosomes becomes twice larger than in the original nucleus. Thus, from diploid cells (2n or 2x) it is formed tetraploid (4n or 4x). The highest practical value of polyploid cells is that they can serve as starting (parent) forms when hybridized with different forms of diploid varieties, and this will create a wealth of polyploid hybrid fund, through which it will be possible selection for triploid level in a variety of ways (parthenocarpy, large-berriedness, early ripeness, and so on).

Induced mitotic autotetraploids can be triggered by exposure to cells with mitosis poisons under experimental conditions. Mitosis poisons include colchicine, vinblastine, acenaphthene, etc. which disrupt the microtubule spindle that makes normal divergence of chromosome impossible (such mitosis is called K-mitosis). In the literature, there are several methods of experimental polyploidy in grapes [7-13]. All spontaneous and experimental polyploids in grapes arised by mitotic means as a result of doubling the number of chromosomes in somatic tissues [14-16]. In experiments at the grapes on the mitotic indexing of polyploids it was used different methods, doses, exposures and the concentration of colchicine when exposed to open buds, seedlings, seeds, seedlings growing point [17-21]. In the scientific literature, there is very little information about biomorphological, chemical and technological features of colchiploid of grapes [4,7,9]. However, there is no information about experimental meiotic autotetraploids kinds of grapes.

Theory Elements of the Work

According to the experimental data, autotetraploids from grapes, like in other plants, can be formed as follows; 1) mitotic: a) spontaneous; b) induced 2) meiotic: a) induced: For genetic studies grapes is unacceptable object. Because, although somatic cells have a small size, they have a number of joint chromosomes. Preparation of new plants from the seeds for genetic studies is difficult, holding a qualifying work requires a lot of time and hard exercise. From the genetic point of view, most importantly, plants derived from somatic diploid cells with existing methods, have a higher rate of fission in comparison with mitotic tetraploid cells, as a result of which the reparation process accelerates and after a while the mutant cells disappear.

Under the new procedure, as a result of the active introduction of colchicine in meiotic division process with water intake during the flowering phase (the period of spermatogenesis and oogenesis), as a result of loss of function of specific proteins, the pushing doubled chromosomes in the field, turns out not be haploid and it assumed the formation of gametes with the diploid number of chromosome, while it is theoretically possible to obtain seeds, in self-pollination with auto tetraploid genotypes, while in free pollination with triploid sets of chromosomes. The advantage of the new technique is that in order to create new forms with positive transgressive signs in the polyploid level, the carried out breeding work have greater genetic opportunities. The manifestation of new qualitative and quantitative agronomic performance, with positive transgressive signs with polygenic inheritance on poliplodnom level, makes actual to conduct breeding work. Unsolved problems of breeding at the diploid level, obviously, is only possible in the transition to a new level of ploidy.

Experimental Part

In world practice, the major genetic pathways of existing methods of obtaining colchiploidy forms in grapes, to create new varieties with different Biomorphological and economic-technological features is described below. We know that colchiploidy form in the grapes obtained with colchicine effects on seedlings, buds and seeds are generally chimeric structure that for obtaining new varieties of them, it is required to carry out the difficult and lengthy breeding work. It should be noted that the true tetrpaploid forms in grapes can be obtained by only from seeds with genotype 4x.or 4p. Given that the experimentally obtained colchiploids have basically chimeric structure, and for the completion of the selection process it requires a number of years, it has forced us to the need to develop a new method for producing autotetraploids from grapes on the stage of gametogenesis.

Methods Induced by Autotetraploids at the Stage of Gametogenesis

We have developed a new method for preparing a genomic mutation in the meiotic stage of gametogenesis in the grapes under the influence of colchicine and it is described briefly below. In the period of 1995-1996 and 2008 it was studied the effect of 0.01;0.05;0.1;0.3;0.5% aqueous solution of colchicine in the generative organs in flowering stage grade of Ag Aldara, Muscat Nakhchivan, and Ag Khalili kinds (Figures 1 & 2). It was used ordinary water in control variants. Fertilization in the inflorescences was carried out by itself and with free pollination. The experiments were started on the eve of the flowering phase during the formation of flower buds. Beginning of meiotic division, that is, the appearance of the gametes in the bud can be visually identified by Belen apex buds. On inflorescences at the tips of whitening buds it is prepared aqueous solutions of colchicine, the concentration of which is indicated above. Corresponding solutions are stored in bottles wrapped in a black paper. Each fertile grape sprout variety that is being tested, is cut with a sharp knife in the middle of the top of the interstices, after the bud, where is inflorescence before flowering phase. Then this part is put special medical rubber tube, corresponding the diameter of the sprout. On the upper part of the rubber tube it is joined to upright funnel a special shape, in a volume of 25 ml. On the day of whitening of buds tips in the funnel it is poured corresponding pre-cooked solutions of colchicine. During the studies, relevant solutions are added, if necessary. In order for the solution not to evaporate in the funnel, the upper edge of the funnel is covered by the filter. The experiments were carried out in five cases for each concentration of each 3 inflorescences. For a good fertilization and determination of the percentage of tying berries it was left 400-450 buds, and the remaining buds were cut off and thrown out with tweezers. Investigations were started 3-5 days before flowering and suspended after uncovering buds 100%. During the study inflorescence was taken from the middle layer of the vine. Duromg the studies, in order for the need to water to increase during the flowering, and for the absorption of mutagenic substances to increase, the irrigation of tested cultivars was deliberately delayed. During the investigations it was found that the corresponding aqueous solutions of colchicine are well soaked by buds. For each inflorescence it was spend about 25-50 ml. colchicine solution. Morphological changes in the structures of androecium and genitsey was not observed. The mechanism of the primary effect of colchicine is observed with some undesirable effects (increase in the flowers fall, darkening in the bottom of the inflorescence). In control options, such effects have not been observed. Phase of flowering took place normally.

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Figure 1: The effect of different concentrations of an aqueous colchicine solution on the inflorescence of grapes.

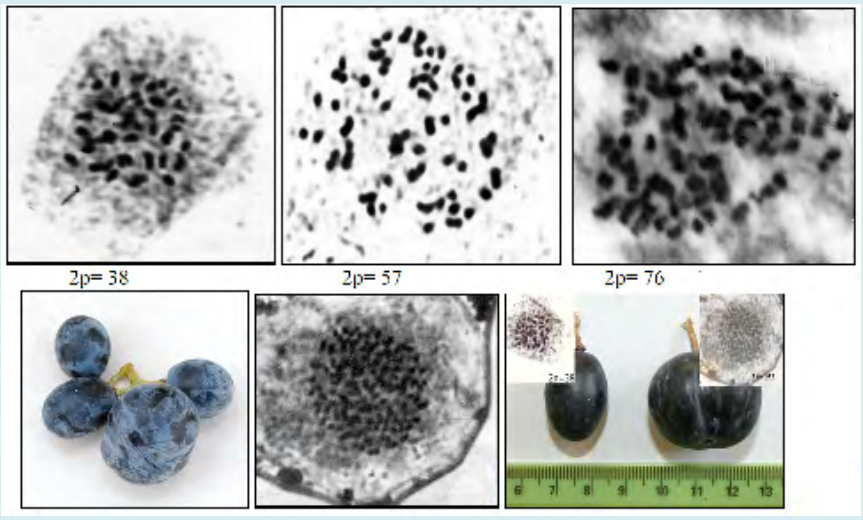

Figure 2: Chromosomes of polyploids, 2p= 38; 76; 152(mixoploid forms).

Anatomical and Histological Features of the Leaves of Autotetraploid and Mixoploid Plantlets

Literature data on the dependence of the size of epidermal and mesophyll cells in grapevine leaves on ploidy are limited. The performed analysis indicates the existence of differences between diploid and tetraploid cultivars in anatomical and histological features of leaves. Autotetraploids possess significantly thicker leaf blades (195-210μm compared to 174.09μm in dip loid cultivars). The length of cells of the upper epidermis (11.9-13.6μm) in all six studied tetraploids was significantly less, while the width (20.9-22.5 μm) was significantly more than in the initial diploid cultivar (14.6-19.6μm). No correlation was established between the size of the lower epidermal cells and the ploidy level. It is suggested that the stomata and epi_ dermal cells of tetraploids are characterized with larger sizes than those of diploids [4]. Measurements of stomata sizes on the lower leaf epidermis in 32 altered plantlets indicated that in 6 tetraploids and 13 mixoploids, the stomatal width was significantly larger than in the initial diploid cultivar: 31.5-34.5 μm in tetraploids, 29.5-30.5 μm in mixoploids, and 20.3-25.0 μm in the initial cultivar. The number of stomata per unit of area of the lower leaf surface on average decreased by 39.5-49.5% when the ploidy level increased in somatic cells (in the case of tetraploidy).

Quantitative and Structural Changes in the Genome

The estimation of the DNA and RNA content, together with calculations of the RNA/DNA ratio for mitochondria, enabled to uncover the different nature of changes in RNA and DNA in seedlings influenced by different doses and expositions of colchicine. It was found that the mtDNA ontent in tetraploids was approximately 1.5 times higher than in diploid culti_ vars, while mixoploids demonstrated

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no differences from the control. The activation of the mitochondrial genetic apparatus in leaves of diploid, mixoploid and tetraploid forms was confirmed by the higher content of mtRNA and the abrupt increase in the RNA/DNA ratio, which can indicate the heightened transcrip_ tional activity of mitochondria. The results of studies indicate that certain changes occur in the genetic apparatus of cytoplasmatic organelles of grapevine leaves, depending on the dose and duration of a mutagenic treatment. The unequal aftereffect of colchicine on the genetic apparatus of the mitochon_ dria and chloroplasts was obvious. Autotetraploid N.80–8/37 with a higher content of cellular DNA and RNA is characterized by earlier fruit ripening (20–25 days), their increased sugariness, and higher yield and larger berries. Another autotetraploid form (N.80–9/6) exceeds the control by almost 2.5 times in the content of the labile DNA fraction and is remark able for its wide leaf blades, robust bushes, and the large elements of flowers and fruits [22,23].

Analysis and discussion of the obtained results: The studies on the options identified totals buds, percentage of setting fruit, the amount of the normal berries and the amount of normal berries seeds at the end of the growing season. During experiments it was determined that by each class it was sucked about 25-30 ml of colchicine solution. It was found out that with increasing concentration of colchicine, the percentage tying berries reduced. Under the influence of a 0,5 % aqueous solution of colchicine, the tying berries reduced to 14,8%. In control options, the reduce of tying berries made up 28.8%. After the final physiological ripening of berry, all clusters were collected on all versions; the seeds were extracted, and collected after drying. The number and weight of seeds were identified according to varieties and options. According to options, a part of the seed samples were grown and it was performed cyto-anatomical analyzes. In the early spring of 1995 and 1996, the seeds were planted in boxes with a nutrient medium 6:3:1 (soil+manure+sand). In the open section, the seedlings received a normal cultural care. From the third year on morphological diagnostic features, the seedlings considered as tetraploid forms, were distributed on the options and it was prepared their sowing materials. The effect of polyploidy on the diagnostic morphological characters as well as cytology and anatomy (the size of the stomata-an indicator of ploidy of the first layer, the size of pollen-an indicator of ploidy of the second layer) analysis was on the options and varieties of Ar Aldara 2,0-16,0%; Nakhchivan Muscat 1,2-28,0%; Ar Khalili 3,5-22,5%/. It is found that in the process of gametogenesis, for polyploidy cells, optimal concentration was of 0,1-0,3%-aqueous solutions of colchicine. After fruiting the obtained polyploid forms of grape were studied ampelographically and it was used in hererogenomic cross breeding in the collection area “of the gene pool of grapes”. As a result, it was obtained about 20 allopoliploid hybrid forms. These polyploids are used to produce new forms by hybridization (Figures 3-5).

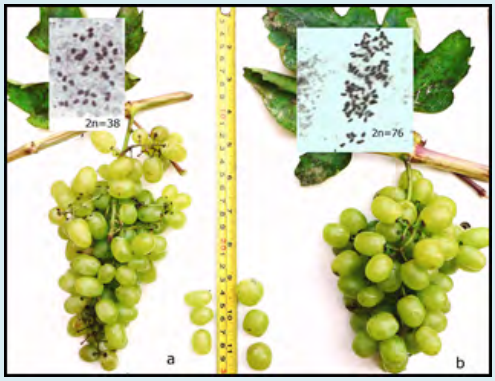

Figure 3: a) Original variety
Ag Khalili (2p= 38)
b) Tetraploid form
Ag Khalili large-berried (2p=76).

Figure 4: Autotetraploid.
H. 81-18/15 (2p=76).

Figure 5: Tripploid.
H. 89-19/10 (2p=57).

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Discussion and Results

Induced meiotic autotetraploids from grapes is caused by non-disjunction of chromosomes during meiosis. Theoretically, this happens as follows:

Nondisjunction of all Chromosomes

Segregation is absent (for example, the complete destruction of the spindle). Then from the original meristem diploid cells it is formed a tetraploid cell, in which there is the entire source chromatin; -The first division of meiosis occurs as mitosis: as a result of the original diploid cell it is formed dyad - two diploid cells with a single chromatid chromosome. The resulting cells are identical to each other and with respect to the mother cell. In this case, each of the diploid cells contains two different chromosomes set (two different genome, for example, x1, x2). These cells lose their ability to divide and give rise to disputes and gametes; -The first division takes place normally (to form haploid cells with dichromatid chromosomes), but the second division is blocked at metaphase II. As a result, it occurs diploidization of haploid cells: each dichromatid chromosome splits into two chromatids, these chromatids do not disperse, and the diploid number of chromosomes is restored. The ending result of this meiosis is also the formation of dyad - two diploid cells, which give rise to sprouts and gametes. At the same time in each of two diploid cells there are found two of the same chromosomal set. Such violations are much rarer than the previous-Revealed cytogenetic and histological mutational variation in forms.

Nondisjunction on Separate Chromosomes

These disorders are similar to the previous one, but segregation violation affects only individual chromosomes. As a result, in some daughter cells there appear redundant chromosomes and the chromosomes are lost in these other cells. According to the cytological data, the polyploid cells in grapes may later give rise to sporogenous cells, and then it is formed polyploid gametes, such as: 4x (sporogenous diploid cells)→meiosis →2x (haploid spores with a double set of chromosomes) giving rise to haploid eggs or sperm[24,25].

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