APOMIXIS IN THE SUGAR BEET REPRODUCTION SYSTEM

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Research on five lines of sugar beet with a tendency towards apomixis showed the presence of facultative apomicts among the studied plants (2.4%). Facultative agamospermy was detected by isozyme analysis and by nuclear DNA amount estimation using flow cytometry. Genetic segregation according to isozymes in seed progenies showed the presence of meiotic agamospermy; its probable mechanism was normal meiosis in tetraploid cells of the female archespor. The occurrence of cytotically unreduced male gametophytes was confirmed in 21% of the plants by the indirect method of determining ploidy level from the number of pore regions in mature pollen grains. These studies identified homozygotic sublines with a tendency towards apomixis, proposed for practical breeding at the Kutno Sugar Beet Breeding Company Ltd., to be included in breeding experiments as components of heterotic hybrids.

Key words: Beta vulgaris, facultative agamospermy, diplospory, parthenogenesis, flow cytometry, isozymes.

INTRODUCTION

Sugar beet is a variety of the species Beta vulgaris L. (Beta vulgaris L. ssp. vulgaris convar. crassa Alef. var. altissima Döll. = var. saccharifera Lange) belonging to sect. Vulgares. Sugar beet reproduces sexually by allogamy. Breeding employs self-fertilization (Dalke, 1980), in vitro multiplication and haploid or doubled haploid production via gynogenesis (Gośka, 1997; Svirshchevskaya and Doležel, 2000). Some authors suggest apomixis (agamospermy) as another mode of sugar beet reproduction for possible use in breeding (e.g., Seilova, 1996; Maletskii, 2000; Bogomolov, 2005).

The interest in apomixis is due in part to the fact that apomictic reproduction leads to maternal (metromorphous) offspring which normally are exact genetic copies of the mother plant (Nogler, 1984). Breeders hope to use apomixis to clone – through seeds – breeding genotypes including genotypes of cultivars. Work on this is in progress. Special attention is paid to crossing sexual plants with apomictic ones with the use of new techniques and technologies such as protoplast fusion, microprotoplast production and monosomic addition line production. Screening methods – flow cytometry and DNA markers – are used in crossing work (Savidan in Savidan et al., 2001), and apomictic development is studied ultrastructurally (Naumova and Vielle-Calzada in Savidan et al., 2001). Work in the last ten years involves comparing sexual and apomictic reproduction in their molecular and genetic aspects (for review see: Savidan et al., 2001). Intensive research on Arabidopsis mutants has sought a way to change sexual reproduction to apomictic reproduction (Ravi et al., 2008). Analysis of mRNA tags of apomictic and sexual ovules in the Boechera holboellii complex implicate polyploid gene dosage in the expression of asexual seed formation, and support hypotheses of deregulation of the sexual pathway (Sharbel et al., 2009). Physical mapping of the apospory-specific genomic region (ASGR) in two apomictic grasses, Pennisetum squamulatum and Cenchrus ciliaris, showed that the ASGR appears to be maintained as a haplotype even though its position in the genome can be variable (Goel et al., 2006), and genetic mapping of the ASGR in P. squamulatum using retrotransposon-based molecular markers is problematic (Huo et al., 2009).

Apomixis has been found within the genus Beta in wild polyploid species of sect. Corollinae (Barocka, 1966; Filutowicz and Dalke, 1970). Apospory and diplospory occur simultaneously in both of the apomictic species B. lomatogona and B. trigyna (Jassem and Jassem, 1970; Jassem, 1976; 1990). Embryos are formed as a result of unreduced parthenogenesis, apogamety or adventitious embry-
ony. Their development may be accompanied by autonomous endosperm. Apomixis in B. lomatogona and B. trigyna seems to be almost obligatory, with a remote possibility of polyhaploid seed formation.

Species of sect. Corollinae are interesting to sugar beet breeders since they are resistant to cold and dry periods as well as to viral diseases of sugar beet, among them mosaic virus and yellow virus. Crossing between species of Corollinae and sugar beet, intended to transmit resistance features (Cleij et al., 1968; 1976; Filutowicz and Dalke, 1970; 1972; Jassem, 1990), did not produce apomictic plants.

The search for apomixis in sugar beet began in the early 20th century. Some signals of the occurrence of apomixis in sugar beet came from Faworski (1928), Zachariév (1975) and Zajkovska et al. (1978) as mentioned by Jassem (1990). In 1981–1985, Jassem herself (1990) searched unsuccessfully for apomictic plants among several thousand male-sterile triploid and tetraploid sugar beets. Then, in an experiment aimed at reproduction of male-sterile and fertile sugar beet plants in alpine conditions, Maleškii and Maleškayâ (1996) showed that as many as 46.14% of male-sterile plants and 75.53% of fertile plants formed seeds under isolation. They concluded that environmental conditions, especially low temperature, promoted successful embryogenensis of apomictically formed embryos.

Seilova (1996) experimentally demonstrated the occurrence of facultative apomixis in sugar beet. She worked out a methodology for detecting apomictic genotypes in cultivar populations of diploid sugar beets and obtaining apomictic inbred lines. She proposed a breeding scheme that employed apomictic inbred lines and took half as long as the traditional 20-year-long scheme. She recommended apomictic inbred lines for different roles in breeding, depending on their qualities: as new initial material, donors inbred lines for different roles in breeding, dependently on their qualities: as new initial material, donors for the formation of heterosis hybrids, and also as pure-line apomictic varieties.


At the Kutno Sugar Beet Breeding Company Ltd. (KHBC), flow cytometric procedures for mass determination of plant ploidy level have yielded some evidence for the occurrence of apomictic processes in breeding material: seeds with haploid embryos, seeds with embryos of increased ploidy corresponding to \( B_m \) hybrids, and seeds formed without pollination. This supported earlier reports by the Dutch breeders Ellerton and Hendriksen (1959) on the formation of sugar beet seeds with unexpected chromosome numbers; in their opinion it showed that male-sterile sugar beet plants produced a certain number of unreduced egg cells.

In Poland, sugar beet is considered one of the most economically important crop species. It is grown for sugar production but can also be used as a source of renewable energy. Thus all research broadening our knowledge of reproduction in this plant may bring measurable economic benefits. The aim of this study was to detect apomixis in sugar beet using standard isozyme electrophoresis, nuclear DNA amount estimation and pollen grain analysis.

MATERIALS AND METHODS

PLANT MATERIALS

Five lines with a tendency to apomixis (four male-sterile and one male-fertile) were selected. Male-sterile line MSMP46/94 and male-fertile line Os96 came from our breeding program at the Kutno Sugar Beet Breeding Company Ltd. Three other male-sterile lines (FC708CMS, FC607CMS, FC403CMS) were obtained from the United States Agricultural Research Station (Salinas, California). They were recommended as variously resistant to sugar beet diseases: cercospora leaf spot, rhizoctonia root rot, and beet curly top virus.

The material examined by laboratory methods consisted of 553 mother plants growing from two-year-old roots of sugar beet, and 343 seed progenies (seed sets from individual mother plants). Each seed progeny was analyzed based on a cluster of from several to 50 plants, most often 25 plants.

FIELD EXPERIMENTS

Field experiments in 2000–2005:

1. 170 diploid male-sterile mother plants reproduced in conditions with no pollen, in the greenhouse in winter or in the field with individual plants covered with isolators;
2. 35 diploid mother plants from male-sterile line were cross-pollinated with marker pollen from a fertile line in a nursery isolated by tall hemp (male-sterile line genotype \( P_gi SS, P_gm FF \times \) male-fertile line genotype \( P_gi FF, P_gm SS \));
3. 77 mother plants – diploid and tetraploid, hemimale-sterile (here: forming up to 10% fertile pollen grains) and male-fertile – were left to self-pollinate in isolation;
4. 271 mother plants – diploid and tetraploid, male-sterile, hemimale-sterile and male-fertile – were left for open pollination;
5. 28 plants (out of 553 analyzed mother plants) with fertile pollen were emasculated and isolated to verify autonomous apomixis.
METHODS

Male-sterility/fertility of plants was determined from anther and pollen morphology and by acetocarmine staining (Owen, 1945).

Ploidy level was determined indirectly from the number of pore regions in mature pollen grains, as described by Kuždowicz (1958). Pollen grains were stained with 2% nigrosin in 22.5% acetic acid. The number of pore regions was averaged from 10–30 pollen grains of each plant.

Plant ploidy was determined by measuring nuclear DNA content in somatic cells in G1 stage, using a Partec PA II flow cytometer. Young leaves or entire seeds were the material examined, and the standard was a cultivar registered in COBORU with stabilized ploidy level of material (young leaves or seeds) corresponding to the examined material. Samples were prepared according to Galbraith et al. (1983) with slight modifications. Cytograms of test results were interpreted according to Śliwińska (1997).

Electrophoresis of isozymes – genetic markers – was run on horizontal starch gel (~12%), based on the methodology described by Scandalios (1969), Wendel and Stuber (1984) and Levites (1986), slightly modified. The tissue examined consisted of seven-day-old germs grown on wet filter paper in sterile conditions in darkness at 20°C, fragments of young leaves, or sometimes entire seeds. The standards were corresponding germs, leaves or seeds of known isozyme spectrum. The plant material was homogenized in extraction buffer, pH 7.5. Electrophoresis was run for ~5 h at 0–4°C. Isozyme staining was performed as described by Vallejos (1983), Van Geyt and Smed (1984) and Levites (1986). The following enzyme spectra were stained: phosphoglucomutase PGM, phosphoglucomutase PGI, phosphoglucoseisomerase PGI, phosphoglucoisomerase PGI, phosphoglucomutase PGM, malate dehydrogenase MDH, isocitrate dehydrogenase IDH, and glutamate dehydrogenase GDH. The corresponding marker loci are Pgi, Pgm, Mdh1, Mdh2, Idh1 and Gdh (Levites et al., 1994). Leucine aminopeptidase and acid phosphastase enzymes were also stained (loci: Lap, Acp2). Diploid plants identified in electrophoretograms as fast homozygotes (fast migration on gel) were marked FF(NN), slow homozygotes SS(PP), and heterozygotes FS(NP).

The conformity of actual genetic segregations with theoretical expectations, according to isozymes, was assessed with the $\chi^2$ test, with significance at 0.05 (Łomnicki, 1995).

RESULTS

Sexual and asexual processes occurred commonly in the same plants of facultative apomict sugar beet (Tab. 1).

### TABLE 1. Results of reproduction experiments

<table>
<thead>
<tr>
<th>Male function of plant/ploidy</th>
<th>Experiment type</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Isolation</td>
</tr>
<tr>
<td>male-sterile/2x</td>
<td>autonomous apomix</td>
</tr>
<tr>
<td>hemimale-sterile/2x</td>
<td>- reduced parthenogenesis</td>
</tr>
<tr>
<td>male-fertile/2x, 4x</td>
<td>- reduced parthenogenesis</td>
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</table>

Apomixis in sugar beet

The whole set of analyses distinguished progenies resulting from hybridization (238), self-pollination (44), cross-pollination with relatives (48) and facultative apomixis (13).

The estimated yield of seeds collected from individual mother plants in the whole experiment ranged from ~0.01 g (1 seed) to 725 g. The seed yield from individual facultative apomicts varied from 0.3 g to 212.4 g (Tab. 2).

The seed production of facultative apomicts was generally higher than that of amphimicts of the same origin developing in the same conditions: on average, 26% higher than that of amphimicts.
Two results are worth considering separately. First, 7 fertile plants formed seeds after self-pollination in conditions of open pollination, without isolation, confirming the self-fertility of these plants (with a tendency towards apomixis). The seed yield (8.0–476.0 g) was double the yield of seeds collected from plants after forced self-pollination in isolation. Second, 12 hemimale-sterile plants formed seeds corresponding to self-pollination (according to isozymes). Moreover, such seeds were formed not only in conditions of forced self-pollination (in isolation) but also in open pollination conditions. The yield of seeds collected from individual hemimale-sterile plants was 0.4–297.0 g, higher than the yield of seeds collected from KHBC breeding plants after forced self-pollination (0.1–230.6 g). For hemimale-sterile plants this is unique. The majority of male-fertile and hemimale-sterile plants in this work were capable of forming unreduced pollen grains (as determined by number of pore regions). The plants presented here were considered potential facultative apomicts.

The tendency towards apomixis differed between lines. The frequency of detected apomicts ranged from 0.6% to 13.8% of examined plants and/or from 0.9% to 16.0% of distinguished seed progenies (Tab. 2).

### Autonomous Apomixis

Autonomous apomixis was shown in seed progenies Ap0012 and Ap037 (Tab. 2). Seeds Ap0012 were formed on a male-sterile mother plant in a greenhouse in winter, in conditions with no pollen. Seeds Ap037 were formed on a male-sterile mother plant in a field under individual isolation. The progenies maintained the ploidy level of the mother plants, and a homozygotic genotype at the enzyme loci that were homozygotic in the mother plants. At the loci that were heterozygotic in the mother plants, the seed progenies showed genetic segregation, indicating the operation of meiotic diplospory (Tab. 3).

Autonomous apomixis was determined in 6 facultative apomicts based on formation of seeds in emasculated flowers kept in isolation. The frequency of apomictically formed seeds ranged from 2.3% to 22.2% (Tab. 4).

### Facultative Apomixis

Facultative agamospermy was detected by flow cytometry based on embryo ploidy level (embryos formed apomictically differed in ploidy level from those formed amphimictically), or by isozyme electrophoresis (apomictic and amphimictic embryos represented the same ploidy level but differed in genotype) (Tab. 5).
Apomictically formed embryos identified by flow cytometry showed gametic ploidy level. Apomictically formed embryos identified by isozyme profile were metromorphous, of maternal type. Apomictically formed seeds constituted from 1% to 61% of the total number of seeds collected from individual facultative apomicts.

MEIOTIC DIPLOSPORY

Genetic segregation of diploid seed progenies Ap0012 and Ap037 originating from diploid mother plants as a result of autonomous apomixis, based on isozyme analysis (ratio: 1FF:2FS:1SS and 3FF:8FS:3SS), suggested meiotic diplospory (Tab. 3). The 1:2:1 ratio might be the result of disturbed meiosis in diploid megaspore mother cells (when the cell wall is formed not after the first but after the second meiotic division), leading to post-meiotic restitution of chromosome number. The 3:8:3 ratio conformed to disomic segregation of chromatid type in a heterozygotic autotetraploid (FFSS). The calculated $\chi^2$ test values are compatible with the co-occurrence of both processes in one mother plant.

In some cases the seed progeny of male-sterile mother plants developing without pollination showed an unexpected change of genotype according to isozyme profile, for example the appearance of alleles absent in the mother plants. Such cases are not presented as apomictic reproduction here.

CYTOLOGICALLY UNREDUCED MALE AND FEMALE GAMETOPHYTES

The occurrence of cytologically unreduced male gametophytes was shown by the counts of pore regions on the pollen grains (Fig. 1a–d). Table 6 shows the frequencies of plants with normal meiosis, according to number of pore regions on the pollen grains, and of plants capable to various degrees of forming unreduced pollen grains, in line FC607CMS and in the five tested lines.

Cytologically unreduced female gametophytes developed in the plants of the diploid male-sterile subline cross-pollinated with tetraploid plant pollen. BIII hybrids appeared at up to 5% frequency among daughter plants, confirming the mother plant's ability to form unreduced embryo sacs.

PARTHENOGENESIS

Reduced parthenogenesis functioned in diploid and tetraploid plants. In diploid mother plants, haploid parthenogenesis was induced by self- and cross-pollination. Seeds Sp012 and Sw01-3 (Tab. 5) as well as seeds obtained after crossing male-sterile diploid plants with tetraploid pollinators contained ~1% haploid embryos.

Reduced diploid parthenogenesis appeared in a tetraploid mother plant after forced self-pollination. Progeny seeds Sp0393 contained 20% diploid embryos and 80% tetraploid embryos.

### TABLE 3. Agamospermous progeny resulting from meiotic diplospory. Male-sterile mother plants flowered in conditions with no pollen. Diploid mother plants and diploid seed progenies

<table>
<thead>
<tr>
<th>Genotype of mother plant</th>
<th>Seed progeny</th>
<th>$\chi^2$ tests and probability P for segregation ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isozyme loci</td>
<td>Symbol</td>
</tr>
<tr>
<td>homzygotic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>heterozygotic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 MDH1 NN, IDH1 FF, GDH SS</td>
<td>PGI FS, PGM FS, MDH2 FS, LAP FS, ACP2 FS</td>
<td>Ap0012</td>
</tr>
<tr>
<td>4 PGM FF, MDH2 SS, IDH1 FF, GDH SS</td>
<td>PGI FS, MDH1 NP</td>
<td>Ap037</td>
</tr>
</tbody>
</table>

### TABLE 4. Autonomous apomixis in emasculated and isolated flowers

<table>
<thead>
<tr>
<th>Line</th>
<th>Mother plant</th>
<th>% of apomictically formed seeds</th>
<th>Collected seed progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC607CMS</td>
<td>a</td>
<td>5.8</td>
<td>P029</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>22.2</td>
<td>not collected</td>
</tr>
<tr>
<td>FC708CMS</td>
<td>c</td>
<td>2.3</td>
<td>P0219</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>14.0</td>
<td>P03104</td>
</tr>
<tr>
<td>O996</td>
<td>e</td>
<td>16.0</td>
<td>P03109</td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>4.6</td>
<td>SP0479</td>
</tr>
</tbody>
</table>
The diploid embryos in seeds Ap0012 and Ap037 described earlier are dihaploids, since they presented genetic segregations in a 3:8:3 ratio (Tab. 5). Unreduced parthenogenesis in diploid mother plants was manifested in the formation of seeds with diploid embryos that did not result from fertilization. The parthenogenetic embryos were morphotypic, of maternal type. They had a homozygotic genotype reflecting the mother plant’s genotype. Progenies Sw0431 and Sw0440 resulted from crossing plants of two sublines (male-sterile and male-ferile) of various genotypes (Pgi SS, Pgm FF × Pgi FF, Pgm SS). Progeny Sw0431 had 10% maternal embryos and 90% hybrid embryos, while progeny Sw0440 had 61% maternal embryos and 39% hybrid embryos (Tab. 5).

The hemimale-sterile diploid heterozygotic mother plant in isolation formed non-hybrid seeds (Ap0316) whose diploid embryos presented genetic segregations in the atypical ratio 1FF:1FS:1SS. The excess of homozygotes (as compared to 1:2:1 for self-pollination), confirmed by the \( \chi^2 \) test (\( \chi^2 = 0.7029; 0.90 > P > 0.70 \)), probably resulted from the functioning of parthenogenetic embryos. Homozygotic embryos could have originated as a result of endoduplication or automixis.

Parthenogenesis, both reduced and unreduced, was induced as a result of diploid male-sterile plant pollinations (line FC607CMS) with pollen of tetraploid pollinators. Such pollinations yielded up to 1% seeds with haploid embryos, up to 19% seeds with diploid embryos, and up to 5% seeds containing BIII embryos. The remaining \( \sim 75% \) were the expected triploid seeds. Haploids and B\( _m \) embryos were discussed earlier. The diploids probably resulted from unreduced parthenogenesis. Only half of them preserved the homozygotic genotype of their mother plant, however; the other diploid plants unexpectedly showed an altered though uniform heterozygotic genotype.

As a result of the studies, three sublines with a tendency towards apomixis were proposed for practical breeding at the Kutno Sugar Beet Breeding Company Ltd., to be included in breeding experiments as components of heterosis hybrids.

**DISCUSSION**

This research showed that apomixis constitutes part of the sugar beet reproduction system. It confirms the suggestion that apomixis occurs in sugar beet (Shiryaeva, 1983; Maletskii and Maleetskaya, 1996; Seilova, 1996; Perfilieva, 2003). Jassem’s failure to find apomixis in sugar beet (Jassem, 1990) may have resulted from her use of other genotypes and also from the lack of access to research methods such as isozyme electrophoresis and flow cytometry.

Based on analysis of isozymes, genetic markers (codominant inheritance), facultative apomixis has been found in Antennaria media (Bayer et al., 1990), Stemonoporus oblongifolius (Murawski and Bawa, 1994), and the European polyploid species Rubus armeniacus and Rubus bifrons (Kollmann et al., 2000). Flow cytometry was used as an efficient screening method allowing reconstruction of different seed formation pathways in studies by Matzk et al. (2001), Savidan (in Savidan et al., 2001) and Taskin et al. (2004).
The methodology and results of the presented analysis conform to a certain extent with earlier work by well-known authors (Shiryaeva, 1983; Maletskii and Maletskaya, 1996; Seilova, 1996; Perfilieva, 2003; Levites, 2002 in Maletskii and Levites, 2005; Bogomolov, 2005). They showed the facultative character of apomixis in sugar beet, as well as the occurrence of parthenogenesis and autonomous development of endosperm.

Meiotic diplospory was found in sugar beet previously by Maletskii and Maletskaya (1996), Maletskii (2000), Szkutnik et al. (2001) and Levites (2002 in Maletskii and Levites, 2005). The 3:8:3 ratio of genetic segregation observed in apomictically formed seed progenies probably resulted from normal meiosis in megaspore mother cells, which underwent endoreduplication. In their cytoembryological analyses of *Allium tuberosum*, Kojima and Nagato (1992) found diplospory, the result of meiosis in megaspore mother cells after endoreduplication. In the same work, endoreduplication occurred in the female archespor much more frequently than in the male archespor. In six cultivars, an average 80% of megaspore mother cells and 3.9% of microspore mother cells had a 4n chromosome number.

In light of these data, what is the type of diplosporous embryo sac in sugar beet? The diplosporous embryo sac of Allium type (Crane in Savidan et al., 2001) is supported by the present results of genetic segregations and also the counts of pore regions on pollen grains. The latter indicate sugar beet's ability to form unreduced male gametophytes. The formation of a diplosporous embryo sac of Allium type in sugar beet seems possible, in view of characteristics given for the *Chenopodiaceae* family (Poddubnaya-Arnoldi, 1982; Johri et al., 1992); those
two sources mention two types of embryo sacs, Polygonum and Allium, as functioning in Chenopodiaceae. Podubnaya-Arnoldi (1982) mentions apomixis – reduced parthenogenesis – among the characteristic features of Chenopodiaceae, precisely in Beta vulgaris. Hybrid line Beta M14, studied by Yan et al. (2007), was shown to be apomictic and of the gametophytic Allium odorum type.

The occurrence of diplospory of Antennaria type in sugar beet is not excluded, though in the present studies it was not shown. Jassem (1990) found this type of diplospory probable in wild apomictic species of Beta.

In considering the functioning of diplospory of Allium type, which is based on tetraploid meiocytes, it needs to be noted that many life processes in sugar beet tetraploids are delayed in relation to diploids (Jassem, 1973; Śliwińska, 1997). Thus the formation of Allium type embryo sacs from tetraploid meiocytes and the development of parthenogenetic embryos may lag in relation to apomeiotic embryo sacs developing from diploid cells and to meiotic embryo sacs originating from diploid meiocytes. In some circumstances having to do with the reproductive capabilities of mother plants and environmental conditions during flowering, however, even late-developed parthenogenetic embryos may appear stronger than amphimictically formed embryos, and they may successfully compete with them in the mother plant. Late-developed parthenogenetic embryos in diplosporous sacs of Allium type may form a component of the pleiotropic effect of apomixis described by Savidan (2001).

Genetic studies showing an unexpectedly frequent occurrence of homozygotic genotypes raise the question of the probability of autonomic parthenogenesis operating in sugar beet, similar to that described by Gerlach (1965) in tetraploid Rubus caesius (Rosaceae).

Here one cannot overlook the possibility of genotype change in apomictically formed embryos, resulting from epigenetic inheritance during apomictic reproduction. In the present experiments in embryos formed without pollination, a new allele not detected in mother plants was identified by electrophoresis, confirming epigenetic inheritance, a phenomenon described in apomictically formed progenies by Maletskii and Levites (2005). Grossniklaus (in Savidan et al., 2001) maintained that our contemporary knowledge of apomixis regulation is entirely compatible with an epigenetic view of this trait. Genomic imprinting resulting from decreased DNA methylation plays an important role in the development of autonomous endosperm. Reports by Rodrigues and Koltumow (2005) indicate the role of genomic imprinting in induction of apomixis.

In this work apomixis was found in consecutive generations only in some individuals. In line with this, Sedlovskiy et al. (2001) stated that agamospermy in sugar beet is not exhibited at the population level, and presented a way of finding individuals with apomictic reproduction.

Contemporary sugar beet breeding can take advantage of facultative agamospermy occurring in self-fertile plants with a homozygotic genotype. In self-fertile facultative apomicts (those that do not prefer foreign pollen), seed formation proceeds in two ways, as a result of apomixis or self-fertilization, which increases seed yield. Unreduced pollen grains formed by facultative apomicts probably stimulated the development of parthenogenetic embryos, thus contributing to increased seed yield. Seeds collected from homozygotic mother plants formed in those two ways have the same homozygotic genotype; they are metromorphous. Hence, homozygotic lines obtained as a result of facultative agamospermy may successfully replace doubled haploid lines, which, according to Gośka (1997), do not form many seeds. Seilova (1996) and Bogomolov (2005) based their suggestions about the use of facultative agamospermy in sugar beet breeding on homozygotic genotypes. In vitro somatic embryogenesis from unfertilized ovules may prove more efficient when ovules are taken from facultative apomicts.

Breeders hope to use obligate apomixis to clone – through seeds – heterozygotic genotypes of cultivars (for review see: Savidan et al., 2001; Sharbel et al., 2009); this probably will not be possible before apomixis is introduced de novo into sugar beet and other crops. Research on gene identification in wild apomicts, gene isolation and gene manipulation is aimed at achieving induction of apomixis in crops by genetic engineering methods (Grimanelli et al. in Savidan et al., 2001). According to Grossniklaus (in Savidan et al., 2001), if apomixis is, for instance, a result of epigenetic interaction between genomes, engineering of apomixis in sexual species will be much more complex. GISH and BAC-FISH studies of apomictic monosomic addition line Beta M14 indicate that a single chromosome is sufficient for transmission of apomixis from the wild species B. corolliflora to the genome of cultivated beet (Yan et al., 2007). The most recent comparative proteomic analysis of apomictic and sexual forms of Beta may help us to better understand the genetic mechanism of apomixis (Zhu et al., 2009).

CONCLUSIONS

The role of apomixis in the sugar beet reproduction system is relatively small, but it should not be neglected. Modern laboratory methods of isozyme (genetic marker) electrophoresis and flow cytometry permit quick and accurate diagnosis of
Apomixis in sugar beet

Apomixis. Facultative agamospermy may be used today in sugar beet breeding to improve the efficiency of homozygotic line production in vitro and in vivo.

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