Limitation of F_1 hybrids uniformity law

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Using isozymes as markers, the data indicative of polymorphism in the progeny produced from crossing two beet plants, homozygous on contrastive alleles of enzyme loci, were obtained.

Keywords: sexual plant reproduction, agamospermy, malic-enzyme, glucosephosphate isomerase, sugar beet.

Introduction

The origin and development of genetics is based on the studies of polymorphism emerging in sexual progenies and conditioned by two independent probable processes: chromosome divergence in meiosis and chromosome integration under gametes merging. Polymorphism in the progenies that form even without meiotic genome transformation of a cell entering embryogenesis (mitotic agamospermy) indicates the existence of one more number of mechanisms that provide variability under seed reproduction (Levites et al., 1998). To explain the polymorphism in agamospermous progenies, a hypothesis suggesting chromosome sites differential polyteny in a cell before its entering embryogenesis and a loss of excessive chromatide regions under the first embryogenetic division (Levites, 2005, 2007) was set forth. According to the suggested hypothesis, only two allelic copies that attached to the nuclear membrane or nuclear matrix are left. The data on induction of inherited changes in wheat and sugar beet by means of affecting nuclear membranes assisted with

detergent Triton X-100 (Makhmudova et al., 2009, Kirikovich, Levites, 2009) are in favor of this supposition. Considerable arguments in favor of this hypothesis were obtained in the analysis of agamospermous progenies of triploid sugar beet plant (Levites, Kirikovich, 2010, 2011). Marker enzyme phenotypes and their ratios revealed in agamospermous progenies are well explained by a high polyteny level of chromosome sites coding marker enzymes in mother plant cells entering embryogenesis. And, finally, a decrease of heterozygotes share in an agamospermous sugar beet progeny (Levites, Kirikovich, 2012) is a weighty argument in favor of polyteny. This decrease is explained herein by a post-meiotic apozygotic combinatorial process that proceeds in heteroallelic (heterozygous) egg cells carrying a doubled chromosome set. This combinatorial process is a randomized equiprobable loss (diminution) of excessive chromatides by a diploid egg cell before entering embryogenesis. As a result of such a combinatorial process, three types of cells are formed, part of which replenishes homozygous classes and only the left part is the base for heterozygous progeny formation.

Thus, studies of agamospermous sugar beet progenies give more and more proofs for the hypothesis about the effect of chromosome polyteny for agamospermous progenies variability. As agamospermy is only a part of existing ways of seed reproduction, the search for an answer to the question — if polytenization of chromosomes, or their separate sites, takes place in plant sexual reproduction — is undoubtedly interesting. In this connection, the aim of the present contribution was studying variability in hybrid beet progenies.

Materials and methods

Seeds of F_1 reciprocal hybrids produced from crossing only two plants: a green plant of sugar beet inbred line KWS-9c and red table beet plant No 19. These plants were differed in alleles of gene *Gpi2* controlling the marker enzyme glucosephosphateisomerase (GPI2, E.C. 5.3.1.9.) and gene *Me1* controlling malic-enzyme (ME1, E.C. 1.1.1.40.). The sugar beet plant had genotype *Gpi2-S/*

Gpi2-S (short - *SS*) and *Me1-S/Me1-S* (short — *SS*) and, accordingly, the slow migrating GPI2 and ME1 isozymes; the red table beet plant had genotype *Gpi2-F/Gpi2-F* (short - *FF*) and *Me1-F/Me1-F* (short - *FF*) and, accordingly, fast migrating GPI2 and ME1 isozymes. These two plants hybridization process was realized on an isolated plot distant from any flowering beet plants at not less than two kilometers. GPI2 and ME1 were analyzed at the seed stage in F_1 progeny set on the green and red plants.

Isozymes electrophoretic separation in starch gel and their histochemical staining were carried out on the earlier-described methods (Vallejos, 1983; Levites, 1986). Scanning electrophoregrams was made using Biodoc device.

Results and Discussion

Isozyme spectrum GPI2 of homozygous beet plants is presented with a one-band spectrum having fast (FF) and slow (SS) electrophoretic mobility (Fig. 1). In heterozygous hybrid plants, spectrum GPI2 has parent homodimeric isozymes FF and SS and one heterodimeric isozyme FS with intermediary electrophoretic mobility (Fig. 1).

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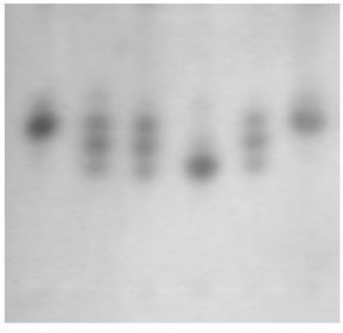

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Fig. 1. Isozyme patterns of GPI2 in beet seeds. Phenotypes: FF (1, 6), SS (4), FS (2, 3, 5). Migration is toward the anode.

Homozygous spectrum of beet ME1 is presented with a one-band spectrum

with fast (FF) or slow (SS) electrophoretic mobility (Fig. 2). Heterozygous hybrid spectrum of beet ME1 has parent homotetrameric isozymes FFFF, SSSS and three heterotetrameric isozymes FFFS, FFSS and FSSS with intermediary electrophoretic mobility (Fig. 2). Electrophoretic distinctions among 5 allelic ME1 isozymes are weak; therefore heterozygous ME1 spectrum is revealed as a regular enzyme activity zone (Fig. 2).

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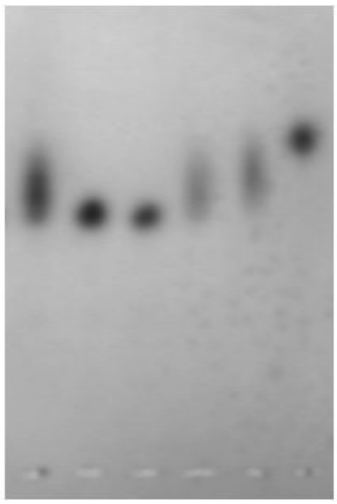

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Fig. 2. Isozyme patterns of ME1 in beet seeds. Phenotypes: FS (1, 4, 5), SS (2, 3), FF (6). Migration is toward the anode.

F_1 progenies of hybrids *sugar beet x red table beet* (short **SB x RT**) and *red table beet x sugar beet* (short **RT x SB**) were analyzed at seed stage. All the seeds **SB x RT** studied on isozyme spectra GPI2 and ME1 were classified as heterozygous. Heterozygousity of hybrids is conditioned by the thing that they were produced from hybridization of plants homozygous on contrastive alleles of genes controlling the present marker enzymes.

The basic part of seed progeny **SB x RT** had a standard three-band isozyme spectrum GPI2 typical of diploid heterozygotes, in which the heterodimeric enzyme manifests itself most intensively. Among 200 analysed F_1 seeds, three seeds were found to have a five-band spectrum GPI2 (Fig. 3) in which,

alongside with isozymes typical of a common heterozygous spectrum, there are two isozymes with a lower electrophoretic mobility.

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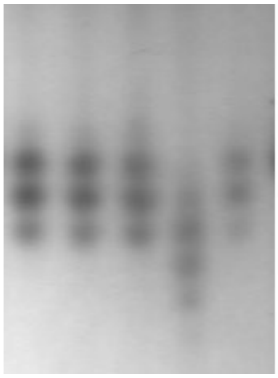

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Fig. 3. Isozyme patterns of GPI2 in beet seeds. Phenotypes: FS (1, 2, 3, 5), FSS^{M} (4). Migration is toward the anode.

It is impossible to explain the appearance of these phenotypes with the pollution of outside pollen as parent plants were growing on a well-isolated plot. It is also impossible to explain by classical mutation as abnormal phenotypic frequency (0.015) is several orders higher than that of spontaneous mutations. On the whole, emerging of abnormal phenotypes in F_1 progeny can be considered as a limitation of F_1 hybrids uniformity law.

As abnormal GPI2 spectra have not only FF, FS and SS isozymes, and also two more isozymes with electrophoretic mobility lower than that in homodimers SS, it is possible to conclude that these abnormalities are conditioned by the capability of allele *Gpi2-S* (short — *S*) to transfer into the new *Gpi2-*S^{M} (short - S^{M}) state. The same phenotypes were found by us when analyzing agamospermous sugar beet progenies (Levites, Kirikovich, 2011). Thus, the presence of five isozymes in spectrum GPI2 points out the thing that the products of three alleles *F*, *S* and S^{M} at once are present in the cell. Symmetricity of enzyme intensity distribution in the five-band spectrum indicates the two relevant moments: 1) the process of monomeres (subunits) integration into

dimeric enzyme molecules proceeds randomly and equiprobably; 2) enzyme subunits synthesized by normal alleles (*Gpi2-F* and *Gpi2-S*) and changed allele *Gpi2-S*^{M} develop in each cell at equal quantities, i.e. activities of each of two initial and one changed allele are equal.

The five-band abnormal spectrum of GPI2 is a unique phenotype in whose appearance two events happened at once: doubling of allelic dosage and transition of one of double-doses into a new state. The frequency of each of these events is not high, but their simultaneity points that the abnormality may emerge in case when an embryogenesis-entering cell has not only allele *Gpi2-F* but also two copies of allele *Gpi2-S,* one of which undergoes changes and transforms into state *Gpi2-S*^{M}.

The electrophoresis technique we use allows us to determine the phenotype of each seed right on several enzymes by means of getting some horizontal slices (sections) of starch gel and their further histochemical staining. The analysis showed that seeds having a changed phenotype on GPI2 have no change of malic-enzyme (ME1), which corresponds to the standard spectrum ME1 revealed in diploids having no abnormalities on GPI2. These data indicate the thing that the *Gpi2* dosage increase is not connected with an increase of the whole genome and/or gametes non-reduction. Appearance of an additional dose of allele *Gpi2-S* in cells of plant generative organs may be a consequence of polytenization of chromosome site carrying this allele. Independence in loci *Gpi2* and *Me1* expression, with the presence of dosal changes in one of them, is in favor of the hypothesis on differential chromosome polyteny in cells of plant generative organs. The obtained result point out the thing that differential polyteny may also manifest itself in gametes. It is this thing that is the reason for the limitation of F_1 hybrids uniformity law.

And, finally, the data obtained when analyzing **RT x SB** hybrid progeny is the most drastic expression of such variability. Among the 37 analyzed **RT x SB** hybrid seeds, a seed with malic-enzyme spectrum which was presented only by mother isozymes — namely FFFF homotetrameres were revealed (Fig. 4).

However, the GPI2 spectrum in this seed was heterozygous (Fig.5).

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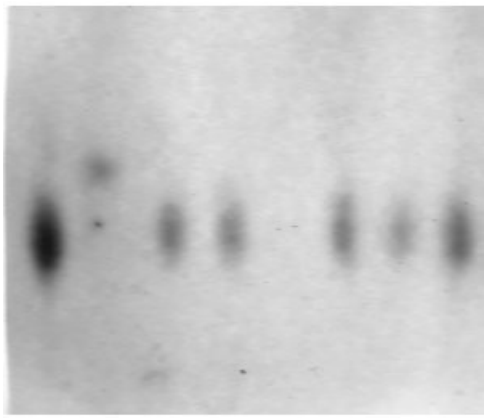

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Fig. 4. Isozyme patterns of ME1 in **RT x SB** hybrid beet seeds. Phenotypes: FS (1, 3, 4, 5 - 7), FF (2). Migration is toward the anode.

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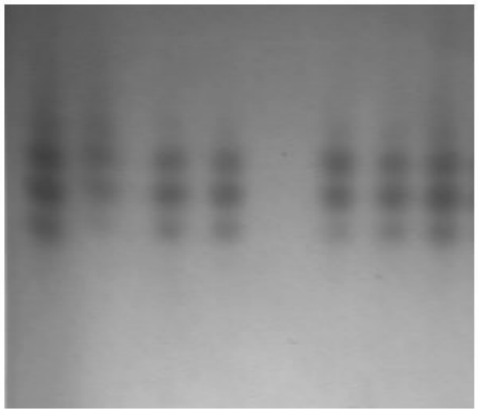

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Fig. 5. Isozyme patterns of GPI2 in **RT x SB** hybrid beet seeds. Phenotypes: FS (1 - 7). Migration is toward the anode.

This event may be explained as the egg cell introduced a high allelic *Me1-F* polyteny level into the zygote. This polyteny led to the combinatorial process proceeding in the zygote before its entering embryogenesis. As a result of this process, paternal allele *Me1-S* was lost.

The polyteny-connected changes we observed well accord with the data of

variability conditioned by gene amplification. For instant, researchers that studied the appearance of gigantic flax plants (genotrophs) characterised by increased habitus came to the conclusion that such changes are conditioned by a 9-10% of DNA content increase that proceeds not by an increase of a certain chromosome number, but by all chromosomes increase simultaneously, their separate sites being proliferated (Evans et al., 1966; Evans, 1968; Durrant, 1971; Durrant, Jones, 1971; Timmis, Ingle, 1974; Cullis, 1973). Cytogenetic amplification is expressed in an increase of certain chromosome parts having homogeneously stained sites. It is known about high mutability of loci that were subjected to amplification (spontaneous or induced) (Ignatova, 1982). This fact well accords with the appearance of abnormal GPI2 spectrum revealed in this experiment.

Amplification is usually understood as an increase of DNA sequences copies by means of repeats formations. But a different way is also possible. It is polytenization of separate chromosome sites. The presence of polytene chromosomes in cells surrounding the embryosac and in the cells of embryosac proper was shown in a number of plant objects (Nagle, 1976; D'Amato, 1984; Carvalheiro, 2000; Morozova, 2002). It is these facts that served as base for the hypothesis explaining sugar beet progenies polymorphism forming by means of agamospermy (Levites, 2005, 2007). Elimination of excessive allelic copies proceeding according to probability laws leads to outlined precised ratios of phenotypic classes in progenies indicating the existence of a combinatorial process underlying variability under agamospermy. It is combinatorial process that allows us to dwell on the thing that polymorphism in an agamospermous progeny is determined not by the emergence of allelic genes linear repeats, but their endoreduplication that leads to separate chromosome sites polytenization.

It is characteristic that the type of abnormal phenotypic classes GPI2 coincides in sexual and agamospermous progenies (Levites, Kirikovich, 2011). For example, in both cases phenotypes FSS^{M} are revealed. The affinity of abnormal phenotypes and their connection with the appearance of an additional allelic

dose allows us to hypothesize on the thing that the emergence of abnormalities is conditioned by chromosome polytenization in both agamospermy and sexual reproduction.

It is worth noting that *Gpi2* changes were added to F_1 by the egg cell. This fact well accords with the known data on the thing that, e.g. *Allium tuberosum* polytenization frequency is 80 % in female generative cells, male being 3.9 % (Kojima, Nagato, 1992). This is an additional argument in favor of the thing that the revealed variability is conditioned herein by chromosome polytenization.

Summing up the results obtained earlier and those in this contribution, it is possible to say that polytenization is a powerful variability factor – in both sexual and agamospermous plant progenies. Considering the dependence of DNA synthesis from an external (exogenic) factor (Cullis, 1973), one can assert the thing that polytenization is one of the ways to record the information about acquired traits.

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