Autosegregation of enzyme loci *Me1* and *Gpi2* in agamospermous progenies of triploid sugarbeet plants

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Ratios of malic-enzyme (ME1) and glucosephosphate isomerase (GPI2) phenotypic classes were studied in agamospermous progenies of triploid sugar beet plants. It was shown that the ratio of enzymes phenotypic classes quite well accords with the calculations based on the supposition about reduplication of chromosome sites carrying alleles of enzyme loci accompanied by a loss of excessive allelic copies in the first cell division under embryogenesis. Polyteny – conditioned allelic dose increase leads to the occurrence of alleles – absent in the initial parent – at a certain frequency in the developing progeny. The notions of "meiotic autosegregation" and "mitotic autosegregation" – typical of meiotic and mitotic agamospermy, respectively, – were introduced; the term locus "polygenotype" characterising the allelic composition of locus, number of chromosomes and a copies number of chromatides sites carrying marker-locus alleles in the cell before its entering embryogenesis was also introduced.

Keywords: agamospermy, autosegregation, malic-enzyme, glucosephosphate isomerase, triploid plants, sugar beet.

Introduction

Agamospermy is a widely spread reproduction way in flower plants which basic feature consists in the thing that seed embryo development proceeds assisted by only one parental genome (Gustafsson, 1946). By the present, quite a big array of data that prove genetic and epigenetic polymorphism in agamospermous plant progenies has been obtained (Richards, 1986; Levites *et al.*, 1998; Maletskii *et al.*, 1998; Chapman *et al.*, 2000; Levites *et al.*, 2001a, b; Verhoeven *et al.*, 2010). Genetic polymorphism was investigated in such progenies using marker enzymes in the whole number of contributions (Richards, 1986; Yahara *et al.*, 1991; Szkutnik *et al.*, 2001; Љtorchovб *et al.*, 2002; Нцrandl, 2004; Kashin *et al.*, 2005).

However, despite a number of publications, the obtained results are often contradictious, and they do not allow us to make ubiquitos conclusions about the variability character in agamospermous plant progenies.

One of the reasons for results uncertainty and contradiction consists, obviously, in the thing that, in these papers, agamospermous plant populations were analyzed, whereas the character and mechanism of genetic variability may be certainly dwelled upon only analyzing individual progenies. Initially, agamospermous plant polymorphism was considered as a consequence of some aberrations and denoted with the term "autosegregation" (Gustafsson, 1946). Further on, autosegregation was implied as meiosis-conditioned segregation (Maletskii and Maletskaya, 1996). Their results prove the existence of such a reproduction type as meiotic diplosporea (meiotic agamospermy) in sugar beet.

However, mitotic agamospermy, (Levites, 2002) under which a seed germ develops out of the cell that did not pass through meiosis, also exists in sugar beet. Theoretically, such progenies are to be homogeneous. However, polymorphism was also revealed in them (Levites *et al*., 1998; Levites *et al*., 2000; 2001a). The nature of this polymorphism expressed as non-standard phenotypic ratios and the occurrence of phenotypic classes, unusual for the progeny, was not clear, and this phenomenon was indicated as "pseudosegregation" (Levites *et al*., 1998) and "redetermination" (Levites *et al*., 2001b). Polymorphism and the occurrence of the third allele in the diploid plant agamospermous progeny were considered as a consequence of epigenetic variability.

At present, a model of the mechanism that determines polymorphism in agamospermous progenies is proposed (Levites, 2005, 2007). The base of this model consists in the supposition about polyteny of chromosomes in embryosac cells, nucellus and integuments, and about excessive chromatide copies elimination from the cell that begins its embryonic development. According to the proposed model, a loss of excessive chromatide copies is random and equiprobable. As a result, only two chromatide copies remain in the cell that begins its embryogenesis out of a multitude of chromatide copies. Due to the thing that there are many independent endoreduplication onset points in each eukaryots chromosome and various sites of one and the same chromosome may have a different endoreduplication degree, it is hypothesized that a loss of excessive DNA quantity may be realised not by whole chromatides, but by separate sites independently (Levites, 2005, 2007). The ratio of phenotypic classes in a developed agamospermous progeny is determined by a chromatide threads ratio in regions of homological chromosomes carrying heterozygous marker-locus alleles. A shortage of some homozygous phenotypic class in such an agamospermous progeny may be the consequence of the thing that the corresponding enzyme locus allele was present in a smaller chromatide number in each of cells getting ready for embryogenesis.

High chromosome endoreduplication level was frequently encountered in tissues of plant reproductive organs, also including embryosac cells (Nagle, 1976; Stozharova, Poddubnaya-Arnoldey, 1977; D'Amato, 1984; Morozova, 2002; Unal, Vardar, 2006). However, the highest polytene chromosome occurrence frequency was observed in polyploid plants (Carvalheira, 2000). Currently accumulated data are indicative of the thing that agamospermy is most frequently expressed in polyploids; apropos, sexual reproduction prevails in even ploidy, agamospermous – in odd (Gadella, 1987; Pogan, Wcislo, 1995). In this connection, it was interesting to add the data on agamospermy in triploid sterile sugar beet plant progenies to the earlier obtained data on diploid plants.

Materials and Methods

Seeds of three sugar beet plants (№ 47, № 55 and № 66), provided by S.I. Maletskii and E.I. Maletskaya, were agamospermous progenies obtained in pollenless regime from pollen-sterile triploid plants of Irys Dutch breeding commercial variety. Malic enzyme (ME1) and glocosephosphate isomerase (GPI2) isozyme spectra controlled by loci *Me1* and *Gpi2*, respectively, (Levites, 1986; Smed *et al*., 1989) were chosen as marker-traits. Electrophoretic analysis was carried out with individual seeds in starch gel according to the earlier-described methods (Cardy *et al.*, 1980; Vallejos, 1983). Electrophoregram scanning was realized using Biodoc device. Theoretical phenotype frequency calculations were made according to the method suggested by Haldane (Haldane, 1930). The correspondence of found ratios to theoretically expected frequencies was evaluated using χ^2 criterion (Sokal and Rohlf, 1995).

Results

Malic enzyme (ME1; E.C.I.I.I.40). It is known that one isozyme with the corresponding electrophoretic mobility – "fast" (FF-phenotype), "slow" (SS-phenotype) or the "fastest" (CC-phenotype) – is revealed in homozygous plant seeds *Me1-F/Me1-F* (short – *FF*), *Me1-S*/*Me1-S* (short – *SS*) or *Me1-C/Me1-C* (short – *CC*).

As malic enzyme is a tetramere in its quartinary structure, five isozymes (CCCC, CCCS, CCSS, CSSS, SSSS) are revealed in heterozygotes, e. g. *CS*. If electrophoretic differences among enzyme allelic variants are less considerable, e. g. heterozygote *CF*, all five isozymes merge to one enzyme activity zone. Malic enzyme polymorphism (Table 1, Fig. 1) was found in investigated plant progenies.

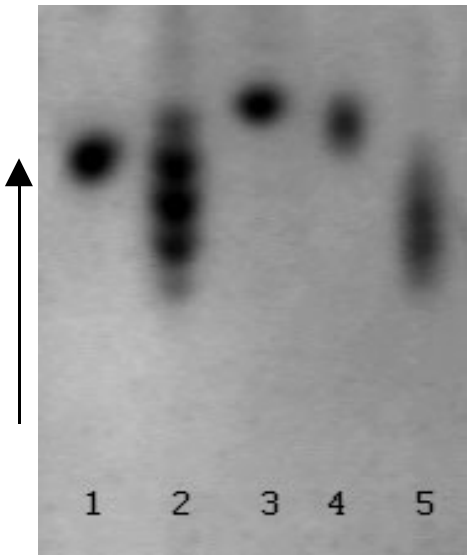

Figure 1. The types of isozyme spectra of malic enzyme (ME1) in sugarbeet seeds. 1 – homozygote *FF*; 2 – heterozygote – *CS*; 3 – homozygote *CC*; 4 – heterozygote *CF*; 5 – heterozygote *FS*. Migration is toward the anode.

Based on the ratio of phenotypic classes in the progeny of plant № 47 (12CC : 48CF : 80FF : 11FS : 24CS), it is possible to calculate and find that allele *Me1-F* frequency is 219/350 (0.626), allele *Me1-C* – 96/350 (0.274), allele *Me1-S* – 35/350 (0.1) in this progeny. It allows us to conclude that initial triploid mother plant № 47 had its genotype on locus *Me1*: *FFC*. Analogous conclusions can be made for plants № 55 and № 66.

Table 1. Phenotypic classes of malic-enzyme in agamospermous seed progenies of triploid sugar beet plants

Progeny	Phenotypic ME1 classes					Seeds, total	Polygenotype *Me1*	Expected ratios of phenotypes	χ^2
	CC	CF	FF	FS	CS				
№ 47 Observed	12	48	80	11	24	175	$F_4F_8C_4$		
№ 47 Corrected	**12**	**72**	**91**	-	-			1 : 8 : 11	1.5506
№ 55 Observed	8	30	35	10	17	100	$F_4F_8C_4$		
№ 55 Corrected	**8**	**47**	**45**	-	-			1 : 8 : 11	4.8431
№ 66 Observed	1	10	18	3	4	36	$F_4F_8C_4$		
№ 66 Corrected	**1**	**14**	**21**	-	-			1 : 8 : 11	0.4393

There arises a necessity to explain the appearance of allele *Me1-S* products in these progenies. The possibility of these plants pollination by the pollen of some plant carrying allele *Me1-S* is excluded not only by pollenless pollination conditions, but on the ground that, under a possible pollination, the ratio of heterozygous phenotypes carrying allele *Me1-S* would have corresponded to the frequencies of alleles *Me1-F* and *Me1-C* in mother plants, i. e. phenotype FS frequency should be higher than that of phenotype CS. But in reality, the observed picture is quite the reverse (Table 1). In this case the occurrence of seeds carrying allele *Me1-S* may be considered as a result of locus *Me1* alleles change of epigenetic expression. It well agrees to the earlier obtained data on epigenetic variability in agamospermous sugar beet progenies (Levites *et al*., 2001b). Resolving the point about which of mother plant allele changes, one should keep to the principle of changed phenotypes minimal share related to the unchanged ones. Allele *Me1-F* variability is adequate to this principle, as the total share of seeds with a changed phenotype (FS + CS) is 69/221 (0.31) in all progenies related to seeds with an unchanged phenotype (FF + CF). Suppose allele *Me1-C* changes, then this share is twice higher and is equal to 69/109 (0.63). Accepting allele *Me1-F* variability, one should conclude on the thing that it is more stable in the homozygous compound than in the heterozygous one, as *FF* → *FS* transfer results only in 24 seeds of phenotype FS, *CF* → *CS* – 45 seeds of phenotype CS.

Let us consider the formation regularities of revealed phenotypic classes ratios with the example of plant № 47 progeny. In this progeny, allele *Me1-F* copies initial frequency is determined by summing the frequencies of alleles *Me1-F* and *Me1-S*. It is 0.726, and that exceeds the frequency of allele *Me1-C* 2.65 times. Allelic ratio 2 : 1 is possible for a heterozygous triploid. The allelic ratio different from that of double in the investigated progeny allows us to hypothesize, in the initial mother plant, alleles of this locus were presented by a different chromatide copies number. To reveal the mother triploid plant chromatides ratio, let us integrate the phenotypic classes into groups that include real initial phenotypes, and the phenotypes that occurred as a result of allele *Me1-F* change of expression. The integrated (corrected) phenotypic class CF, that includes the revealed phenotype CF and phenotype CS that appeared as a result of allele *F* → *S* transfer, consists of 72 seeds; integrated (corrected) class FF, that includes the revealed phenotype FF and phenotype FS, that appeared as a result of allele *F* → *S* transfer, is presented with 91 seeds (Table 1). The final corrected ratio 12CC : 72CF : 91FF obtained for the progeny of plant № 47 well corresponds to the theoretically expected ratio 1 : 8 : 11 under locus *Me1* chromatide sites autosegregation, also provided that allele *Me1-F* is presented by 4 chromatides in one chromosome and 8 chromatides in the other, and allele *Me1-C* is presented in one chromosome by 4 chromatides (χ^2=1.551; P > 0.05).

To indicate the state of endoreduplicated locus, let us introduce the notion of "locus polygenotype" characterizing its allelic composition, number of chromosomes and chromatides that carry these alleles. The polygenotype may be presented as $F_4F_8C_4$ for locus *Me1* of plant № 47. Analogous analysis of phenotypic classes ratios of ME1 in progenies № 55 and № 66 shows the thing that, in these plants, locus *Me1* polygenotype has also formula: $F_4F_8C_4$ (Table 1).

Glucosephosphate isomerase (GPI, E.C. 5.3.1.9.). In its structure, GPI is a dimere enzyme. In sugar beet, GPI is found with the method of electrophoresis as two zones of enzyme activities – fast- (GPI1) and slow-migrating (GPI2). Genetic control was analysed for zone GPI2; it was established that it is controlled by locus *Gpi2* having two alleles (Smed *et al*., 1989). In homozygotes *Gpi2-F*/*Gpi2-F* (short – *FF*) and *Gpi2-S*/*Gpi2-S* (short – *SS*), this zone is presented either by fast-migrating (FF phenotype) or slow-migrating isozyme (SS phenotype). In heterozygotes *Gpi2-F*/*Gpi2-S* (short – *FS),* alongside with homodimeres FF and SS, heterodimere isozyme FS is revealed in this zone.

In the progenies derived from triploid plants, polymorphism on GPI2 – presented by homozygous (FF and SS) and heterozygous phenotypes (FS, FFS, FSS, FSS^M, SS^M и SS^MS^M) (Fig. 2) – was revealed.

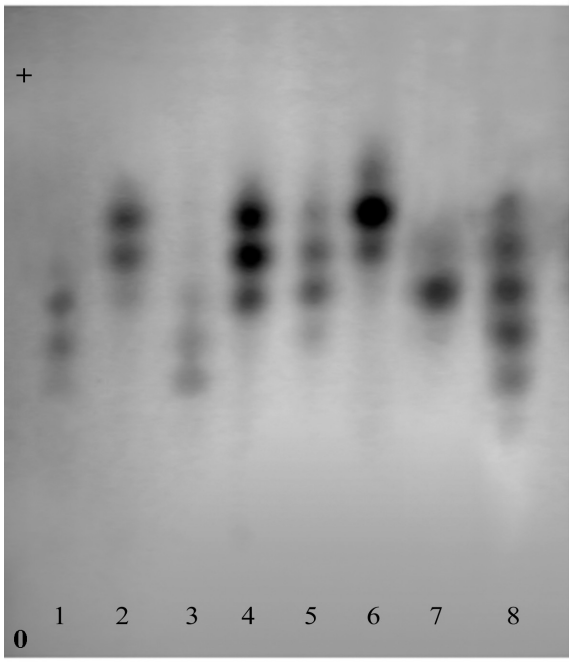

Figure 2. The types of isozyme spectra of glucosephosphate isomerase (GPI2) in sugarbeet seeds. 1 – heterozygote SSS^M; 2 – heterozygote *–FFS*; 3 – heterozygote SS^MS^M; 4 – diploid heterozygote *FS* with standard phenotype; 5 – heterozygote *FSS*; 6 – homozygote *FF*; 7 – homozygote *SS*; 8 – heterozygote FSS^M. Migration is toward the anode.

Table 2. Phenotypic classes of glucosephosphate isomerase-2 in agamospermous seed progenies of triploid sugar beet plants

Progeny	Phenotypic GPI2 classes								Seeds, total	Poly-genotype *Gpi2*	Expected ratios of phenotypes	χ^2
	FF	FS	SS	FFS	FSS	FSS^M	SS^M	SS^MS^M				
№ 47 Observed	51	68	24	2	4	6	8	4	167	$F_4F_4S_8$		
№ 47 Corrected	51	80	36	-	-	-	-	-	-		7 : 16 : 7	4.8648
№ 55 Observed	28	52	10	-	-	2	3	-	95	$F_4F_4S_8$		
№ 55 Corrected	28	54	13	-	-	-	-	-	-		7 : 16 : 7	5.5451
№ 66 Observed	12	18	4	-	1	1	2	-	38	$F_4F_4S_8$		
№ 66 Corrected	12	20	6	-	-	-	-	-	-		7 : 16 : 7	2.0375

Standard heterozygous phenotype (FS) is presented by a symmetrical three-banded pattern in which heterodimere is the most intensive isozyme. Such isozyme pattern is the basic one among diploid heterozygous GPI2 spectra, its frequency is 90 % among heterozygotes. This fact is suggestive of the thing that the basic part of agamospermous progeny obtained from triploid sugar beet plants is presented by diploid plants. We classified the spectra having a shifted intensity (asymmetrical) (FFS, FSS) as trisomic or trisomal. The notion of "trisomal" characterises the genome locus dosage, – unlike trisomy that does so for chromosome dosage. Also noteworthy is the thing that, in these progenies, alongside with phenotypes usual for GPI2, isozyme patterns indicative of allele *Gpi2-S* changed expression are revealed. We designated the allele with a changed expression as *Gpi2-*S^M. Among the patterns FSS^M, SS^M и SS^MS^M, conditioned by the presence of allele *Gpi2-*S^M, the trisomal phenotypic share is 50 % (13/26) in all three progenies, whereas the share of trisomal phenotypes is less than 5 % (7/145) among normal heterozygous patterns. It is indicative of the thing that an allelic dosage change of enzyme loci is decisive in the occurrence of abnormal patterns.

Regularities in the occurrence of phenotypic classes ratio may be analyzed with the example of plant № 47 progeny. Based on the phenotypic classes ratio, we find that allele *Gpi2-F* frequency is 184/350 (0.526) in the investigated progeny (Table 2). The frequency of allele *Gpi2-S* is the result of a sum of allele *Gpi2-S* and its modified form *Gpi2-*S^M frequencies; it is equal to 166/350 (0.474). Despite the thing that one of this triploid plant alleles is to be present in two chromosomes and the other allele – only in one, both these alleles were presented in the initial mother plant by an approximately equal number of copies, as the differences in

frequencies of alleles *Gpi2-F* and *Gpi2-S* are not significant in the progeny. However, an exceeding tendency of allele *Gpi2-F* frequency over that of *Gpi2-S* is observed in this agamospermous progeny. Therefore, it is possible to hypothesize that the mother plant had its genotype *Gpi2-F/Gpi2-F/Gpi2-S* (short – *FFS*). If we integrate the phenotypic classes having both these alleles, including the modified (FS, FFS, FSS, FSS^M) into group FS, and phenotypic classes SS, SS^M и SS^MS^M – into group SS, then the following corrected phenotypic ratio will be obtained: 51FF : 80FS : 36SS (Table 2). The theoretically possible phenotypic ratio at 8 copies of each of alleles is: 7FF :16FS : 7SS. The observed ratio well corresponds to this one: (χ^2=4.8648; P > 0.05). The polygenotype on locus *Gpi2* may be indicated as $F_4F_4S_8$. The ratios of GPI2 phenotypic classes in the progenies of plants № 55 and № 66 also correspond to polygenotype $F_4F_4S_8$.

Discussion

The polymorphism revealed in investigated sugar beet progenies well agrees to the known scientific-literary data on variability of agamospermously produced plants. The following traits are typical of the found polymorphism. The major part (90 %) of revealed heterozygous enzymes phenotypes have traits typical of diploid plants. It is suggestive of the thing that a triploid plant is capable of producing a diploid agamospermous progeny. The ratio of enzymes phenotypic classes quite well corresponds to calculations based on the supposition about allelic copiness conditioned by polyteny of chromosome sites carrying alleles of enzyme loci. It proves the proposed hypothesis about polyteny and diminution of excessive chromatide copies (Levites, 2005, 2007) as factors of variability in agamospermous progenies determining both the presence of polymorphism proper and the ratio of phenotypic classes in a progeny. The predominant share of diploid phenotypes points out the thing that diminution of excessive chromatide sites can affect the regions that have centromeres leading, this way, to a lower ploidy level.

Polyteny-conditioned increase of allelic dosage in the cells, before their embryogenesis, leads to the appearance of alleles in the developing progeny that were absent in the initial parental plant. It is seen both in phenotypes GPI2 (transfer of allele *Gpi2-S* to $Gpi2\text{-}S^M$) and ME1 (transfer of allele *Me1-F* to *Me1-S*). However, the sequential relation is vividly revealed only on GPI2, where, among usual diploid heterozygotes, the frequency of trisomal phenotypes is 10 times lower than that among heterozygotes with an abnormal allele. It is dosage effects that may be the reason for the earlier-revealed allelic redetermination in agamospermous sugar beet progenies (Levites *et al*., 2001).

As is seen from the presented tables, polyteny degree is different in chromosome sites independently of the thing if they carry different alleles or those with the same phenotypic manifestation. Different endoreduplication degree of alleles having the same phenotypic manifestation, but located in different chromosomes of triploid exists, e.g., in cells having polygenotype $F_4F_8C_4$ on locus *Me1*. It well accords with such a known fact as the existence of 14 *Adh1-F* allelic variants of alcoholdehydrogenase locus and 6 variants of *Adh1-S* alleles in maize (Woodman and Freeling, 1981). It is not excluded that predisposition to a certain polyteny degree depends on alleles structural peculiarities.

An important point in this is the thing that the revealed ratios are well explained by the polyteny level expressed in the power of number two, – namely 2, 4, 8 and 16. It reflects the process of successive duplication of chromatide sites carrying marker-locus alleles. The calculated polyteny degree is different in various loci, and it reflects the independence of endoreduplication process in different chromosome parts. Such an endoreduplication type may be called differential endoreduplication.

The considered mechanism of excessive chromatide sites diminution is possible both in meiotic and mitotic agamospermy. In meiotic agamospermy, the ratios corresponding to free combination of chromatide sites are found out in the marker-locus located at the distance of 50 % from the centromere. There is no free chromatide combination in close linkage of marker-locus and centromere, and polymorphism is determined by means of chromosome segregation complemented by the combinatory process expressed in only heterozygous cells, i. e. those that got two different alleles as a result of meiosis. Let us indicate the combinatory process that leads to polymorphism in progenies obtained by meiotic agamospermy as “meiotic autosegregation”. Thus, there may be two types of “meiotic autosegregation”: chromatide autosegregation conditioned by free combination of endoreduplicated chromatide regions and chromosome autosegregation complemented by the combinatory process expressed only in heterozygous cells.

In mitotic agamospermy, free combination of endoreduplicated chromatide sites is possible in any marker-locus location on the chromosome. Let us denote the combinatory process leading to polymorphism in mitotic agamospermy as “mitotic autosegregation”.

It is possible to differentiate meiotic and mitotic autosegregation only then when chromosome autosegregation is revealed at least on one marker-trait in an agamospermous progeny produced from an individual plant. In this case, it is possible to assert the thing that, in this progeny, any phenotypic ratios – including those corresponding to chromatide site free combination – are conditioned by meiotic autosegregation.

Thus, the results presented in the contribution are an additional argument in favor of the thing that, besides the commonly known combinatory process conditioned by chromosome random divergence in meiosis and gametes random gathering in pollination, there exists one more combinatory process conditioned by a random equiprobable diminution (loss) of excessive copies of endoreduplicated chromatide sites in the cell that entered embryogenesis.

Dependence of DNA reduplication on external factors (Gendreau *et al*., 1998; Lee *et al*., 2007) allowed us earlier to make a conclusion on the thing that differential polyteny is a way of inherited information record about the acquired traits (Levites, 2005, 2007). The unique combination of endoreduplication dependence on external conditions and its effect for the ratio and composition of progeny phenotypic classes is an effective factor of plant evolution.

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