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Genetic Proof of Chromatin Diminution under Mitotic Agamospermy

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Abstract- **The previously published data are examined on the base of the hypothesis about the existence of chromosomes differential polyteny and excessive chromatin diminution during the first stages of sugar beet plant embryogenesis. It has been concluded that available data provide the genetic proof of that chromatin diminution is one of the mechanisms underlying the origin of polymorphism in sugar beet agamospermous progenies.**

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I. Introduction

A bulk of evidence has been obtained for polymorphism in agamospermous diploid sugar beet plant (*Beta vulgaris* L.) progenies. One of the explanations of such polymorphism is based on the recognition of the important role of mixoploidy in plants. Mixoploidy is manifested by an admixture of tetraploid cells among the bulk of diploid archespore mother plant cells (Maletskii, Maletskaya, 1996; Maletskaya E.I., Maletskaya, S.S., 1999). The entering of a tetraploid cell into meiosis leads to the diploid embryo sac formation and, accordingly, to the formation of a diploid egg cell, capable of entering into embryogenesis without fertilization. This mechanism is characteristic for meiotic diplospory which can be also designated as meiotic agamospermy (Levites, 2002). In this case polymorphism is a natural consequence of meiosis and can be designated by the known term "autosegregation" (Gustafsson, 1946-1947; Maletskii et al., 1998). Genetic and cytological data support this hypothesis (Szkutnik, 2010).

At the same time, an additional mechanism has been proposed to explain polymorphism in agamospermous sugar beet plant progenies (Levites, 2005, 2007). It suggests that polymorphism occurs mostly due to the polytenization of chromosomes regions carrying marker loci. Differential polyteny could subsequently lead to a random equiprobable loss of excess chromatin by a cell before it enters embryogenesis.

Theoretical calculations indicate that the differential chromosome polytenization and subsequent diminution of excess chromatin are possible both under meiotic agamospermy and mitotic agamospermy (adventive embryony) when an offspring arises from the somatic cells which have not undergone meiotic genome transformations. There is also genetic evidence that polytenization can occur in egg cells chromosome regions under sexual plant reproduction (Levites and Kirikovich, 2013a). A genetic proof of this hypothesis has been obtained along with the proof that the polytenization process depends on external conditions (Levites and Kirikovich, 2013b).

The studies of agamospermous progenies, as well as the consideration of chromosome polytenization, provide a new insight into many genetic processes and the causes of numerous variations in the genotype and phenotype ratios of the resulting offspring. A characteristic feature of polymorphism under agamospermy is the mismatch between the identified ratios and the normal Mendelian ratios.

Accounting for the effect of polytenization of chromosome regions carrying marker genes on the respective marker traits segregation expands the boundaries of genetics. At present, trait segregation can be attributed both to changes in the cell chromosomes number (meiosis and gamete fusion) and to other process not attributable to such changes (chromosome endoreduplicated sites diminution).

The facts collected since the early studies have contributed to a gradual shift in our view at the mechanisms underlying agamospermy. At this stage it is necessary to review our earlier data, which is the aim of this article.

Under discussion will be the data presented in the article entitled "Pseudosegregation in the agamospermic progeny of male sterile plants of the sugar beet (*Beta vulgaris* L.)" (1999), (Authors: Levites E.V., Shkutnik T., Ovechkina O.N. and Maletskii S.I.). Isozymes were used as genetic marker traits in this work.

A wonderful peculiarity of isozymes is their codominant inheritance due to which the hybrid plant isozyme spectrum is different from each parent isozyme spectrum (Schwartz, 1966; Scandalios, 1969). For instance, one isozyme with fast (phenotype FF) or slow (phenotype SS) electrophoretic mobility, which corresponds to the genotype of a given locus, is revealed in the electrophoregram for the homozygote *FF* or *SS* on the gene controlling this marker enzyme. But both enzyme allelic variants (isozymes) and also hybrid isozymes are revealed in the heterozygote (phenotype

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FS). This allows one to reveal all 3 phenotypic classes in the progeny of plant heterozygous at the isozyme locus: two homozygous (FF and SS) and one heterozygous (FS) (Schwartz, 1966; Scandalios, 1969).

In the considered paper the genetic methods were used to show that the analyzed sugar beet agamospermous progenies were formed from somatic cells (Levites et al., 1999). This conclusion was based on the monomorphism of the KWS1-5A offspring by heterozygous isozyme spectrum of marker enzyme alcohol dehydrogenase (ADH1). Interesting, the study also revealed the dimorphism of the analyzed progenies, including KWS1-5A, for other marker enzymes. In the progeny the enzymes dimorphism was expressed by the presence of only two phenotypic classes: one homozygous and one heterozygous. Of all the data from the cited article, let us consider two offsprings: KWS1-5A and KHBC2-78A (Table 1).

In the cited article it was assumed that the dimorphism was due to the inactivation in a part of the offspring of one of the alleles at a heterozygous locus. As a result, the seeds with the phenotypes similar to the homozygous one carry one active allele which determines the electrophoretic mobility of the enzyme and one inactivated allele. However, later it was found that phenotypes similar to those of the homozygous are conditioned by homozygous genotypes indeed (Levites, Kirikovich, 2003).

The findings allowed us to hypothesize that the dimorphism of agamospermous progeny is due to the heterozygosity at the marker enzyme locus with one allele represented by a single copy and the other allele represented by three copies arising as a result of polytenization (Levites, 2005, 2007). Polyteny of chromosomes in plants – a well known fact (Carvalheira, 2000). The somatic cells with genotype *FFFS* lose excessive allelic copies equiprobably before entering embryogenesis. The calculations assisted with hypergeometric distribution formulas (Feller, 1950) indicate that, in this case, only two genotypes, *FF* and *FS,* in the ratio of 1:1 are theoretically possible.

The calculation is as follows:

For homozygotes *FF* - $C^2_3 \times C^0_1 / C^2_4$ - the number of combinations of the choice two out of three, multiplied by the number of combinations 0 out of 1 and divided by the number of combinations 2 out of 4, i.e., 3x1/6.

For heterozygotes *FS* - $C^1_3 \times C^1_1 / C^2_4$ - the number of combinations of the choice one out of three multiplied by the number of combinations one out of one, and divided by the number of combinations 2 out of 4, i.e., 3x1/6.

In the reduced form, this ratio expressed in integers is 1:1.

The *SS* genotype is not formed because it requires two allelic copies while only one copy is present in the genome.

The equiprobable diminution process requires a free exchange of chromatides between chromosomes. The existence of such exchange was demonstrated later on the base of the phenotype ratio observed in the agamospermous colchicine-treated plant progeny (Levites, Kirikovich, 2012).

Therefore, it is interesting to consider the phenotype ratios of the marker enzymes isocitrate dehydrogenase (IDH3) and malate dehydrogenase (MDH1) in agamospermous progeny KHBC2-78A. This offspring is of particular interest because it combines two distinctive traits that are inherent to the agamospermous progeny: the phenotype class ratio for IDH3 corresponding to 3FF:8FS:3SS and dimorphism for MDH1. The MDH1 phenotype class ratio 1FF:1FS indicates that this progeny has originated from somatic cells with different alleles doses at locus *Mdh1.* The somatic origin of these cells implies that no meiotic genome transformations have occurred in such cells nuclei.

Mathematically, ratio 3:8:3 is known to be possible derived if 2 elements are selected randomly out of a sample containing 4 elements of one type and 4 elements of the other type (Feller, 1950).

This occurs, for example, when a heterozygous tetraploid cell of genotype *FFSS* enters into meiosis. Since, at this moment, each chromosome is represented by two chromatids, 8 allelic copies are presented in the nucleus by 4 copies of each of the two alleles. If the frequency of crossing-over between the marker locus and the centromere is 50%, all allelic copies behave independently and the random selection of two copies obeys the probability laws. The frequencies of the resulting gametes can be calculated by the hypergeometric distribution formulas (Feller, 1950). For the above example the gamete frequencies in units fractions can be determined as follows:

For homozygotes *FF* - $C^2_4 \times C^0_4 / C^2_8$, the number of combinations of the choice 2 out of 4 multiplied by the number of combinations 0 out of 4 and divided by the number of combinations 2 out of 8, i.e., 6x1/28.

For heterozygotes *FS* - $C^1_4 \times C^1_4 / C^2_8$, the number of combinations of the choice 1 out of 4 multiplied by the number of combinations 1 out of 4 and divided by the number of combinations 2 out of 8, i.e., 4x4/28.

For homozygotes *SS* - $C^0_4 \times C^2_4 / C^2_8$, the number of combinations of the choice 0 out of 4 multiplied by the number of combinations 2 out of 4 and divided by the number of combinations 2 out of 8, i.e., 6x1/28.

In the reduced form, this ratio expressed in integers is 3:8:3.

From the above-mentioned it can be concluded that the ratio 3:8:3 for *Idh3* observed in the KHBC2-78A

offspring implies that: 1) the offspring emerged from the cells with an increased copies number of each allele at locus *Idh3;* 2) the number of allelic copies decreases in the moment before cells entering into embryogenesis.
On the other hand, the presence in the same seeds of this progeny of two phenotypic classes for MDH1 indicates that this progeny originates from the cells which have not undergone meiotic genome transformations. Therefore, the reduction in the number of allelic copies at the *Idh3* locus is not a consequence of meiosis, but it is the result of chromatin diminution only.

Moreover, the phenotype class ratios in both progenies described here can be explained precisely by chromatin diminution.

The presence in one offspring of two complementary traits (somatic origin of the cells entering into embryogenesis and an increased dose of alleles in the cells capable of embryogenesis) confirms both the agamospermous origin of the offspring and the process of chromatin diminution from the cells at the moment before their entering into embryogenesis.

In conclusion, it should also be added that, according to the proposed hypothesis, the equiprobable diminution process of the number of redundant allelic copies is a consequence of equiprobable allelic copies attachment to the nuclear membrane (Levites, 2005, 2007). It is assumed that only one copy from each chromosome out of two homologous ones in a diploid plant attaches to the cell nuclear membrane before its entering embryogenesis. The attached allelic pair determines the genotype of a developing embryo while the unattached allelic copies are lost.

Thus, a new analysis of the previously published data gives a new insight on the complementary genetic facts, which confirm the model describing a specific mechanism of the origin of polymorphism in agamospermous progenies. The available data provide the genetic proof of that chromatin diminution is one of the mechanisms underlying the origin of polymorphism in sugar beet agamospermous progenies.

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Table1: Phenotypic classes of marker enzymes in agamospermous progenies obtained from pollen sterile sugar beet plants (Levites et al., 1999)

Progeny	Marker enzyme phenotypes of progenies		
	ADH1	IDH3	MDH1
	FF : FS : SS	FF : FS : SS	FF : FS : SS
KWS1-5A	0:78:0	23:41:0*	9:0:0
KHBC2-78A	-	9:47:10**	45:57:0*

The probability of affinity with theoretically expected ratio 3:8:3 - *- $P<0.001$; ** - $P>0.05$